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Author

Corporate Author JRB Associates, McLean, Virginia

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**Volume II
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**Review of Literature
on Herbicides, Including
Phenoxy Herbicides and
Associated Dioxins**

**Annotated Bibliography
Volume II**

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Prepared for Contracting Officer's Technical Representative:
Barclay M. Shepard, M.D.
Special Assistant to the Chief Medical Director
for Environmental Medicine (102)
Department of Medicine and Surgery
Veterans Administration
810 Vermont Avenue, N.W.
Washington, D.C. 20420

Submitted by:
JRB Associates
8400 Westpark Drive
McLean, Virginia 22102

FOREWARD

Public Law 96-151 enacted December 20, 1979, mandated the Veterans Administration to conduct "...a comprehensive review and scientific analysis" of the worldwide literature on Agent Orange and other phenoxy herbicides. The need to conduct such a review was in response to an increasing awareness among veterans the Congress and the public of the potential long-term health consequences of exposure to these herbicides and the contaminant dioxin.

This report was prepared wholly and exclusively by JRB Associates, Inc., and represents an independent assessment of the current state of science relating to the herbicides used in conjunction with the Vietnam conflict. The publication of this document does not signify that the contents necessarily reflect the views and policies of the Veterans Administration.

PREFACE

The controversy surrounding the tactical use of herbicides in Southeast Asia during the Vietnam conflict has now extended into its third decade. Few environmental or occupational health issues have received the sustained national attention that has been focused on "Agent Orange". The controversy centered first on the actual employment and subsequent ecological effects of herbicides in South Vietnam, then on the question of the safe disposal of surplus herbicide following the conflict, and lastly, on whether herbicides were responsible for health problems reported among Vietnam veterans. As each facet of the controversy came to the attention of the public, more government agencies were tasked to deal with the associated issues. Hence, today a great many branches and agencies of the Federal government are involved in seeking resolutions of the scientific, medical, legal and social problems surrounding "Agent Orange." In addition, many state governments have enacted legislation relating to this highly controversial issue.

The basis for resolving the Agent Orange controversy must in large measure stem from the results of scientific inquiry. Appropriately, the preparation of extensive reviews and summaries of the scientific literature have appeared in the past, e.g., Midwest Research Institute Report (1967), National Academy of Science Report (1974) and United States Air Force Technical Report (1978). The present report therefore has benefited from the information developed in previous reports as well as from recently published scientific data. The volume of available scientific literature pertaining to the herbicides used in Southeast Asia has increased almost geometrically since the 1967 report. Especially important has been the increase in the scientific data on the toxic contaminant 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD). The present report is published in two volumes. Volume I presents a detailed scientific assessment of the literature, and Volume II contains an annotated bibliography of the relevant literature.

Although much is known about the toxicity of the herbicides used in Vietnam, a number of gaps in the scientific knowledge are still present. It is hoped that this two-volume report may serve to focus the continuing public dialogue and future scientific inquiry on those aspects of the problem in which substantial doubt or gaps in information remain. This report should also assist researchers in identifying opportunities for the systematic development of new knowledge based on what is now known and accepted as fact.

VETERANS ADMINISTRATION
October 1981

INTRODUCTION

This annotated bibliography represents about 1,200 documents identified as relevant to the review of the scientific literature on the environmental fate and toxicity of herbicides, including phenoxy herbicides, and associated dioxins, used in South Vietnam. Over 900 of these citations, those dealing primarily with health effects, are accompanied by annotations; relevant abstracts, summaries, review articles, editorials, and documents used for background material are included in the bibliography, but are not annotated.

The bibliography is arranged alphabetically by author. A subject index is included in the back of the bibliography.

Herbicides described in this literature include: Herbicides Orange, Orange II, Purple, Pink, Green, White, Blue (cacodylic acid), Dinoxol, Trinoxol, Bromacil, Diquat, Tandex, Monuron, Diuron, and Dalapon. The key constituent compounds include: 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, 2,3,7,8-tetrachlorodibenzo-para-dioxin, cacodylic acid, and picloram.

The following abbreviations are used in the annotations:

2,4-D	2,4-dichlorophenoxyacetic acid
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
TCDD	2,3,7,8-tetrachlorodibenzo-para-dioxin or 2,3,6,7-tetrachlorodibenzo-para-dioxin
ppm	parts per million
ppb	parts per billion
ppt	parts per trillion
kg	kilogram (doses expressed per kg always refer to kg of body weight unless otherwise designated)
(u)g; ug	microgram
mg	milligram
mM	millimolar

ANNOTATED BIBLIOGRAPHY

1. Aberg, B., and Eliasson, L. The herbicidal effects of phenoxy compounds. In Chlorinated Phenoxy Acids and Their Dioxins. ed. C. Ramel (Ecol. Bull. no. 27, Stockholm: Swedish Natural Science Research Council, 1978) pp. 86-100.

[Review article.]

2. Abou-Donia, M. B., Charles, J. M., and Menzel, D. B. (1976) Uptake and metabolism of pesticides by the isolated perfused rat lung. Pharmacologist 18(2):247.

[Abstract, only.]

3. Abo-Khatwa N., and Hollingworth, R. M. (1974) Pesticidal chemicals affecting some energy-linked functions of rat liver mitochondria in vitro. Bull. Environ. Contam. Toxicol. 12(4):446-454.

The effects of diuron, 2,4-D, 2,4,5-T and 2-(2,4,5-trichlorophenoxy) propionic acid (2,4,5-TP) on rat liver mitochondrial function in vitro were described. The herbicides were added to mitochondrial suspensions and 4 energy-linked functions were measured. These functions were the efficiency of respiratory chain phosphorylation (measured as the ADP to oxygen ratio), state 4 and state 3 respiration rates (the rates of oxygen uptake in the absence and presence of phosphate acceptor, respectively), and the respiratory control index (the ratio of succinate oxidation with ADP present to the rate after the acceptor is depleted). At 10^{-4} M, 2,4,5-TP reduced the respiratory control index, stimulated the state 4 respiration rate, and reduced the ADP to oxygen ratio. Diuron caused these effects at 10^{-4} M, and 2,4-D and 2,4,5-T reduced the respiratory control index and stimulated the state 4 respiration rate at 10^{-4} M. The effects of a total of 47 pesticides were studied on mitochondrial function. The authors concluded that all four compounds were uncouplers of respiratory chain phosphorylation.

4. Adamoli, P., Angeli, E., Bandi, G., Bertolotti, A., and Bianchi, E. Analysis of 2,3,7,8-tetrachlorodibenzo-para-dioxin in the Seveso area. In Chlorinated Phenoxy Acids and Their Dioxins, ed. C. Ramel. (Ecol. Bull. no. 27, Stockholm: Swedish Natural Research Council, 1978) pp. 31-38.

The authors present information on the analytical methods used in the analysis of TCDD after its release from a manufacturing plant accident in Meda, Italy on July 10, 1976. Several laboratories participated in the analysis of a large number of samples collected in two phases. During the first phase, which lasted from July 22-August 6, 1976, environmental sampling was performed to determine the distribution and concentration of TCDD in the environment. Levels of 0.1 Mg in 100g of

soil were measured using a gas chromatograph - mass spectrometer and a Finnigan 3000 instrument. The sensitivity of the instruments was 0.1 ppb. In the second phase, more precise measurements were made to determine the amount of TCDD in the environment in order to plan decontamination procedures. A sampling scheme was developed and soil samples were collected and analyzed using an analytical procedure with sensitivity reaching 1 ppt. The author concludes that samples analyzed simultaneously at several laboratories showed TCDD levels that were closely comparable.

5. "Agent Orange" - Bibliography. (1980) Personal Communication with Dr. J.A. Moore, National Institutes of Environmental Health Sciences, Research Triangle Park, NC. 76 pp.

[Bibliography.]

6. Ahling, B., Lindskog, A., Jansson, B., et al. (1977) Formation of polychlorinated dibenzo-p-dioxins and dibenzofurans during composition of a 2,4,5-T formulation. Chemosphere 33:461-468.

[Background material.]

7. Ahmed, F. E., Hart, R. W., and Lewis, N.J. (1977) Pesticide induced DNA damage and its repair in cultured human cells. Mutat. Res. 42:161-174.

The authors investigated the potential of 2,4-D, diquat and 11 other pesticides to induce unscheduled DNA synthesis in human cells in culture. The cell line VA-4, a human fibroblast cell line transformed by the virus SV-40 grown on cover slips was used as the cell culture system. Diquat and 2,4-D were dissolved in water and added to the cell cultures at 1, 10, 100 and 1000 uM for 1, 3, 5, 8, or 12h. Unscheduled DNA synthesis measured by [³H]-TdR incorporation and autoradiographic analysis was determined in cell cultures with and without (S9) metabolic activation. Silver grains in 30 cells per cover slip were counted to determine unscheduled DNA synthesis. The number of replicate slides counted was not reported. Cytotoxicity at experimental herbicide concentrations was not reported. Negative controls were included in the experiment, but no positive controls were mentioned. Results of the assay were analyzed by a t-test comparing the mean number of silver grains in controls versus treated cells. Both diquat and 2,4-D treated cultures had statistically significant increases in mean numbers of silver grains at all concentrations tested. No metabolic activation was necessary for this effect. In addition, the authors also measured 2,4-D-induced 313 nm photolysis of BudR containing repaired regions of DNA to determine the form of DNA repair induced. At 1, 10, and 100 uM, 2,4-D induced 0.7, 1.1 and 1.3 breaks per 10⁸ daltons DNA, respectively. The shape of the curve of induced breaks for 2,4-D resembled the shape of curves obtained in similar experiments using ionizing radiation and alkylating agents such

as ethyl methane sulfonate. This result suggests a similarity between alkylating agents and 2,4-D in the mechanism of induction of unscheduled DNA synthesis.

8. Ahmed, F. E., Lewis, N. J., and Hart, R. W. (1977) Pesticide induced ouabain resistant mutants in Chinese hamster V79 cells. Chem.-Biol. Interactions 19:369-374.

The authors studied the mutagenicity of 2,4-D in an assay for detection of forward mutations in Chinese hamster V79 cells. This system measures the induction of ouabain resistant mutants in an ouabain sensitive cell line. 2,4-D was tested in triplicate at a concentration of 10 uM which yielded about 40% survival. A negative control but no positive control was included in the assay. Metabolic activation was also not included in the study. The forward mutation frequency of 2,4-D exposed cells was calculated to be $25.5/10^6$ survivors while mutation frequency of controls was $1.8/10^6$ survivors. The authors concluded that 2,4-D was a weak mutagen in this test system.

9. Air Force will study men who sprayed Agent Orange. [editorial] (1980) JAMA 243(2):102-103.

[Editorial.]

10. Aitio A., and Parkki, M. G. (1978) Organ specific induction of drug metabolizing enzymes by 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat. Toxicol. Appl. Pharmacol. 44(1):107-114.

The effect of TCDD on 6 different enzyme activities from 5 rat tissues is described. Male Wistar rats were administered a single oral dose of 20 ug/kg TCDD in acetone-corn oil (1:90). After 7 days, rats were killed and microsomes were prepared from the liver, kidney, lung, intestine, and testis and assayed for various enzyme activities. Ethoxycorimarin deethylation was induced by TCDD 20 fold in the kidney, and 5 fold in the liver and lung. Benzo(a)pyrene hydroxylation activity was increased 60 fold in the kidney, 7 fold in the lung, and 5 fold in the liver. VDP glucuronosyltransferase activity was induced 7 fold in the liver and 1.5 fold in the lung and kidney by TCDD. Increased liver and kidney VDP Glucuronosyl-transferase activity was elicited in control and TCDD-treated microsomes by addition of digitonin, trypsin or phospholipase A to the incubation mixture. TCDD did not induce cytochrome C reductase activity in the liver or kidney, epoxide hydratase or glutathione s-transferase activities (with styrene oxide as substrate for these enzymes) in any tissue or any of the assayed enzymes activities in intestine or testis. The authors concluded that TCDD produced a different patterns of induction of enzymes than the patterns reported for phenobarbital and polycyclic hydrocarbon induction.

11. Akamine, E. K. (1951) Persistence of 2,4-D toxicity in Hawaiian soils. Botany 112:312-319.

The author studied the persistence of 2,4-D toxicity in soils and the influence of physical and chemical factors on persistence. Indicator crops were planted and harvested every 2 weeks from 2,4-D treated agricultural soils at five locations. Higher soil temperatures resulted in a more rapid rate of 2,4-D dissipation. High pH values also resulted in more rapid dissipation. High aerobic bacterial counts were associated with shorter retention times of 2,4-D in the soils. Organic matter content was not correlated with 2,4-D persistence. The author concluded that there was a considerable variation of 2,4-D toxicity persistence among the soils.

12. Akermark, B. Photochemical reactions of phenoxy acids and dioxins. In Chlorinated Phenoxy Acids and Their Dioxins. ed. C. Ramel (Ecol. Bull. no. 27, Stockholm: Swedish Natural Science Research Council, 1978) pp. 75-81.

[Background material.]

13. Albright, R., Johnson, N., Sanderson, T. W., Farb, R. M., Melton, R., et al. (1974) Pesticide residues in the top soil of five west Alabama counties. Bull. Environ. Contam. Toxicol. 12(3):378-384.

The authors reported on the occurrence of 2,4,5-T in five west Alabama counties. In three of the counties, Greene, Sumter, and Marengo, over 60% of the land area was farmland, while in Tuscaloosa County 27% was farmland and in Pickens County 42% was farmland. Amounts and types of pesticides applied to the sampling areas could not be documented. At least 5-100 g soil samples were collected in each county and analyzed by gas-liquid chromatography. 2,4,5-T was found in 24% of the samples analyzed. Concentrations of 2,4,5-T were less than 0.1 ppm. The authors noted that heaviest concentrations of pesticide residues were found in new fields, old farm fields, run-offs and drainage ditches.

14. Aldred, J. E., Belcher, R.S., Christophers, A.J., Clements, A., Danks, D.M., et al. (1978) Report of the consultative council on congenital abnormalities in the Yarram district. Presented to both Houses of the Australian Parliament.

The relationship between birth defects that occurred in the Yarram district of Australia and use of the herbicides 2,4-D and 2,4,5-T were investigated. Between 1975 and 1978 8 cases of abnormal (human) births and birth effects occurred in the yarram district and were investigated. The incidence of abnormal births in this time period was compared statistically to incidences between 1960 and 1977 for this district. Veterinarian reports of abnormal births and a review of scientific literature related to the teratogenicity of 2,4-D, 2,4,5-T and TCDD were used to evaluate the significance of the abnormal human

births related to herbicide use. The abnormal births included 2 premature births (at 22 and 27 weeks of gestation), phocomelia of a child whose mother resided outside of Yarram until after the infant was born, cystic hygroma of a child born in 1978, 2 cases of spin bifida, 1 case of anencephalus, and 1 case of renal agenesis. The incidence of 4 neonatal deaths due to birth defects (the last 4 cases listed) among 278 births between 1975 and 1977 exceeded the expected rate of 2.7 per 1000 births. From the case records, no direct herbicide exposure could not be established for any of the 8 mothers. The times of conception also did not correlate with seasons of heavy herbicide usage and to usage was not notably elevated in 1975-1977. From animals studies doses below teratogenic levels for 2,4-D, 2,4,5-T and TCDD were estimated, as well as food residue levels. Consumption of enough contaminated food to reach an effective herbicide level required that a 60 kg pregnant woman ingest unreasonable volume of food (6,000 kg of meat or 60,000 liters of water). No evidence of teratogenic effects in animals was reported in Yarram in 1975-1977. The 3 birth defects related to neural tube closure were considered to be non-specific, with 53 other potential etiologies, suggested by the authors. The committee concluded that available information did not include any evidence of birth defects that could be attributed to the use of 2,4-D or 2,4,5-T in Yarram.

15. Allebone, J. E., Hamilton, R. J., and Ravenscroft, B. (1975) Environmental organic chemistry of 2,4-dichlorophenoxyacetic acid. Environ. Chem. 1:160-190.
[Review article.]
16. Allen, A. M. (1977) Skin Diseases in Vietnam, 1965-72. In Internal Medicine in Vietnam. U.S. Army, Washington, D.C. pp. 1-13, 29-51, 125-129.
[Background material.]
17. Allen, J. R., Barsotti, D. A., Lambrecht, L. K., and Van Miller, J. P. (1979) Reproductive effects of halogenated aromatic hydrocarbons on nonhuman primates. Ann. N.Y. Acad. Sci. 320:419-425.
[Review article.]
18. Allen, J. R., Barsotti, D. A., Van Miller, J. P., Abrahamson, L. J., and Lalich, J. J. (1977) Morphological changes in monkeys consuming a diet containing low levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Fd. Cosmet. Toxicol. 15:401-410.

The subacute toxicity of TCDD in the monkey is described. Eight young female Rhesus monkeys were fed a diet containing 500 parts per trillion (ppt) TCDD for 9 months. Monkeys were observed daily, food intake was

monitored daily, hematology and blood chemistry were analyzed monthly, and autopsies were performed, including gross and microscopic examinations only on the monkeys that died. Five of the eight animals died; these deaths occurred between months 7 and 12. By 3 months, periorbital edema, loss of facial hair and eyelashes, and dry scaly skin were observed and worsened during the next 3 months. Food intake was unaltered, but all monkeys lost weight. The hematological alterations were decreased hemoglobins and hematocrits by 6 months in all animals and, prior to death, slight increase in albumin and decrease in glutamic-pyruvic transaminase levels. Four monkeys died between 7 and 11 months from the start of TCDD exposure, and all four developed anemia, thrombocytopenia, and leukopenia prior to death. One survivor developed the last two symptoms at 12 months, and all three survivors continued to lose hair and show periorbital edema after exposure ended. Additional observations at necropsy included ascites, edema and numerous hemorrhages in many organs, abnormal nail growth, lymph node atrophy, bile duct distention, degeneration or atrophy of bone marrow and lymphopoietic tissues, and cellular hypertrophy and hyperplasia of epithelia, metaplasia of mucus-secreting cells, including sebaceous glands, and keratinization of hair follicles and adjacent sebaceous glands.

19. Allen, J. R., and Carstens, L. A. (1967) Light and electron microscopic observations in Macaca mulatta monkeys fed toxic fat. Amer. J. Vet. Res. 28(126):1513-1526.

The effects of toxic fat, which was lethal to exposed chickens, were studied in monkeys. Rhesus monkeys were fed diets containing from 0.125 to 10% toxic fat. Monthly analyses of blood for hematologic and biochemical parameters, liver biopsies and body weights were evaluated. Gross and light and electron microscopic examinations were performed when death occurred. Mean survival times were related to dose and varied for 91 days for groups fed the 5% and 10% toxic fat diets to 445 for the group fed 0.125% toxic fat. The clinical and pathologic findings were the same for all groups. Alopecia and subcutaneous edema developed 1-2 months prior to death. Edema appeared on the lips and eyelids, then included the face and finally the whole body. Anorexia and diarrhea occurred, along with ascites, hydrothorax and hydropericardium. Hematologic changes included decreased erythrocyte and leukocyte counts. Total serum protein levels decreased. Cardiac dilation, myocardial hypertrophy and edema, reduced hematopoiesis, reduced spermatogenesis, blood vessel degeneration, focal necrosis of the liver and gastric ulcers occurred to exposed monkeys. Detailed descriptions of these findings are provided at the light and electron microscopic levels. No evidence of acne was observed and the thymus was not examined. The authors concluded that hepatic parenchymal cells, endothelium and myocardium are the most sensitive tissues to the toxicity of toxic fat. At the time of publication, the authors were unaware of the identity of the toxic components in toxic fat, which have been identified subsequently and include several dioxins, including TCDD.

20. Allen, J. R., and Van Miller, J. P. (1978) Health implications of 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure in primates. In Pentachlorophenol, K.R. Rao, ed. (New York: Plenum Publishing Co.) pp. 371-379.

[Review article.]

21. Allen, J. R., Van Miller, J. P., and Norback, D. H. (1975) Tissue distribution, excretion, and biological effects of [¹⁴C] tetrachlorodibenzo-p-dioxin in rats. Fd. Cosmet. Toxicol. 13:501-505.

The tissue distribution, clearance, and toxicity of TCDD was studied in the rat. Male Sprague-Dawley rats (40 per group) were administered 50 ug/kg [¹⁴C]-TCDD in corn oil by gastric intubation or vehicle only. After 1-21 days, 5 rats per group were killed, tissues were weighed, and portions were prepared for light and electron microscopy and analyzed for radioactivity. Hepatic microsomal enzymes were assayed and liver homogenates were analyzed for RNA, DNA, and protein. Of 10 rats per group that were not scheduled to be killed at specified times after TCDD exposure, half had died within 25 days and the survivors had lost an average of 76 g of 200 g initial body weight. Anorexia did not occur and all hematologic and blood chemistry tests were normal. During the first 3 days, 25% of the dose of radioactivity was excreted in feces and 1-2% per day was excreted thereafter. By day 21, 4.5% of the dose had been excreted in urine. Histological changes included lipid droplets and proliferation of the smooth endoplasmic reticulum of the liver and a paucity of cortical thymocytes. Grossly, liver enlargement and thymic atrophy were observed. The levels of radioactivity were 6-7 times higher in the liver than in other tissues. The levels in the liver remained constant for the first 2 weeks and dropped by 50% in the subsequent week. Most of the hepatic radioactivity was associated with the microsomal fraction. The fat had the second highest concentration of radioactivity and the skin accounted for the second highest portion of the body burden. The half-life of TCDD clearance was estimated at 16-21 days. Liver homogenates from exposed animals had decreased protein content and decreased esterase, glucose-6-phosphatase and aromatic hydroxylase activities. The authors concluded that the proliferated endoplasmic reticulum was the site of TCDD localization and that hypoactivity of the associated enzymes occurred. The authors also concluded that TCDD tissue distribution contrasted that of polychlorinated biphenyl, concentration of TCDD in fat remained constant while the fat depots were depleted, and the concentration of TCDD in the liver declined substantially.

22. Allred, P. M., and Strange, J. R. (1977) The effects of 2,4,5-trichlorophenoxyacetic acid and 2,3,7,8-tetrachlorodibenzo-p-dioxin on developing chicken embryos. Arch. Environ. Contam. Toxicol. 6(4):483-489.

The effects of 2,4,5-T and TCDD on chicken embryo viability and liver weight were evaluated. Test compounds were administered in acetone

into the airspace of eggs and on day 18 the eggs were opened, embryos were examined for viability and weighed. Livers were weighed and the DNA and RNA contents were analyzed. The LD₅₀ values were 133.1 mg of 2,4,5-T per kg of egg weight 2.4×10^{-4} mg/kg for TCDD. TCDD contamination of 1.8 ppm in the LD₅₀ dose of 2,4,5-T would be required to account for the effect of 2,4,5-T. A synergistic effect was observed on embryo viability when the 2 compounds were administered together. The slopes of the probit plots of the data for each compound were substantially different, indicating (along with the synergistic effect) that the compounds probably have different modes of action. Liver weights relative to egg weights were significantly increased in 2,4,5-T treated eggs but not in TCDD-treated eggs. The hepatic nucleic acid levels did not increase in relation to increases in liver size, and did not support the hypothesis that hyperplasia accounted for increased liver size, but rather suggested that hypertrophy from increased lipid content causes liver cell enlargement. The changes in RNA and DNA content were examined at 1 dose of 2,4,5-T and of TCDD, a dose-related response has not been demonstrated and concomitant changes in the vehicle controls complicate the interpretation of these results.

23. Althouse, R., Tomatis, L., Huff, J., and Wilbourn, J. (1979) Chemicals and Industrial Processes Associated with Cancer in Humans. International Agency for Research on Cancer, World Health Organization, Lyon. pp. 46-47.

[Review article.]

24. Altom, J. D., and Stritzke, J. F. (1973) Degradation of dicamba, picloram and four phenoxy herbicides in soils. Weed Sci. 21(6):556-560.

The authors determined the degradation rates of 2,4-D, 2,4,5-T, picloram, and several other pesticides in three soils. Two of the samples were forest soils and one sample was a grassland soil, but all of similar composition. Collections were made of the top 2.5 cm of soil from the three sampling areas. Each soil sample was mixed and 130 g aliquots were placed in styrofoam cups. The cups were placed in a growth chamber and kept moist to allow for good microbial growth before pesticide application. Herbicides were applied to triplicate soil samples at 4.79 ppm. Soil samples treated with 2,4-D, or 2,4,5-T were removed at days 0, 5, 10, 20, and 40 for gas chromatographic analysis, while soils treated with picloram were sampled on days 0, 20, 40, 80, and 100. The degradation of 2,4-D was the most rapid of the three herbicides with a half-life of 4-5 days. Degradation of 2,4,5-T occurred more slowly, the half life being 14 days in grassland soil and 21-24 days in forest soil. Picloram was the most persistent of the three herbicides. No half-life could be calculated, because degradation was less than 50% by the end of the experiment at 100 days.

25. Aly, O.M., and Faust, S.D. (1964) Studies on the fate of 2,4-D and ester derivatives in natural surface waters. J. Agric. Food Chem. 12:541-546.
- [Background material.]
26. American Council on Science and Health. The health effects of 2,4,5-T. March, 1981. 16 pp.
- [Background material.]
27. American Legion (1980) Statement before the Interagency Work Group to Study the Possible Long-Term Health Effects of Phenoxy Herbicides and Contaminants. Sept. 22, 1980. 4 pp.
- [Testimony.]
28. Andersen, K. J., Leightty, E. G., and Takahashi, M. T. (1972) Evaluation of herbicides for possible mutagenic properties. J. Agric. Food Chem. 20(3):649-656.

The authors evaluated the mutagenicity of cacodylic acid, 2,4-D, dalapon, diquat, diuron, monuron, picloram and 2,4,5-T in four in vitro mutagenicity assays in bacteria and viruses. The test systems used were: 1) eight strains (unspecified) of Salmonella typhimurium that revert from histidine dependence to histidine independence when exposed to a mutagenic chemical; 2) T₄ bacteriophage in Escherichia coli B cells that form morphologically distinguishable mutant plaques when exposed to a mutagen; 3) and 4) mutants of T₄ bacteriophage, AP 72 and N17 that revert to the wild type with mutagen treatment. The S. typhimurium assay was carried out as a "spot test" in which the herbicide to be tested was added as a liquid or as crystals to the surface of the agar plate. No metabolic activation system was used, therefore only direct acting mutagens could be detected. Incubation of the test compound in bacteriophage assays was in liquid suspension followed by plating. All of the herbicides tested were negative in the 4 assays. Several omissions, however, detract from the quality of the experiment: number of replications of experiments and platings were not reported; strains of S. typhimurium used were omitted; concentration of compounds tested in S. typhimurium was not included; only 1 test concentration of herbicide in the bacteriophage assays were reported; and no criteria for positive result were defined by the authors.

29. Anderson, D., McGregor, D. B., and Purchase, I. F. (1976) Dominant lethal studies with paraquat and diquat in male CD-1 mice. Mutat. Res. 40:349-358.

The authors tested diquat in a dominant lethal test in CD-1 mice. Male mice 10-12 weeks old (15 per treatment group) were given 5 daily oral doses of 0.1, 1.00, or 10.00 mg diquat in 0.5% Tween 80/kg body weight. The high dose, 10 mg/kg, was determined to be the maximum tolerated dose for these animals by a range finding experiment. Negative controls received 10 ml 0.5% Tween 80 per kg body weight. Positive control groups (15 animals per treatment) received either 5 daily oral doses 100 mg ethyl methane sulfonate in water per kg body weight or one intraperitoneal dose of 200 mg cyclophosphamide in water. Each treated male was placed with 2 virgin 8-10 week old females. After 7 days, males were transferred to cages containing a second batch of females. This process was repeated for a total of 8 weeks to insure that all phases of spermatogenesis were represented by matings. Fifteen or 16 days after caging females with males, the female mice were killed and each uterus examined for live implantations, early fetal deaths, and late fetal deaths. Data were analyzed according to analysis of variance and chi-square analysis. Number of males which successfully mated was not affected by any of the treatments according to a chi-square test. Similarly, numbers of females that became pregnant showed no statistically significant variations using a chi-square test. This indicates that diquat did not decrease fertility at the doses tested. The total implants per pregnant female and number of early deaths per pregnancy were compared statistically using an analysis of variance and Dunnett's t-test. No statistically significant differences between negative controls and the diquat treated groups were observed. However, the positive control groups had statistically significant decreases in total implants per pregnant female and increases in the number of early deaths per pregnancy. Furthermore, no statistical difference in percentage of total implants recorded as early deaths per pregnancy and percentage induced pre- and post-implantational dominant lethality was observed between negative control and the diquat treated groups. Positive controls did, however, show statistically significant differences. Late deaths were distributed evenly throughout all treatment groups. The authors concluded that there was no evidence of a dominant lethal effect in male CD-1 mice orally administered diquat.

30. Appleman, S. M. (1980) Public information aspects of Agent Orange. Presented at the 2d Continuing Education Conference of Agent Orange, Washington, DC, May 28-30.

[Background material.]

31. Aquatic Chemistry. An introduction emphasizing chemical equilibria in natural waters. ed. by Stum, W. and Morgan, J.J. (New York: Wiley-Interscience, 583 pp., 1970)

[Background material.]

32. Arkhipov, G. N., and Kozlova, I. N. (1974) Research on the carcinogenic properties of the herbicide amino salt 2,4-D. Vop. Pitan. 5:83-84.

Forty-five female and 120 male nonparous rats, weighing 80-100 g at the beginning of the study, were administered amino salt 2,4-D at .10 LD₅₀ (dose not stated) in their feed. A control group received the same diet without 2,4-D. In the test group, two rats developed tumors at 1 year 11 months; one was a fibroadenoma of the mammary gland, and the other was a hemangioma in the abdominal cavity and mesentery. Four months later, one control rat also developed fibroadenoma of the mammary gland. Three groups of approximately 100 female OSVA X S57/VL mice, weighing about 18-20 g at the beginning, were also tested. One group received .10 LD₅₀ of the herbicide in their food. A second group was administered two drops per week of a 10 percent solution in acetone of the amino salt 2,4-D on a cutoff part of the skin between the shoulder blades. The third group of mice was kept as a control. Testing was continued through the animals' lifetimes, and no tumors developed. Approximately 200 OSVA X S57/VL mice in two groups were also tested throughout their lifetimes (1 year, 8 months). Neither the age nor the weight of the mice at the time testing began is stated. In the first 3 weeks of testing, both groups were pretreated on the skin between the shoulder blades, with 1 drop of a .5 percent benzol solution of 3-methylcholanthrene. One group was then treated with a 10 percent solution in acetone of amino salt 2,4-D (purity not stated). Among the test group mice 17.7 percent developed papillomas; no changes were noted in the control group. The authors concluded that these results provided evidence of oncogenic activity in amino salt 2,4-D. However, serious reporting deficiencies in the account of this study make it difficult to assess accurately the conclusions of the authors.

33. Associate Committee on Scientific Criteria for Environmental Quality. (1978) Phenoxy Herbicides--Their effects on environmental quality. (Ottawa, Canada: National Research Council of Canada) 440 p.

[Review article.]

34. Aulicino, F., Bignami, M., Carere, A., Conti, G., Morpurgo, G., and Velcich, A. (1976) Mutational studies with some pesticides in Aspergillus nidulans. Mutat. Res. 38(2):138.

[Abstract, only.]

35. Axelson, O., and Sundell, L. (1974) Herbicide exposure, mortality and tumor incidence: An epidemiological investigation on Swedish railroad workers. Work Environ. Health 11(1):21-28.

The authors performed an historical prospective study of 348 Swedish railroad workers exposed to combinations of herbicides to determine the effect of amitrol and/or phenoxy acid exposure on mortality or tumor

incidence. The authors compared 4 groups of non-exclusive cohorts exposed to combinations of non-specified herbicides with amitrol and/or phenoxy acid. The authors also performed an age matched case-control retrospective study of phenoxy acid and amitrol to "attempt to evaluate a possible dose response relationship" and to investigate "possible co-carcinogenicity from smoking." The authors calculated the relative risk using age and sex-specific death and cancer incidence for expected rates. Data were analyzed using the Poisson distribution and one-tailed P-values. The authors observed an excess tumor incidence in the amitrol and combinations groups and an increased but not significant tumor incidence in the phenoxy acids and combinations groups. Smoking was found to have a co-carcinogenic influence. The authors failed to indicate specifically what herbicides and what combination of each herbicide was used. The authors used a limited population of 348 workers and compared them in non-exclusive groups where an individual could be in more than one group. It is not possible to determine the effects of specific herbicides on the population due to the confounding factor of mixed and multiple exposures to other herbicides.

36. Axelson, O., and Sundell, L. (1977) Phenoxy acids and cancer. Lakartidningen 74(35):2887-2888.

[Review article.]

37. Axelson, O., Sundell, L., Anderson, K., Edling, C., Hogstedt, C., and Kling, H. Herbicide exposure and tumor mortality: An updated epidemiological investigation on Swedish railroad workers. Unpublished paper. 10 pp.

A followup historical prospective study of 348 railroad workers with occupational exposure of greater than 45 days to phenoxy acid and/or assitual was performed. The population was followed from 1957 to 1978. Data were abstracted from death certificates and the National Central Bureau of Statistics. The expected number of total deaths and deaths from different tumors were calculated by multiplying the person years of observations by the cause and age-specific national death rates for males during the respective calendar years. The statistical methodology utilized was the Poisson distribution with one tailed p-values. An over mortality of 6 tumors was observed among persons with combined exposure to phenoxy acid and amitrol where 1.78 were expected. Based upon three and six observed cases, the risk ratio calculated for amitrol was 1.5 and 1.9 for phenoxy acid. Persons exposed to phenoxy acid were found to have a higher rate of tumor incidence than that demonstrated in an earlier study. This increase was associated with the increased latency period which allowed for tumor development. The authors were non-specific in detailing which and what amount of herbicides the workers were exposed to. Therefore, it is not possible to determine a specific causal association between tumor development/mortality and a specific herbicide.

38. Baader, E. W., and Bauer, H. J. (1951) Industrial intoxication due to pentachlorophenol. Ind. Med. Surg. 20(6):286-290.

Ten cases of chloracne in workers who were exposed to trichlorophenol and tetrachlorophenol are described. The plant produced pentachlorophenol only between August, 1948 and February, 1949; experimentation with trichlorophenol production in this plant was mentioned also, but the dates were not indicated. The first case of chloracne began in December, 1948 and the last case began in May, 1949. Four of the 10 men had severe lesions and 4 others had moderate lesions in the summer of 1950, and only 1 case had cleared. The skin lesions were typical of those reported for other cases of chloracne. Neuralgic pain of the lower extremities was experienced by 8 of the 10 workers, although definite signs of neuritis were not observed during neurological exams. Four workers complained of heart disorders, including palpitation and shortness of breath, 4 complained of libido, and 4 of bursitis of the elbow. Laboratory tests, which were only performed after the maximum clinical symptoms had subsided, revealed nothing significant. Irritation of the eyes and respiratory tract during exposure was attributed to pentachlorophenol. The authors concluded that the symptoms experienced by the workers resulted from their occupational exposure to a noxious substance, and proposed pentachlorophenol to be responsible for the observed toxicity. From other industrial incidents that occurred since this report was published, TCDD produced during the trichlorophenol manufacturing experiments is a likely candidate, at least as the cause of chloracne.

39. Baars, A. J., Jansen M., and Breimer, D. D. (1978) The influence of phenobarbital, 3-methylcholanthrene and 2,3,7,8-tetrachlorodibenzo-p-dioxin on glutathione S-transferase activity of rat liver cytosol. Biochem. Pharmacol. 27:2487-2494.

The enzyme kinetics of TCDD-induced glutathione-S-transferase of rat liver cytosol are described. Male SPF-Wistar rats were administered 10 ug/kg TCDD in dioxane-sesame oil (1:40) intraperitoneally on days 1 and 7. On day 13, the animals were killed and the hepatic microsomal supernatant fraction was prepared and assayed for GSH S-transferase activity using 3 different substrates, 1,2-butylene oxide (BOX), 1-chloro-2,4-dinitro-benzene (CDNB), and styrene oxide (STOX). TCDD treated rats and control rats gained weight at the same rate and liver weights were not altered by TCDD treatment. GSH S-transferase activity per gm body weight with each of the 3 substrates were significantly elevated in TCDD pretreated rats, although enzyme activity per mg hepatic protein were not significantly increased over vehicle controls. TCDD pretreatment resulted in increased apparent V_{max} values with the substrates BOX and CDNB, an increased apparent K_m^{max} value with CDNB and a decreased K_m with STOX. The effects of methylcholanthrene and phenobarbital pretreatments on GSH S-transferase activity were also described. The authors concluded that TCDD probably induced different types of GSH S-transferases than those induced by the other 2 agents.

40. Backstrom, J. The phenoxy acid problem in Sweden. In Chlorinated Phenoxy Acids and Their Dioxins, ed., C. Ramel (Ecol. Bull. no. 27, Stockholm: Swedish Natural Science Research Council, 1978) pp. 108-121.

[Review article.]

41. Babbitt, B., Risch, S., Choffnes, E., Kalis, J., Zupanic, M., et al. (1973) Effects of 2,4,5-T on human chromosomes. Genetics 71(Suppl):53.

[Abstract, only.]

42. Bage, G., Cekanova, E., and Larsson, K. S. (1973) Teratogenic and embryotoxic effects of the herbicides di- and trichlorophenoxyacetic acids (2,4-D and 2,4,5-T). Acta Pharmacol. Toxicol. 32:408-416.

The teratogenic effects of 2,4,5-T and of 2,4,5-T with 2,4-D were evaluated in mice exposed during days 6 to 14 of gestation. Daily subcutaneous doses of 110 or 50 mg/kg of the butoxy ethyl ester of 2,4,5-T with less than 1 ppm dioxin contaminant or of 2,4-D and 2,4,5-T (2:1; both as acids) were administered in dimethyl sulfoxide. On day 18 of gestation, the numbers of dead, resorbed and viable fetuses were determined. Living fetuses were examined for gross malformations. Skeletal defects were observed in alizarin red-stained fetuses and internal malformations were observed in fetuses fixed in Bouin's fluid. The only adverse effect observed in the pregnant dams was necrosis at the site of injection. All treatment groups had more litters with at least one resorbed fetus (75-100%) than the vehicle control group (58%). Substantial increases in the percentage of resorbed implantation sites and incidence of cleft palate and decreases in fetal weights were produced in the treatment groups. The incidences of rib and vertebral malformations and of hemorrhages increased 2-3 times in the high dose groups, compared to the vehicle controls. Hemorrhages were observed in various locations, but not in the gastrointestinal tract. No cystic kidneys were observed. The data was not analyzed statistically. The authors indicated that the doses of phenoxyacetic acid compounds that were teratogenic in animals were extremely high compared to levels of likely human-exposure.

43. Baker, D. L., Ramsey, F. K., and Sylvester, E. P. (1953) Suspecting poisoning of dogs from eating grasses treated with 2,4-D. North Am. Vet. 34:194.

The effects of oral administration of 2,4-D to dogs is reported. Grass was sprayed with 4 lb per acre of 2,4-D butyl ester and was harvested 2 days later. The grass was then mixed with dog food and administered to 2 dogs for 2 days. No decrease in food consumption or change in behavior was noted in either animal over the next 2 days. The dogs were then administered 500 mg/kg 2,4-D butyl ester in a gelatin capsule. No changes were seen in 1 dog over the next 82 days or in the

other dog over the 4-day period after feeding or at necropsy at this time. The authors concluded that suspected poisoning of dogs admitted to clinics was unlikely to be caused by ingestion of 2,4-D.

44. Baldwin, R. C., Pasi, A., MacGregor, J. T., and Hine, C. H. (1975) The rates of radical formation from the dipyridylum herbicides paraquat, diquat, and morfamquat in homogenates of rat lung, kidney, and liver: an inhibitory effect of carbon monoxide. Toxicol. Appl. Pharmacol. 32(2):298-304.

The formation of diquat-derived free radicals was determined in various rat tissues in vitro. The lungs, liver, and kidneys of male Sprague-Dawley rats were removed, homogenized, centrifuged, and incubated in the presence of 10^{-6} M diquat. Formation of diquat free-radicals was monitored by an increase in absorbance (at 435 nm). The inhibitory effects of carbon monoxide, 1.0 mM potassium cyanide, and 1.0 mM SKF 525-A on radical formation were tested. The rates of radical formation were 5.4, 2.1, and 1.1 mol/liter/min/mg protein for lung, lung, and kidney, respectively, and in the presence of carbon monoxide were 0.2 to 0.4 times these rates. No other inhibitors altered the rate of radical formation. The formation of radicals from paraquat and morfamquat were also described. The authors concluded that the organ selectivity observed for the 3 cations studied did not result from differences in radical formation by different tissues and that diquat radicals were the only radicals formed by a carbon-monoxide-sensitive enzyme system.

45. Bamesberger, W. L., and Adams, D. F. (1966) An atmospheric survey for aerosol and gaseous 2,4-D compounds. Advan. Chem. Ser. 60:219-227.

[Background material.]

46. Bankowska, J., and Bojanowska, A. (1973) Induction effect of diuron and linuron in short-term chronic experiments in adult rats. Rocz. Panstu. Zabl. Hyg. 24(4):493-505.

[Foreign language.]

47. Barnett, A. P., Hauser, E. W., White, A. W., et al. (1967) Loss of 2,4-D in washoff from cultivated fallow land. Weeds 15:133-137.

[Background material.]

48. Barr, M. Jr., Keller, C. A., Rogan, W. J., and Kline, J. (1979) Summary of the workshop on perinatal and postnatal defects and neurologic abnormalities from chemical exposures. Ann. N.Y. Acad. Sci. 320:458-472.

[Review article.]

49. Barsotti, D. A., Abrahamson, L. J., and Allen, J. R. (1979) Hormonal alterations in female rhesus monkeys fed a diet containing 2,3,7,8-tetrachlorodibenzo-p-dioxin. Bull. Environ. Contam. Toxicol. 21(4-5):463-469.

Steroid hormone levels were monitored in female monkeys that were administered TCDD-contaminated diets for 9 months. Female rhesus (Macaca mulatta) monkeys (8 per group) were administered feed containing 500 ppt TCDD or a control diet for 9 months. One treated monkey had an unsuccessful mating history and was eliminated from the study; all other treated animals had previously given birth to normal offspring. Food intake was monitored daily. Hematologic parameters and blood chemistries were assayed monthly. Serum progesterone and estradiol levels were monitored for a complete menstrual cycle, 1, 3, and 6 months after the experimental diets were started. Mating with control males was initiated 6 months after TCDD began and was conducted during the appropriate time of the menstrual cycle. Pregnancy was confirmed by a mouse bioassay for monkey chorionic gonadotropin and by palpation of the uterus (Morphological changes were reported separately; see Allen et al, 1977). The length and duration of the menstrual cycle was not altered in monkeys fed TCDD for 6 months. Of the treated animals that remained in the study, 3 had reduced progesterone levels and 2 of these 3 had reduced estradiol levels as well, compared to pretreatment levels; 2 had anovulatory patterns and 2 had normal patterns. All of the control monkeys and 1 treated monkey with normal steroid patterns conceived and delivered healthy infants. After the treated infant was weaned, normal menstruations were re-established. The second monkey with normal steroid patterns and the monkey with only decreased progesterones both conceived and then aborted (on days 46 and 62). Both of these monkeys showed prolonged implantation bleeding. The other monkeys failed to conceive. The 2 monkeys with both steroid levels diminished also had intense and sporadic menstruations. Except for 1 anovulatory monkey and the monkey that delivered normally, all other treated monkeys died between the seventh and twelfth months of the experiment. After the anovulatory monkey was returned to a control diet, both steroid patterns returned to normal and this animal was bred and delivered a normal infant. Food consumption of the treated monkey that delivered normally was not reduced. The authors concluded that reproductive toxicity from TCDD was reversible in the monkey that survived TCDD intoxication and suggested that the effects of TCDD on steroid hormones were related to placental and maternal hepatic microsomal enzyme effects of TCDD.

50. Bashirov, A. A. (1969) Health conditions of workers producing herbicides of amine salt and butyl ester of 2,4-D acid. Vrach. Delo. 10:92-95.

A summary is given of a survey of the health status of 292 workers from a factory that produces the amine salt and butyl esters of 2,4-D. The group surveyed included 248 men, 264 of the 292 workers were between 21 and 40 years of age, and 194 were exposed for less than 36 years. Frequent headaches that intensified at the end of the work-day were

reported by 63% of the workers, 33% reported dizziness, 27% had respiratory problems during physical stress, 18% reported pain in the region of the heart, 52% complained of digestive problems, and 61% had functional disturbances related to the nervous system. An objective health examination was conducted on 50 workers who were free of illnesses prior to employment in the 2,4-D plant and the results were compared with those of a control group of 20 subjects who were not exposed to toxic substances. Cardiovascular parameters of the experimental group (but not the control group) were reported. Parameters related to the digestive system were examined and revealed compromised function in the exposed group, although the methods used and data were not reported. The author concluded that employment in herbicide plants can lead to diminished health of the digestive and cardiovascular systems.

51. Basrur, S. V., Fletcher, R. H., and Basrur, P. K. (1976) In vitro effects of 2,4-dichlorophenoxyacetic (2,4-D) on bovine cells. Can. J. Comp. Med. 40(4):403-415.

The effects of 2,4-D treatment in vitro on bovine fetal muscle cells were examined histologically. Limb muscles from 2.5-4.5 month bovine fetuses were cultured as cell suspensions for 1 day and then aliquots of cells were cultured in the presence of 2 or 20 mg/liter 2,4-D (99.6% purity). After a 24-96 hour culture period, cell counts were taken and cells were stained for light microscopy. Counts of differentiating cells (multinucleated cells with linear striations), degenerating cells (interphase cells with pyknotic nuclei or abnormal mitotic cells) and polyploid cells (with over double the nuclear size of the majority of cells in the field) were counted. In control cultures, fibroblast-like cells on day 1 became confluent by day 2-3 and resembled fusing myoblasts by day 3. Cells treated with the higher dose of 2,4-D contained more differentiating cells and were in more advanced stages of differentiation (more nuclei per myotube) than controls, at 72 hours. The numbers of metaphase cells, polyploid cells and degenerating were higher in cells treated with either concentration of 2,4-D than in control cells. Abnormalities of treated cells included unipolar and tripolar spindles of mitotic cells, malorientation of the mitotic apparatus and mitosis of myoblasts undergoing myogenesis. The authors concluded that 2,4-D exerted a mitostatic effect on dividing cells and a mitogenic effect on postmitotic myogenic cells.

52. Bastomsky, C. H. (1977) Enhanced thyroxine metabolism and high uptake goiters in rats after a single dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Endocrinology 101:292-296.

The effect of TCDD pretreatment on thyroid hormone metabolism and excretion were studied in the rat. Rats were administered 25 ug/kg TCDD in acetone-corn oil (1:9) by stomach tube 9 days prior to measuring biliary excretion. L-[¹²⁵I]T₄ was injected intravenously and bile was collected from cannulated bile ducts for 1 hour. Plasma was collected at the end of the hour. All samples were counted for

radioactivity [^{125}I] triiodothyronine (T_3) was administered by the same procedure and bile was collected for 30 minutes. ^{131}I was administered to a group of rats and 4 hours later blood was collected and analyzed for T_3 by competitive protein-binding analysis and T_3 by radioimmunoassay. Thyroids were removed, weighed and analyzed for ^{131}I uptake. TCDD treatment produced a 10-fold stimulation in biliary clearance rate of [^{125}I] T_4 . Excretion of [^{125}I] T_4 increased 4-fold, with no change in bile flow rate. The proportion of biliary radioactivity as T_4 glucuronide increased from 50 to 84% with less as T_4 and I^- after TCDD treatment. T_3 excretion in bile was unaltered. Both thyroid weight and ^{131}I uptake increased and both weight of TCDD treated rats decreased. Serum T_4 was half of control and T_3 and TSH levels were elevated in serum of treated rats. Sephadex uptake of [^{125}I] T_3 from the serum of treated rats was lower than control uptake. The author concluded that TSH-stimulated thyroid secretion produced in treated rats caused elevated T_3 levels and that T_4 itself has intrinsic hormone activity rather than serving solely as a precursor.

53. Batten, C. R. (1978) The phenoxy herbicides in California, a history of the conflict. California Forest Protective Assn. 12 pp.

[Background material.]

54. Bauer, H., Schulz, K. H., and Spiegelberg, V. (1961) Arch. Gewerbepath. 18:538-555.

The clinical states are described for 9 of 31 workers, 5 years after they were involved in the manufacture of 2,4,5-T in Hamburg, Germany and had developed chloracne. Nine workers described were those who remained under medical care, 5 years after exposure ended. The dermatologic symptoms of the workers resembled chloracne in workers exposed to various chlorinated aromatics that were not present in the Hamburg factory. Chloracne was resistant to treatment and left severe scarring in some patients. All patients complained of pronounced tiredness and weakness in the legs, although polyneuropathy was not diagnosed in any of the cases. Six patients had abnormal electroencephalograms, 5 had signs of vegetative over excitability, 5 had gastrintestinal disorders, 3 cases of pathological liver biopsies were reported. All patients exhibited signs of psychological disorders, characterized by depression, fatigue, increased irritability, loss of appetite, loss of potency, and decreased mental efficiency. These symptoms were revealed by the patients and some were confirmed by physicians who conducted interviews and psychological tests of these patients. Some of these tests were briefly described. The authors summarized the dermatologic and psychologic disturbances common to chloracne that appeared in 100 workers in 3 work populations: the group described in this report, a second group of workers manufacturing 2,4,5-T in Middle-Rhein (not described further in this report) and a group of 17 workers exposed to perchlorinated naphthalenes. The authors reviewed previous reports on animal testes that identified TCDD as the probable acnegenic agent in the 2,4,5-T manufacturing process.

The authors concluded that small amounts of manufacturing byproducts were shown to produce severe industrial disease, which upon identification of the etiology, was eliminated by changing manufacturing processes.

55. Baughman, R., and Meselson, M. (1973) An analytical method for detecting TCDD (Dioxin): Levels of TCDD in samples from Vietnam. Environ. Health Perspect. 5:27-35.

[Background material.]

56. Baur, J. R., Bovey, R. W., and McCall, H. G. (1973) Thermal and ultraviolet loss of herbicides. Arch. Environ. Contam. Toxicol. 1(4):289-302.

The authors studied the thermal and ultraviolet losses of 2,4,5-T and picloram under laboratory conditions. Solutions of 100 (u)g 2,4,5-T and picloram-free acids were prepared in acetone and evaporated in beakers. Aqueous solutions of 100 (u)g K⁺ salts of the two herbicides at pH 4.0 and 7.0 were treated in the same manner. All six herbicide preparations were exposed to temperatures of 30°C and 60°C for 7 days in the dark to determine thermal loss. Measurement of thermal loss included both degradation and volatilization of the compounds. The authors made no attempt to separate the two components. Residual herbicide after 7 days was determined by gas-liquid chromatography. Thermal losses of the free acids of 2,4,5-T and picloram at 60°C were 55% and 24%, respectively. No losses of other herbicide were observed at 30°C. Exposure of the potassium salts of 2,4,5-T to 30°C at pH 7.0 resulted in a 31% loss of herbicide. No significant losses were observed at 30°C for picloram at pH 4.0 and 7.0 or 2,4,5-T at pH 7.0. At 60°C, only 2,4,5-T at pH 7.0 was thermally degraded. The herbicides were also exposed to two levels of long wave (356 nm) UV irradiation: low intensity in which the light source was 98 cm from the herbicide and high intensity in which the light source was 3 cm from the herbicide. The wavelength used simulates sunlight. Three concentrations of each herbicide were used: 10, 100, and 1,000 (u)g/dish. The potassium salt herbicides were at pH 7.0. After 7 days of low intensity exposure, the free acid and salt of both 2,4,5-T and picloram were photo-decomposed. Results of high intensity UV exposure were complicated by the fact that at such an intensity the exposure temperature reached 60°C. However, degradation of the herbicides was greater with high intensity UV irradiation than with 60°C alone. The significance of such laboratory studies to a real world situation is not known. However, the authors concluded that picloram and 2,4,5-T are degraded by both temperature and UV light under laboratory conditions.

57. Baur, J. R., and Bovey, R. W. (1974) Ultraviolet and volatility loss of herbicides. Arch. Environ. Contam. Toxicol. 2:275-288.

[Background material.]

58. Beale, M. G., Shearer, W. T., Karl, M. M., and Robson, A. M. (1977) Long-term effects of dioxin exposure. Lancet 1(8014):748.

The clinical follow-up examinations of three females exposed to TCDD are described. Following the spraying of a farm horse arena with waste-oil contaminated with TCDD, a 6-year-old female, who was in the area daily, developed nosebleeds, headaches, diarrhea, and painful micturition. Her urine was found to contain protein and red and white blood cells, and she had a contracted edematous bladder, a renal caliceal diverticulum, and a systolic murmur. Following identification of TCDD on the arena floor, the patient was removed from exposure, and her symptoms resolved within one week. The child's mother and 10-year old sister also reported symptoms of abdominal pain, diarrhea, and headaches shortly after the TCDD was sprayed. Five years later all three females received thorough examinations, which included neural, renal, hepatic, and thyroid tests, and no health problems were identified except for persistence of the heart murmur in the younger child. No other human exposures during this incident were mentioned. Many animals on the farm, including horses, dogs, cats, and birds, died.

59. Beatty, P. W. (1977) Studies of the metabolism and possible mechanisms of toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Dissertation Abstracts International Section B: Physical Sciences and Technology 38(8):3665-B.

[Abstract, only.]

60. Beatty, P. W., Lembach, K. J., Holscher, M. A., and Neal, R. A. (1975) Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on mammalian cells in tissue cultures. Toxicol. Appl. Pharmacol. 31(2):309-312.

The effects of TCDD on cell growth and morphology of cultured cells are reported. Cultures of human epithelial carcinoma cells (HeLa), normal mouse (Balb-3T3) fibroblasts, virus-transformed mouse cells (SV101), normal human diploid fibroblast cells (FS), and normal human lymphocytes (NC-37) were cultured in the presence of 10^{-6} M TCDD (98% purity). After 4 days of treatment, cells were trypsinized and counted. Aliquots of lymphocyte suspensions were removed during the treatment period, and counted or grown to confluency and prepared for electron microscopy. FS and SV101 cells were cultured in medium that contained 0.32 ug/ml [14 C]-TCDD (99% purity), lysed with sodium hydroxide and the cell lysates were counted for radioactivity. TCDD treatment did not alter cell growth rate for any of the 4 cell lines examined and produced no changes in cell morphology. The TCDD concentration for FS cells was 310 ppb and for SV101 cells was 637 ppb; the corresponding

media concentrations were 2.3 and 5.2 ppb, respectively. The authors concluded that cultured cells took up TCDD, but their growth was unaffected by the compound.

61. Beatty, P., and Neal, R. A. (1978) Factors affecting the induction of DT-diaphorase by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Biochem. Pharmacol. 27(4):505-510.

Induction of diaphorase activity in various tissues of the rat and guinea pig by TCDD is examined. Sprague-Dawley rats and Hartley guinea pigs were administered TCDD (98% purity) in olive oil, intraperitoneally. Tissues were removed 12 hours to 9 days after TCDD treatment. Liver cells were fractionated into mitochondria, microsomes and cytosol. The mitochondrial fraction was assayed for NADH-diaphorase activity and the other 2 fractions for NADPH-diaphorase activity. Brain, heart, lung, kidney, spleen, and thymus tissues were assayed for NADPH-diaphorase activity as well. Diaphorase activity in all rat extraembryonic tissues except the brain was elevated 2 days after 50 ug/kg TCDD was administered compared to vehicle controls, and all levels were higher at 9 days than at 2 (thymic atrophy at 9 days precluded analysis of thymic levels). A dose of 45 ug/kg TCDD induced rat hepatic microsomal and cytosol levels 12-24 hours later and actinomycin D pretreatment blocked this effect. A dose of 50 ug/kg TCDD to male rats or 25 ug/kg to females produced induction of diaphorase levels in all 3 subcellular fractions up to 17 times the control value by 21 days after treatment. Doses of 0.6-6.0 ug/kg TCDD to guinea pigs failed to produce a dose-related elevation in diaphorase levels of liver tissue over 7 days or of extrahepatic tissues in 48 hours, except for a doubling in lung activity. A dose of 1000 ug/kg of octachlorodibenzo-p-dioxin was required to produce a significant elevation in rat hepatic diaphorase levels 7 and 16 days after treatment. The authors concluded that the role of enzyme induction by TCDD in eliciting toxic effects is unknown, since TCDD did not induce diaphorase activity in the species most sensitive to TCDD toxicity.

62. Beatty, P. W., and Neal, R. A. (1976) Induction of DT-diaphorase activity of rat liver by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol. Appl. Pharmacol. 37(1):189.

[Abstract, only.]

63. Beatty, P., and Neal, R. A. (1976) Evidence for a role for DT-diaphorase induction in the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Pharmacologist 18(2):211.

[Abstract, only.]

64. Beatty, P. W., Vaughn, W. K., and Neal, R. A. (1978) Effect of alteration of rat hepatic mixed-function oxidase (MFO) activity on the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol. Appl. Pharmacol. 45(2):513-519.

The toxicity of TCDD was compared in male and female rats, in rats of different ages, and in rats given stimulators or inhibitors of mixed-function oxidase (MFO) activity. TCDD was administered to Sprague-Dawley rats intraperitoneally in olive oil at 4 doses, including the LD₅₀. Adult male rats were given 20-80 ug/kg doses, adult females were given 10-60 ug/kg and weanling males were given 5-50 ug/kg. The LD₅₀ values of TCDD for each group of rats given TCDD or pretreated with testosterone (100 mg/kg/day for 3 days), phenobarbital (50 mg/kg/day for 3 days), 3 methylcholanthrene (40 mg/kg), TCDD (5 ug/kg), cobaltous chloride (20 mg/kg/day for 3 days), hemin (20 mg/kg/day for 4 days), or piperonyl butoxide before TCDD and for 2 days afterwards) intraperitoneally. Hepatic microsomes were isolated from pretreated and non-treated rats and several MFO activities were measured. Adult male rats had 2-3 fold more MFO activity than adult females or weanling males (cited from another publication) and LD₅₀ value was 60 ug/kg, compared to 25 ug/kg for both adult females and weanling males. Castrated males showed significantly lower aminopyrine demethylase and aniline hydroxylase activities and a lower TCDD LD₅₀ than non-castrated males, while testosterone treated females had higher enzyme levels and a higher LD₅₀ than non-testosterone-treated females. Pretreatment of male weanling rats with phenobarbital or TCDD increased both aniline hydroxylase and benzpyrene hydroxylase activities as well as the TCDD LD₅₀ values, while 3-methylcholanthrene pretreatment elevated only the benzopyrene hydroxylase activity and the LD₅₀. Pretreatment with cobaltous chloride, hemin, or piperonyl butoxide caused decreases in the TCDD LD₅₀ which were not statistically significant and the first 2 compounds significantly decreased benzpyrene hydroxylase activity, as well. The authors concluded that the inverse relationship between MFO activity and susceptibility to TCDD toxicity probably indicates that TCDD was metabolized by the MFO system to a less toxic metabolite. Other explanations exist and verification of this conclusion awaits a demonstration of TCDD biotransformation by MFO enzymes.

65. Beck, J. Emotional struggle on Agent Orange. (1980) Akron Beacon Journal, Akron, Ohio. March 24.

[Editorial.]

66. Beck, S. (1981) Assessment of adult skeletons to detect prenatal exposure to 2,4,5-T or Trifluralin. Teratology 23:33-55.

The teratologic effects of 2,4,5-T in the mouse are described and the frequencies of 88 skeletal deviations in exposed offspring at 2 months of age are presented. CD-1 mice were administered 20 or 100 mg/kg 2,4,5-T (less than 0.5 ppm dioxin contaminant) in corn oil, by oral gavage on days 6 to 15 of gestation. Litter size, mating efficiency,

frequency of gross malformations, and fetal and neonatal deaths were recorded. At 62+2 days of age, mice were killed and skeletal structures were visualized in cleared, alizarin red stained specimens. Bones were cleared of surrounding tissue, dried, and weighed. Some measurements and observations were made from photographs of the stained articulated skeletons. Each specimen was examined for a total of 88 skeletal variants and the frequencies of each variant were determined for the 2,4,5-T-treated groups and compared to groups of untreated and of vehicle controls. Significant reductions in numbers of live newborns, mean birth weights, mating efficiency, and increased numbers of stillborns and of malformations occurred in the higher dosage 2,4,5-T group, compared to the untreated controls. Nineteen of the 88 skeletal parameters had significantly different incidences in the high dosage group than in the untreated group; (17 had increased frequencies) for the low dosage group and vehicle control group, none and one, respectively, of the variants occurred more frequently than in the untreated controls. The average difference in incidence of each variant for the treated group from control incidence was 23.7%. Types of variants that occurred more frequently in the treated group were presence of an interfrontal bone, parted frontals, fusion of the frontals, variants in cervical vertebrae, reduction in the number of cardal vertebrae, and loss of the prominent dorsal spine of the second thoracic vertebra. The authors concluded that the skeletal variant assay system they used provides a useful method for postnatal detection of prenatal exposure to potentially noxious substances. Considering the small increases in postnatal frequency of variants, the non-specific nature of the variant and the high and maternally toxic doses of 2,4,5-T administered to produce these effects, the potential of this method as a device for predicting exposure is dubious.

67. Becker, D. (1973) The effect of folate overdose and of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on kidney and liver respectively of rat and mouse embryos. Teratology 8:215.
[Abstract, only.]
68. Becroft, D. M. O. (1977) The safety of the herbicide 2,4,5-T. New Zealand Medical Journal. 35-36.
[Abstract, only.]
69. Benedetto, A. V., and Taylor, J. S. (1978) Porphyria cutanea tarda. Cutis 21:483-488.
[Review article.]
70. Benes and Scram. (1969) Ind. Med. 38:50-62.
[Not available.]

71. Benigni, R., Bignami, M., Carere, A., Conti, G., Conti, L., Crebelli, R., Dogliotti, E., Gualandi, G., Novelletto, A., and Ortali, V. A. (1979) Mutational studies with diquat and paraquat in vitro. Mutat. Res. 68:183-193.

The authors studied the mutagenicity of diquat in several in vitro mutagenicity assays, ranging from prokaryotic bacterial cells to eukaryotic mammalian cells. This battery included: 1) Ames test, Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538, with and without metabolic activation, measuring reversion from histidine dependence to histidine independence; 2) forward mutation test in S. typhimurium measuring induction of 8-azaguanine resistance in a sensitive strain; 3) DNA repair test in S. typhimurium measuring preferential killing of DNA repair deficient cells compared to DNA repair proficient cells; 4) forward mutation test in Aspergillus nidulans measuring induction of 8-azaguanine resistance and methionine suppression; 5) induction of recessive lethals in A. nidulans; and 6) induction of unscheduled DNA synthesis in a human cell line. A thorough Ames test was performed. Diquat dissolved in water was tested at 0.25, 0.5, 1, 2.5, and 5 (u)g/plate in triplicate platings. No increase in numbers of revertants was observed at any dose, either with or without metabolic activation. Both positive and negative controls were present. An adequate dose range was selected, i.e., doses ranged over several logs and included a toxic dose. A less complete experimental design was employed in the repair test in S. typhimurium. Killing of bacteria was measured in strains TA 1538, a repair deficient strain, and TA1978, a repair proficient strain. Diquat was tested at only one concentration (10 (u)g/plate), with and without metabolic activation. Data for positive controls but not negative controls were reported. Diquat had a relative activity (zone of killing of repair deficient/zone of killing of repair proficient) of 1.07, without metabolic activation, and 1.91, with metabolic activation, which was classified as a weak positive response. A forward mutation test measuring 8-azaguanine resistance was also performed in S. typhimurium strains hisG46, TA92, and TA1535. Diquat induced dose-dependent forward mutations at 0.1 and 0.25 (u)g/plate, without metabolic activation. Toxicity, evident by decreased numbers of revertants, was present at the highest concentration tested, 0.5 (u)g/plate. Results from both positive and negative controls were included. Diquat also induced forward mutations in the haploid strain 35 of Aspergillus nidulans, a yeast, in both a plate incorporation assay and a liquid suspension test. No exogenous metabolic activation system was included. Results from 5-8 replicate plates were analyzed by a student's t test. Diquat was tested for 8-azaguanine resistance in three separate experiments at 20, 100, 500, and 1,000 (u)g/plate, at 400 and 500 (u)g/plate, and at 600 and 700 (u)g/plate. Statistically significant increases (p less than 0.01) in 8-azaguanine mutants were induced by diquat treatment at concentrations of 400-600 (u)g/plate. However, in the methionine suppression plate assay using A. nidulans, no increase in mutants was observed at concentrations of diquat from 2-100 (u)g/plate. Concentrations higher than 100 (u)g/plate were toxic. In the liquid suspension test, diquat was tested at 10 mg/ml in both the 8-azaguanine resistance assay and the methionine suppression

assay. Six replicate plates were scored per treatment level. Treatment times for both assays were 0, 1, 2, and 4 hours. At 1, 2, and 4 hour treatment times, diquat significantly increased the induction of 8-azaguanine resistant mutants (p less than 0.001) and at 2 and 4 hours treatment time, significantly increased (p less than 0.001) numbers of mutants in the methionine suppression assay. Data for negative controls, but not positive controls were reported. Another strain of A. nidulans, P₃, which is diploid, was used as the indicator organism in a test for induction of recessive lethals. Diquat (10 mg/ml) was tested at treatment times of 2, 4, and 8 hours. Recessive lethals increased with increasing treatment times from 4 to 24 times that of the negative control. No positive controls were reported. To complete the test battery, induction of unscheduled DNA synthesis by diquat was studied in human cell cultures. The cell line EVE, an epithelial-like cell derived from human skin and muscle explants was used as the indicator cell. Diquat was tested at 20, 100, 1,000 and 2,000 (μ)g/ml for 1 hour of treatment time. Cells were labeled with [³H] thymidine for 4 hours. Results for both positive and negative controls were reported. Incorporation was detected by autoradiography. The mean number of grains per nucleus was 4-6 times higher in diquat-treated cells than in negative controls. Grains per nucleus increased with increasing diquat concentrations. This study represents a thorough in vitro test battery to study the mutagenicity of diquat and revealed much valuable information. Diquat was mutagenic in forward mutation tests in bacteria and yeast, DNA repair tests in bacteria and mammalian cells, and a recessive lethal test in yeast. Reverse mutation tests in bacteria were negative. The authors suggested the reason for these results may be that diquat is unable to induce frameshift or base-pair substitution type mutations, but may be able to cause other damage such as deletions, strand breaks or cross links at the gene level. Diquat was shown to cause damage at the chromosomal level (recessive lethal test) and to damage DNA (unscheduled DNA synthesis).

72. Bennett, P. N., Davies, D. S., and Hawkesworth, G. M. (1976) In vivo absorption studies with paraquat and diquat in the dog. Br. J. Pharmacol. 58(2):284P.

[Abstract, only.]

73. Berkley, M. C., and Magee, K. R. (1963) Neuropathy following exposure to a dimethylamine salt of 2,4-D. Arch. Int. Med. 111:133-134.

The health condition of a 39-year old farmer that had been exposed to 2,4-D is described. Two weeks prior to the clinical examination, the farmer had been spraying weeds with a liquid solution of 2,4-D. The solution contained about 40% 2,4-D that was 98 to 99% pure. He had repeatedly used his hands to unplug the sprayer, so came into direct contact with the solution. Initial symptoms were constant prickling of the fingers, toes and midabdomen. During the first week, this progressed into a general numbness of the extremities accompanied by muscular aches and stiffness. Despite the numbness, however, the

patient complained of hypersensitivity to touch. By the second week, manual dexterity and coordination had become unstable. Clinical examination revealed hypoactive biceps, triceps and ankle reflexes, hand uncoordination, and sensory impairment in the distal extremities. Laboratory tests were conducted, including blood analysis, x-rays, urinalysis, and EEG, but all results were within normal limits. The patient was treated with daily doses of thiamine hydrochloride and vitamin supplementation in the diet. The authors concluded that cutaneous exposure to 2,4,4D produced a primary sensory neuropathy in this patient.

74. Berlin, A. (ed.) (1976) Proceedings of the expert meeting on the problems raised by TCDD pollution. Commission of the European Communities, Milan, 1976. 178 p.

[Background material.]

75. Berndt, W. O., and Koschier, F. (1973) In vitro uptake of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) by renal cortical tissue of rabbits and rats. Toxicol. Appl. Pharmacol. 26(4):559-570.

Some characteristics of 2,4-D and 2,4,5-T accumulation by renal cortical slices from the rabbit and rat are described. Renal cortical slices were incubated in the presence of ^{14}C -2,4,-D or ^{14}C -2,4,5-T and the uptake of radioactivity by the tissue was determined at the end of the incubation period. The time-course for uptake and effects of various inhibitors and nutrients added to the incubation medium were established. Data were expressed as the ratio of slice to medium (S/M) radioactivity. The concentrations of herbicides used were 10^{-5}M (except kinetic competition studies). The S/M ratios for 2,4-D uptake were 37 for rabbit slices and 16 for rat slices and for 2,4,5-T were 59 and 30, respectively. Uptake of both compounds by rabbit slices was linear for 5 hours, and for both species was substantially reduced in the presence of 2,4-dinitrophenol, iodoacetamide, probenecid, and nitrogen. Uptake of both substances studied in rabbit slices was increased significantly in the presence of acetate, pyruvate, and lactate was significantly decreased by alpha-ketoglutarate and succinate and was unaltered by glucose and citrate. Lactate and succinate caused the same effects in rat slices as rabbit slices, but acetate was ineffective in altering uptake by rat slices. The only difference in uptake between slices from male and female rats was increased 2,4,5-T uptake by slices from males (S/M ratio = 23) than by those from females (S/M ratio = 16). From Lineweaver-Burke plots of data for both species and compounds in the presence of para-amino hippurate, transport was concluded to be mediated by a carrier, as Michaelis-Menton-type kinetics were involved. Uptake of both herbicides, studied in liver slices, approached a steady state and was inhibited by 2,4-dinitrophenol and, for only 2,4,5-T, by probenecid. The authors concluded that both phenoxy acids were transported by the

classical organic anion transport process but non-specific tissue binding also occurred because a small degree of uptake occurred in the presence of metabolic inhibitors that blocked the transport process.

76. Bernstein, J. Z. (1980a) Statement before the Committee on Veterans' Affairs, United States Senate. [Agent Orange] Feb. 21, 1980. 16 pp.

[Testimony.]

77. Bernstein, J. Z. (1980c) Statement at the Public Meeting of the Interagency Work Group to Study the Possible Long-Term Health Effects of Phenoxy Herbicides and Contaminants, Sept. 22, 1980. 10 pp.

[Testimony.]

78. Berry, D. L., DiGiovanni, J., Juchau, M. R., Bracken, W. M., Gleason, G. L., and Slaga, T. J. (1978) Lack of tumor-promoting ability of certain environmental chemicals in a two-stage mouse skin tumorigenesis assay. Res. Commun. Chem. Pathol. Pharmacol. 20(1):101-108.

The authors tested TCDD and several other chemicals for tumor promoting ability in the mouse skin tumorigenesis assay. Female CD-1 mice 6-8 weeks old, were shaved 2 days before treatment. Only mice in the resting stage of the hair cycle were used in the experiment. Pre-shaven mice (30 per group) received 200 nmol dimethyl benzanthracene (DMBA) in 0.2 ml acetone topically. One week after DMBA initiation, the positive control group received TPA in 0.2 ml acetone. Test groups received a topical application 0.1 (u)g TCDD in acetone/mouse. TCDD was one of several compounds tested. The concentration of TCDD was determined by a range-finding histological study. At 2 (u)g/mouse TCDD increased intrafollicular epidermis from 1 to 4 layers, and caused severe gastrointestinal damage, and death in 30% of the animals. At 1 (u)g/mouse TCDD caused only slight increases in intrafollicular epidermis, which the authors did not consider significant. Each compound was applied twice weekly for 30 weeks. Papillomas and carcinomas were observed weekly until the end of the experiment at 30 weeks. The positive control, DMBA, followed by TPA promotion, produced 8.1 papillomas/mouse, while the negative control, TPA promotion without the initiator DMBA, produced 0.03 papillomas/mouse. DMBA followed by TCDD promotion and TCDD alone did not induce any papillomas at the concentration tested. The authors also noted skin rashes on animals (numbers unspecified) treated. From these data, it appears that TCDD at the concentration tested does not act as a tumor promoter.

79. Berry, D. L., Slaga, T. J., DiGiovanni, J., and Juchau, M. R. (1979) Studies with chlorinated dibenzo-p-dioxins, polybrominated biphenyls, and polychlorinated biphenyls in a two-stage system of mouse skin tumorigenesis: Potent anticarcinogenic effects. Ann. N.Y. Acad. Sci. 320:405-414.

[Review article.]

80. Berry, D. L., Slaga, T. J., Wilson, N. M., Zachariah, P. K., Namkung, M. J., Bracken, W. M., and Juchau, M. R. (1977) Transplacental induction of mixed function oxygenases in extra-hepatic tissues by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Biochem. Pharmacol. 26(15):1383-1388.

The effects of TCDD on extra-hepatic maternal and fetal enzyme levels are described. Pregnant Sprague-Dawley rats were administered a single dose of 0.2-6.0 ug/kg TCDD in corn oil, intraperitoneally on day 17 of gestation. On day 20 of gestation, maternal and fetal tissues were removed and microsomes were prepared and analyzed for various enzyme activities. Dose-related increases in aryl hydrocarbon hydroxylase activities were demonstrated for fetal tissues, including lung, kidney, and skin, placental, and maternal lung, kidney, adrenal and liver. Formation of some metabolites of benzpyrene, identified by high pressure liquid chromatography were increased in TCDD-treated fetal and maternal tissues. Epoxide hydratase activity in maternal and fetal lung and fetal skin were induced almost 3 fold by TCDD. In vitro covalent binding of [³H]-benzpyrene to DNA was enhanced by tissue homogenates of TCDD-treated rats. The authors concluded that maternally administered TCDD produced a potent broadspectrum induction of carcinogen-transforming enzymes in extra-hepatic fetal tissues.

81. Berry, D. L., Zacharian, P. K., Namkung, M. J., and Juchau, M. R. (1976) Transplacental induction of carcinogen hydroxylating systems with 2,3,7,8 tetrachlorodibenzo-p-dioxin. Toxicol. Appl. Pharmacol. 36(3):569-584.

Effects of TCDD administration to pregnant rats on microsomal enzyme activities and histopathology of fetal and maternal tissues is described. Pregnant female rats were administered 0.2-6.0 ug/kg TCDD in corn oil, intraperitoneally on day 17 of gestation. On day 20 of gestation, maternal livers and adrenals, placentas, and fetal livers were prepared for light and electron microscopy and microsomes were isolated and assayed for various enzyme activities. Spectral analyses of microsomal chromosomes were performed on fetal and maternal samples. Maximum induction of fetal aryl hydrocarbon hydroxylase (AHH) was produced by 2.5 ug/kg of TCDD and this dose was used for the remaining experiments. Ring and N-hydroxylation of N-2-fluorenylacetamide (FAA) were elevated 3-20 fold in maternal liver and 30 to 300 fold in fetal liver; Placental levels were also elevated. AHH activity was increased 6-100 fold in fetal livers after TCDD treatment and 15 fold in adult liver; placental levels were also elevated. Adrenal AHH levels were not induced by TCDD and cytochrome P-450 levels increased in hepatic tissues and decreased in placenta. Formation of benzpyrene metabolites, formed by liver homogenates and identified by high-pressure liquid chromatography, was increased by TCDD in fetal and maternal tissue. After TCDD treatment, formation of doils relative to phenols was favored. Cytotoxic effects were evident in fetal hepatic tissue and in placental and maternal liver and included cellular necrosis and placental hemorrhages. the adrenals were not altered ultrastructural changes in fetal liver cells included enlarged mitochondria and rough

endoplasmic reticulum and glycogen deposits. The authors concluded that the fetus contained the genetic potential to respond to hepatic mixed-function enzyme inducers, although such fetal responses are not usually elicited.

82. Berwick, P. (1970) 2,4-Dichlorophenoxyacetic acid poisoning in man. JAMA 214(6):1114-1117.

The clinical symptoms of 2,4-D poisoning are described. A 46-year-old farmer ingested an estimated 30 ml of Knoxweed containing 7200 mg of 2,4-D, 14,700 mg of S-ethyldipropyl thiolcarbamate and 150 mg of epichlorohydrin. The formulation caused oral burning, flushing of the face, gastritis, vomiting, muscular and chest pains, and eventually respiratory, renal, and neurological disorders which subsided after several weeks. Muscular effects included fibrillary twitching, myotonia and muscle weakness, but no cardiac complications. Skeletal muscle damage was evident from elevated serum enzyme levels, and myoglobinuria. No peripheral neuropathy was ever observed, although the patient complained of insomnia and was moderately depressed. The authors concluded that the toxic effects observed in the patient were produced by 2,4-D because the normal serum cholinesterase level was not consistent with poisoning by S-ethyl-dipropylthiolcarbamate, a cholinesterase inhibitor, and only a small amount of epichlorohydrin was ingested.

83. Bethel, J. S., Turnbull, K. J., Briggs, D., and Flores, J. (1975) Military defoliation of Vietnam forests. American Forests 81(1):26-30, 27-61.

[Review article.]

84. Bezuglyi, V. P., Kokina, K. V., Komarova L. I., Sivitskaia, I. I., Ilina, V. I., Gorskaia, N. Z. (1979) Clinical manifestations of long term sequelae of acute poisoning with 2,4-dichlorophenoxy acetic acid. Gig. Tr. Prof. Zabol. 3:47-48.

The clinical symptoms of 11 workers 2 years after they were acutely exposed to 2,4-D is described. All workers were female with a mean of 42.4 years of age and with 17-32 years of field crop working experience. Exposure was described as slight for 8 workers, moderate for 2, and severe for one. Symptoms described by the patients included periodic headaches, general weakness and rapid fatigue, dizziness in 7 patients, the appearance of circles in the visual field, numbness and pain in hands and legs (8 cases) and neck (2 cases), increased irritability (5 cases) and loss of memory (2 cases). In 7 cases, headaches were accompanied by heart palpitations, shortness of breath, cooling of the extremities, profuse sweating, increased urination and sudden weakness. Dysnia occurred in 5 patients and heart irregularities and pain, in 6. Physical examinations revealed a soft grade I murmur in all patients and a systolic murmur in 4. Five patients had elevated

arterial blood pressure and 1 had low blood pressure. Various neural parameters were altered in the subjects; the methodology used for the tests and any tests in which no deficiencies were identified were not given by the authors. Gynecological examinations revealed menstrual cycle upsets in 9 of the women, but the parameters measured and types of upsets were not explained. Monocytosis, lymphosytosis, hypercholesterolemia lowered blood phospholipid levels and lowered activities of leukocyte oxidizing enzymes were mentioned. Chronic conjunctivitis in 8 subjects and toxic hepatitis in 2 subjects, remained 1 1/2 years after the exposure. The patients were treated with various therapies and all were reported to have been discharged in satisfactory condition. The authors concluded that the principal disorder in these patients was pathology of the nervous system, involving polyneuritis and dystonia. This report the reader with a dim clinical picture of the consequences of an unknown dose of 2,4-D by an unknown route because no description of the conditions of 2,4-D exposure nor an adequate description of the clinical findings, beyond summary statements is provided.

85. Bibliographic References: Dioxins. (1980) Environmental Sciences Laboratory, Mt. Sinai School of Medicine of the City University of New York, Jan. 18. 48 pp.

[Bibliography.]

86. Bignami, M., Aulicino, F., Velcich, A., Carere, A., and Mopurgo, G. (1977) Mutagenic and recombinogenic action of pesticides in Aspergillus nidulans. Mutat. Res. 46:395-402.

The authors studied the mutagenicity of dalapon, picloram, tordon, and other pesticides in Aspergillus nidulans. Three types of mutagenic events were studied: forward point mutation from 8-azaguanine sensitivity to 8-azaguanine resistance in haploid strain 35; mitotic crossing over in the diploid strain P; and mitotic nondisjunction in the diploid strain P. In all three, a spot test was employed; that is, the chemical to be tested was added to a filter disc which was placed on top of the agar surface of the test plates which contained the indicator organism. 8-Azaguanine resistance is measured by growth of colonies on medium containing the inhibitor. Both mitotic crossing-over and mitotic nondisjunction are determined by growth of mutant colonies on medium containing fluorophenylalanine. The colonies derived by crossing-over are pale green, while the nondisjunctional ones are yellow, and the tester strain colonies are white. In the forward mutation test, all three chemicals were negative according to the authors. Doses used, negative and positive controls, were not reported by the authors. No numerical data were presented. Ability of the compounds to induce nondisjunction was also tested by a non-selective test in which fluorophenylalanine was omitted. The endpoint was measured by screening colonies arising on complete medium for colored sectors. The authors reported testing a range of doses for each of the pesticides but only reported on the highest dose tested.

Dalapon, up to 0.8 mg/ml, picloram, up to 0.8 mg/ml, and tordon, up to 1.5 mg/ml, did not induce nondisjunction. Postive controls were not reported.

87. Bignami, M., and Crebelli, R. (1979) A simplified method for the induction of 8-azaguanine resistance in Salmonella typhimurium. Toxicol. Lett. 3:169-175.

The authors tested diquat and several other chemicals in a forward mutation assay and in the standard reverse mutation assay (Ames test) in *Salmonella typhimurium*. In the standard Ames test, mutagenicity is detected by induction of histidine independent revertants in a histidine dependent strain. The forward mutation assay measures the induction of 8-azaguanine resistant mutants in a sensitive strain. Diquat dissolved in water was tested at 0.1-1.0 ug/plate in a plate incorporation assay in strains his G46, TA92, TA1535, TA1538, TA100. Data reported were averages of triplicate plates; at least three experiments were performed. The herbicide was not mutagenic in the standard Ames test. In the forward mutation assay, however, diquat induced significant dose dependent increases in 8-aza guanine mutants in strains TA1535 and TA92.

88. Bimber, D. L., Boening, R. W., and Sharma, M. L. (1976) Respiratory stress in yellow perch induced by subtoxic concentrations of diquat. Ohio J. Sci. 76(2):87-91.

The authors measured respiratory stress in fish exposed to diquat. Two-year-old yellow perch were and obtained from an area untreated by herbicide at Chautaugua Lake, NY. Buccal catheters were implanted in the fish to monitor respiratory distress by the "cough reflex." Fish were placed in test chambers, and after a 24-hour acclimation period diquat was added at 1 and 5 ppm. Continuous recording of the cough response was made for 48-72 hours. One ppm diquat was, according to the authors, the dose commonly applied to the lake for weed control. Local concentrations of up to 10 ppm were often found in the lake immediately after spraying. At both concentrations of diquat tested, statistically significant (p less than 0.05) increases in cough frequency were observed. The authors concluded that toxic effects from diquat occur in yellow perch at concentrations routinely used in weed control.

89. Binns, W., and Balls, L. (1971) Non teratogenic effects of 2,4,5-tri-chlorophenoxyacetic acid and 2,4,5-T propylene glycol butyl ester herbicides in sheep. Teratology 4:245.

[Abstract, only.]

90. Bionetics Research Laboratories, Inc. (1968a) Evaluation of carcinogenic, teratogenic, and mutagenic activities of selected pesticides and industrial chemicals. Vol 1: Carcinogenic study. NTIS PB 223159.

A compilation of experimental results presented in Innes et al. (1969).

91. Bionetics Research Labs. of Litton Ind. (1968b) Progress report on program of carcinogenesis studies Vol. 2 - Teratogenic study in mice and rats. National Cancer Institute.

The teratology of 2,4-D and 4 esters, 2,4,5-T, monuron, diuron and a proprionate were studied in mice and 2,4,5-T was studied in rats. A/Ha, AKR, C3H, and C57BL6 strains of mice and BL6AK hybrid mice were administered test compounds in doses of 22-215 mg/kg subcutaneously in DMSO or orally by gavage in honey-water (1:1) on days 6-15 to AKR mice and days 6-14 for all other mice. Additional studies of 2,4,5-T were conducted in C57BL6 mice given 113 mg/kg 2,4,5-T in DMSO subcutaneously on days 9-17 and in rats orally administered 5-47 mg/kg 2,4,5-T in honey on days 10-15 or 22-47 mg/kg on days 6-15. On day 19 for AKR mice and day 18 for all other mice, fetuses and placentas were removed and weighed. The amount of amniotic fluid was calculated from the difference between gravid uterus weight and the sum of the placentas, fetuses, and uterine muscle weights. Fetuses were examined grossly and then fixed in Bouin's solution and examined for visceral anomalies or stained with alizarin red and examined for skeletal anomalies. Maternal liver and body weight were determined and fetal liver weights of mice treated with 2,4,5-T on days 9-17 were determined. Postnatal examinations were performed on some mice by recording body weights of neonates at 1 and 8 days of age and performing the gross examinations of 1 day-old neonates and necropsy and skeletal examinations on 8-day-old neonates. Results of treated animals were compared to vehicle controls. 2,4-D administration caused a decrease in fetal weight and an increased number of abnormal fetuses. No consistent, reproducible, dose-related effects were observed for the butyl, isopropyl or isooctyl esters of 2,4-D and no adverse effects followed administration of the methyl or ethyl esters. 2,4,5-T caused an increase in malformations and increases in fetal and maternal liver weight in rats and mice. Both monuron and diuron produced increases in the numbers of abnormal C57BL6 fetuses and in mortality of C3H fetuses. Alpha-(2,5-dichlorophenoxy)-propionic acid treatment yielded an increase in fetal mortality and decrease in fetal weight. The teratogenicity of a total of 48 compounds was reported in this study. The authors ranked the teratogenic potential of each compound and concluded that 2,4,5-T is probably dangerous, 2,4-D and 2,4-D isooctyl ester are potentially dangerous but need further study, 2,4-D butyl ester is fetotoxic but probably not teratogenic, 2,4-D isopropyl ester, monuron, diuron, and alpha-(2,5-dichlorophenoxy) propionic acid are unclassifiable, needing further study and the methyl and ethyl esters of 2,4-D are essentially inactive. The postnatal study was considered inconclusive because the numbers of mice of the same strain given the same treatment were too low for statistical evaluation, and in some groups no neonates survived for 8 days. The causes of death for these

neonates were unknown, but cannibalism was identified as contributing to fetal loss.

92. Bionetics Research Laboratories. (1968c) Evaluation of carcinogenic, teratogenic, and mutagenic activities of selected pesticides and industrial chemicals. Vol. 3: Mutagenic Study. NTIS PB-223 161.

The authors evaluated the mutagenicity of 2,4-D isooctyl ester, diuron, monuron, 2,4-D and several other pesticides. Mutagenicity tests were not specified, and no results were presented for these compounds.

93. Birmingham, D. J. (1964) Occupational dermatology: current problems. Skin Feb. 1964:38-42.

[Review article.]

94. Biro, P. (1979) Acute effects of the sodium salt of 2,4-D on the early developmental stages of bleak, Alburnus alburnus. J. Fish. Biol. 14(1):101-109.

The authors studied the effects of 2,4-D on fertilized eggs and freshly hatched larvae of bleak, a freshwater fish. Fish were exposed to concentrations of 0, 25, 50, 100, 200, 400, 800, 1,600 and 3,200 mg/liter 2,4-D in triplicate. Mortality in eggs and larvae was monitored every 2 hours. At 12 hours, LC_{50} for embryos was 159.4 mg/liter 111.2 mg/liter. At 48 hours, the LD_{50} for embryos was 12.9 mg/liter and for larvae 51.6 mg/liter. Effects of 2,4-D exposure included slowing or stopping of development of embryos, malformations of hatched larvae, and behavioral changes. The authors concluded that to prevent injury to littoral fish the concentration of 2,4-D in shallow freshwater lakes should not exceed 0.5-1.0 mg/liter.

95. Bjorklund, N. E., and Erne, K. (1971) Phenoxy-acid-induced renal changes in the chicken. I. Ultrastructure. Acta Vet. Scand. 12:243-256.

The subacute toxicities of 2,4-D and 2,4,5-T were studied in the chicken. Cornish chickens (15 per group) were administered 1,000 ppm 2,4-D triethanolamine salt (commercial preparation) in drinking water 1,000 ppm 2,4,5-T triethanolamine salt (a combination of technical and analytical grades) in drinking water from 5 days of age to 7 months. After 20 weeks of exposure 2 chickens from each treatment group were removed from exposure for the subsequent 8 weeks. Beginning at 18 weeks of age, 2 control chickens were administered 1,000 ppm 2,4-D for 7 months. Chickens from each group were killed after 14-201 days of exposure and tissues were removed; kidney tissue was prepared for light and electron microscopic examinations and several tissues were analyzed for herbicide levels. The only clinical sign of toxicity was general weakness, observed after both treatments. Decreased food intakes and

growth rates as well as increased relative kidney and liver weights of the treated groups were not compared statistically to the result for the control group. Grossly, the kidneys were enlarged in both treatment groups but not in the chickens that were returned to (control) water for 8 weeks. No other gross pathological changes occurred. Hypertrophy of the renal proximal convoluted tubules was observed. This lesion, and the renal ultrastructural changes were more severe after 2,4,5-T than 2,4-D exposure and occurred in chickens first exposed at 8 weeks of age, but not in chickens fed 2,4-D and then returned to water for 8 weeks prior to death. Ultrastructural changes included the presence of nuclear bodies and electron opaque regions of the nucleus, circular arrays and fusion of mitochondria and more microbodies than controls. Tissue levels reported for the liver, lung, kidney, gastrointestinal contents and egg yolk revealed relatively high kidney and egg levels for both phenoxy acids. The authors concluded that both compounds produce severe lesions in the proximal convoluted tubule, the region of the kidney probably involved in phenoxy acid excretion.

96. Bjorklund, N., and Erne, K. (1966) Toxicological studies of phenoxy-acetic herbicides in animals. Acta Vet. Scand. 7:364-390.

The acute and subacute toxicities of 2,4-D were studied in pigs, calves, rats, and chickens. The triethanolamine salt or the potassium-sodium salt of 2,4-D, or 2,4-D-butyl ester or the triethanolamine salt of 2,4,5-T orally as a single dose or repeatedly for up to 51 doses. Some animals were fed diets with 500-1000 ppm 2,4-D amine chronically. Peak plasma levels, general condition, and for some experiments histopathology and organ weights were determined. Two calves were given each salt and ester of 2,4-D as single doses of 100 mg/kg with a 4 week period separating each administration. The 2,4-D amine salt also was administered at doses of 50 and 200 mg/kg to each calf. The peak plasma levels of 2,4-D were 100, 150 and 250 ug/ml after 50, 100, and 200 mg/kg doses, and 24 hours after treatment, plasma levels were 10, 20, and 70 ug/kg, respectively. Dysphagia, anorexia, and hind muscle weakness were observed within the first 4 days of treatment. Two pigs were given a single 50, 100, 500 or 1000 mg/kg dose of 2,4-D amine. Peak plasma levels ranged from 120 to 525 ug/ml 2,4-D and levels after 24 hours were between 35 and 580 ug/ml in accordance with the magnitude of the dose given. Vomiting, severe muscle weakness and general depression occurred in pigs given the 2 highest doses. Autopsy 2-3 days after dosing revealed pneumonia and renal and hepatic congestion and, at all doses, gastro-intestinal irritation. Subacute doses of 2,4-D administered to pigs caused lesions of the gastrointestinal tract, lungs, and excretory organs. Plasma levels of 200-400 ug/ml after 24 hours were observed in affected pigs while pigs tolerating 50 mg/kg/day doses (the lowest given) had 24 hour plasma levels of 10 ug/ml. After chronic exposure to 500 ppm 2,4-D in feed for up to 12 months, 3 of 5 pigs developed locomotory disturbances, 2 had elongated lateral hoofs, and all showed depressed growth rates. A pregnant sow was fed 2,4-D during her seventh pregnancy which resulted in her first abnormal litter. One stillborn and 10 deaths of 15 neonates occurred

within 1 day of parturition. Maternal anorexia was evident throughout the pregnancy. The surviving piglets were maintained on a diet of 500 ppm 2,4-D and developed locomotory disturbances. Small alterations occurred in blood analysis. The results of blood analyses showed small alterations, but no statistical analysis was performed. Albuminuria occurred in treated animals and multinucleated hepatocytes were observed. No acute toxicity was observed in rats given 100 mg/kg 2,4-D or 2,4,5-T. No reproductive effects were observed in 5 rats given diets with 1,000 ppm 2,4-D. No malformations or toxicity was observed in the offspring. Chronic treatment of the 22 offspring with dietary levels of 1,000 ppm 2,4-D for 2 years resulted in decreased growth rate and food intake and an increased mortality which was not associated with any specific cause. Toxic effects of 2,4-D administered to chickens involved the same organs as those affected in other species. Plasma levels of 2,4-D were reported for rats and chickens after several dosage regimens, but no clinical signs were observed in these animals. The authors concluded that a threshold plasma level of 200-300 ug/ml of 2,4-D in the pig and calf is required to produce clinical symptoms. The butyl ester of 2,4-D was not absorbed well after oral administration. 2,4-D was concluded to produce moderate chronic toxicity.

97. Bjorn, M. K., and Northen, H. T. (1948) Effects of 2,4-dichlorophenoxyacetic acid in chicks. Science 108:479-480.

A brief report of the toxicity of 2,4-D to chickens is given. White Rock chicks (5 per group) were administered from 0.3 to 280 mg/kg acid equivalent of 2,4-D alkanolamine salt by gavage 3 times per week for 4 weeks or a single dose of 380 or 765 mg/kg of the 2,4-D salt. Body weights were recorded of chicks that received subacute exposure, and mortality and gross pathology were described after acute exposure. None of the subacute doses caused a significant decrease in body weight compared to controls that did not receive 2,4-D. The lower acute dose produced no mortality and the higher dose produced 100% mortality. Hemorrhagic gastroenteritis, and fatty degeneration and mottling of the liver, spleen, kidney, and heart were observed at necropsy. The authors concluded that 2,4-D was not a cumulative poison, because the highest subacute dose, equivalent to 3,360 mg/kg total was ineffective while an acute dose of 765 mg/kg produced 100% mortality. The authors estimated that a 1 kg chicken would have to consume all of the 2,4-D applied to 72 sq. ft. at the rate of 1 lb. per acre in 1-2 days to consume a lethal dose.

98. Blackman, G. E., Fryer, J. D., Lang, A., and Newton, M. The Effects of Herbicides in South Vietnam. Part B: Working papers: Persistence and disappearance of herbicides in tropical soils. National Academy of Sciences--National Research Council. NTIS Publication No. AD779025.

The authors measured levels of herbicides including 2,4-D, 2,4,5-T and picloram in South Vietnam soils to determine persistence and disappearance of these chemicals. They sampled sites that had been sprayed

during major herbicide operations during the war and sites that were sprayed at the time of the study. Analysis of surface and subsurface samples indicated levels of 0.007-0.08 lb/acre of 2,4-D, 0.004-1.35 lb/acre of 2,4,5-T, and 0.001-1.19 lb/acre of picloram in soils sprayed during military operations. Water samples analyzed for picloram had levels of 0.07 - 0.03 ppb of water and 2.2 to 0.8 pp of dry weight of sediment, the picloram being detected in the sediment filtered from the water. Agricultural sites in South Vietnam and the Philippines were sprayed with herbicides and crops were planted for this study. It was found that Agent White (with picloram) had longer lasting effects than Agent Orange (with 2,4,5-T). Both agents persisted longer in the Philippines. Areas of forest soil in the Philippines were sprayed with the herbicide and soil samples showed the levels of 1.6 lb/acre of picloram applied decreasing to less than 0.02 lb/acre in 31 days, and a more rapid disappearance rate for 2,4,5-T. The author notes that volatilization, leaching, runoff, and uptake by plants are responsible for removing the herbicides unchanged from the system. Chemical breakdown by biological and nonbiological processes results in the decomposition of the herbicides within the system. The author concludes that herbicides applied in massive doses may still be present at levels that would induce phytotoxic symptoms in some species. Herbicide use during the Vietnam war did not result in making the soil "sterile."

99. Bleiberg, J., Wallen, M., Brodtkin, R., and Applebaum, I. L. (1964) Industrially acquired porphyria. Arch. Dermatol. 89:793-797.

The results of physical examinations of 29 workers with chloracne who were involved in the manufacture of 2,4,-D and 2,4,5-T were reported. Eleven of these workers had acquired porphyria cutanea tarda, evidenced by increased urinary excretion of uroporphyrins. In addition, hyperpigmentation of the sun-exposed areas of the head, neck, and hands was seen in 17 workers, and hirsutism, which always involved the temples, was seen in 14. The severity of both of these symptoms, unlike the severity of the porphyria, was proportional to the severity of chloracne. Neither the severity of chloracne or of porphyria corresponded to the degree of exposure to chemicals. Instead, previous liver damage seemed to predispose workers to porphyria, and adolescent acne predisposed workers to severe chloracne. The authors concluded that one of the chemical intermediates or final products in the manufacture of 2,4-D or 2,4,5-T caused both chloracne and porphyria.

100. Bodai, J., Tamas, L., and Vegh, A. (1974) Reproductive consequences of Dikonirt toxicosis in cattle. J. Hungarian Veterinar. 29(5):319-322.

Reproductive toxicity is described for cattle that grazed on pastures or fed corn that was sprayed with 2,4-D. The authors described 8 incidents that involved inappropriate mating behavior and premature births after cattle were exposed to Dikonirt, comprised of 80% 2,4-D. Methods for examining herds, and in some instances, numbers of cows per herd or numbers adversely afflicted, amount of herbicide sprayed, and

time between spray and consumption of sprayed foliage were not reported. The symptoms described in cattle resembled those caused by estrogens and not those reported from acute poisoning of 2,4-D in man. Pregnant cows exhibited behavioral and physiological characteristics of animals in heat and abortion occurred. Fetuses of various ages were aborted and no fetal or placental abnormalities were observed. In 2 of the 8 instances of poisoning described, anestrus and ovarian atrophy occurred justifying the slaughter of 14% to 42% of the herds. Disturbances in the timing of the normal estrous cycle were observed in non-pregnant animals. Samples of fodder and green feed were found to contain from 0.075 to 0.8 mg/kg 2,4-D but no indication was made of which herds had received this feed. The authors concluded that the reported reproductive effects resulted from ingestion of 2,4-D and that sprayed fields were used for grazing without allotting enough time for degradation of herbicide to occur. In other instances, the authors attributed poisoning to low temperatures and high humidity which resulted in a decreased rate of degradation and increased persistence of the herbicide. Confounding exposure to other chemicals, other components in the herbicide preparation that was sprayed, and analysis of relevant pasture foliage for 2,4-D were not reported. The lack of similarity of the symptoms reported here with symptoms reported by others emphasizes the importance of residue levels and confounding factors to evaluate the present results.

101. Boeri, R., Bordo, B., Crenna, P., Filippini, G., Masetto, M., and Zecchini, A. (1978) Preliminary results of a neurological investigation of the population exposed to TCDD in the Seveso region. Riv. Pat. Nerv. Ment. 99:111-128.

Clinical examinations were conducted of representative populations from the Seveso region of Italy to determine neurologic effects resulting from exposure to TCDD. This study was initiated 7 months after the accident that released TCDD into the surrounding regions. Populations from two zones were chosen for investigation: 470 subjects from zone A (high risk for acute exposure) and 152 subjects from zone R (low risk). The study populations included children and adults of both sexes. Each subject was examined twice, 6 months apart. Results reported here were mainly from the first set of examinations since the second sets were not completed. Medical histories were compiled for each subject prior to clinical examinations. Neurophysiologic examinations of motor nerve conduction velocities of the right ulnar and left peroneal nerves, as well as an electromyogram of the uterossseus muscle of the right hand, were also conducted on each subject. Clinical symptoms of peripheral neurologic disorder included numbness and paresthesia of the extremities, muscle fatigue, decreased tendon reflexes and abnormal position sense of fingers and toes. Clinical symptoms were manifest in populations from both zone A and zone R, but a significantly higher prevalence of symptoms were evident in subjects from zone A. The zone A subjects also had a greater frequency of psychological symptoms and visual coordination problems than zone R subjects. In the 6 months between the first and second set of examinations of the zone A subjects, the neurologic condition of 12.7% of the adults and 11.1% of

the children worsened or declined. Results of the condition velocity tests and electromyograms indicated no difference between zone A and zone R populations. The authors tentatively concluded that although both populations (zone A - high risk and zone R - low risk) exhibited a high frequency of neurologic disorders, the zone A population had a significantly greater incidence of polyneuropathy than the zone R population. These disorders were assumed to be caused by exposure to TCDD.

102. Boffey, P. M. (1971) Herbicides in Vietnam: AAAS study finds widespread devastation. Science 171:43-47.

[Editorial.]

103. Bogen, G. (1979) Symptoms in Vietnam veterans exposed to Agent Orange. JAMA 242(22):2391.

[Review article.]

104. Bonaccorsi, A., Fanelli, R., and Tognoni, G. (1978) In the wake of Seveso. AMBIO 7(5-6):234-239.

[Review article.]

105. Bonderman, D. P., Mick, D. L., and Long, K. R. (1971) Occupational exposure to aldrin, 2,4-D and 2,4,5-T and its relationship to esterases. Ind. Med. 40(6):23-27.

The levels of erythrocyte esterases in 20 herbicide formulators were determined and compared to levels in matched controls. Twenty workers and 20 unexposed controls matched for age (within 3 years), sex and race were studied. Seven formulators exposed to 2,4-D and 2,4,5-T were included. Blood drawn from each subject was separated into plasma and erythrocyte fractions. Erythrocyte esterases, including tributyrinase, acetylcholinesterase, and cholinesterase and plasma tributyrinase were assayed. No statistically significant differences between any enzyme-activities of the group of 2,4-D and 2,4,5-T exposed workers and their matched controls were observed. Data was also analyzed according to duration of employment and no differences between pesticide (including aldrin)-exposed workers and controls existed.

106. Bongso, T. A., and Basrur, P. K. (1973) In vitro response of bovine cells to 2,4-dichlorophenoxyacetic acid. In Vitro 8(5):416-417.

[Abstract, only.]

107. Bovey, R. W., and Baur, J. R. (1972) Persistence of 2,4,5-T in grasslands of Texas. Bull. Environ. Contamin. Toxicol. 8:229-233.

[Background material.]

108. Bovey, R. W., Burnett, E., Richardson, C., Baur, J. R., Merkle, M. G., and Kissel, D. E. (1975) Occurrence of 2,4,5-T and picloram in subsurface water in the blacklands of Texas. J. Environ. Qual. 4(1):103-106.

The authors describe the persistence and movement of 2,4,5-T and picloram in soil and vegetation and the occurrence of these herbicides in subsurface water after repeated herbicide application. The study areas were located near Riesel and Temple, Texas, and were composed of deep, dense, slowly permeable, montmorillonite clay soils. The first study area was 3.1 ha, consisting of a cattle feedlot, a cultivated area, and an area of native grasses. The area was sprayed five times in 3 years with a 1:1 mixture of 2,4,5-T triethylamine salts and picloram at 2.24 kg/ha (four times) and 1.12 kg/ha (one time). Determination of 2,4,5-T in soil was made by gas chromatography, while water samples were analyzed by gas-liquid chromatography. In the first study area, herbicide concentration in the soil profile immediately after treatment did not exceed 144 ppb 2,4,5-T or 162 ppb picloram. Concentrations in grass cover, however, were high soon after treatment, e.g. less than 30,000 ppb 3 days after the second spraying. Dissipation of the herbicide occurred rapidly. After 145 days, no herbicide was detected in grass. Similar results were observed after the 1st and 4th treatments. The authors believed that loss of pesticide was due mainly to photodegradation. Neither herbicide appeared to accumulate in soils or vegetation. Herbicide levels in subsurface water from the treated area were not significant 2 weeks, and 6, 7, 9 and 11 months after the last herbicide treatment. The second study area was a drainage lysimeter (213 cm diameter x 132 cm deep) which received a single application of 2,4,5-T triethylamine salt and picloram (1:1) at 1.12 kg/ha. No 2,4,5-T was detected in 122 water samples from the drainage lysimeter for 1 year after herbicide application. Picloram (1-4 ppb) was detected up to 9 months after application, but was not detected after 9 months. The authors concluded that the herbicide tested did not accumulate in plants or soil and degraded and disappeared even after repeated application.

109. Bovey, R. W., and Diaz-Colon, J. D. (1978a) Selected Bibliography of the Phenoxy Herbicides, Vol. VIII: Effects on Higher Plants. U.S. Department of Agriculture, 58 pp.

[Bibliography.]

110. Bovey, R. W., and Diaz-Colon, J. D. (1978b) Selected Bibliography of the Phenoxy Herbicides, Vol. VI: Methods of Extraction and Analysis. U.S. Department of Agriculture, 34 pp.
- [Bibliography.]
111. Bovey, R. W., and Diaz-Colon, J. D. (1978c) Selected Bibliography of the Phenoxy Herbicides, Vol. IV: Ecological Effects. U.S. Department of Agriculture, 28 pp.
- [Bibliography.]
112. Bovey, R. W., and Diaz-Colon, J. D. (1977) Selected Bibliography of the Phenoxy Herbicides, Vol II: The Substituted Dibenzo-p-Dioxin. U.S. Department of Agriculture, 57 pp.
- [Bibliography.]
113. Bovey, R. W., Dowler, C. C., and Merkle, M. G. (1969) Pesticides in soil. The persistence and movement of picloram in Texas and Puerto Rican soils. Pestic. Monit. J. 3(3):177-181.

The authors reported on the persistence and movement of picloram in sandy and clay soils. Five soil types were used, two in Texas and three in Puerto Rico: Nipe clay (Puerto Rico) in which water penetrates rapidly and is poorly retained; Fraternidad clay (PR) which drains slowly; Catano sand (PR) which is poorly drained; and Erving clay loam (TX) and Lakeland sand (TX). Picloram was applied to each plot (three replicates per soil type) at rates of 1, 3, and 9 lb/acre. Texas plots were analyzed for picloram by gas chromatography and plant bioassay immediately after application, and at 3, 6, and 18 months after application. Puerto Rican plots were analyzed at 3, 6, and 12 months. In Texas, picloram was applied to dry soils which received little rainfall during the first 6 weeks. Loss of picloram was rapid and most likely due to photodecomposition. By gas chromatography, approximately 20% of the 1 lb/acre application was found in the clay soil at 3 months, while no picloram was detected in the sandy soil at 3 months. At 3 and 9 lb/acre, picloram persisted for 18 months in clay soil but only for 6 months in sandy soil. A bioassay at 18 months using beans as the indicator organism yielded similar results: in sandy soil, picloram was only detected at 9 lb/acre, while in clay soil, picloram was detected at 3 and 9 lbs/acre. Picloram applied at 1 lb/acre was only detected in the top 0-6 cm of soil, while the higher picloram applications were detected at the greatest depths tested (36-48 cm) at all three application rates. In Puerto Rican soils, at 3 months picloram, occurred throughout the soil profile (0-48 cm) at all three application rates. However, in the sandy soil, picloram was only detected at 21-27 cm (1 lb/acre), 21-39 cm (3 lb/acre), and 6-39 cm (9 lb/acre). Picloram in sandy soil was undetectable at all application

rates 6 months after application. The authors concluded that the rapid disappearance was probably due to the heavy rainfall occurring in this area. Picloram was most persistent in Fraternidad clay where rainfall was lowest and was detected throughout the soil profile at 3 and 9 lb/acre applications one year after application. Nipe clay also had detectable levels of picloram after 1 year at the 9 lb/acre application rate. The authors concluded that leaching by rainfall and soil type are important to the movement of picloram through soil.

114. Bovey, R. W., and Young, A. L. (1980) The Science of 2,4,5-T and Associated Herbicides. (New York: John Wiley and Sons.)

[Review article.]

115. Boyd, E. M., and Dobos, I. (1969) Acute oral toxicity of monuron in albino rats fed from weaning on different diets. J. Agr. Food Chem. 17(6):1213-1216.

The acute toxicity of monuron was compared in rats fed dietary protein levels of 0-26% casein. Male weanling Wistar rats were fed diets with no protein, 3.5%, 9.0% or 26% protein for 28 days. The control group was fed standard laboratory chow. Monuron (94% purity) in cottonseed oil was administered by gastric intubation and the subsequent effects of mortality and gross and histopathologic changes were observed. The LD₅₀ of monuron was 1.48 g/kg for controls, 0.25 g/kg for rats fed a protein-free diet, 0.95 g/kg for rats fed 3.5% casein, 1.58 g/kg for rats fed 9% casein, and 2.88 g/kg for rats fed 26% casein. All LD₅₀ values were significantly different from controls except for the value of the group fed 9% casein. The hours to death were significantly shorter (11 hours) for the rats in the protein-free diet and longer (41 hours) for rats fed 26% casein than for controls (27 hours). Monuron produced the same clinical signs of toxicity in all groups of rats, including anorexia and central nervous system disorders. Gross pathological changes included gastric ulcers, gastroenteritis, renal and splenic pallor and hepatic necrosis. Degenerative changes were observed histologically in the kidneys, liver, muscle, salivary glands and testes, congestion was observed in the brain, heart, and lungs; the spleen, adrenals and thymus showed evidence of stress. Most organ weights were decreased after monuron treatment. Survivors were free of clinical signs of toxicity within 4 days of treatment. The authors concluded that rats fed diets low or free of protein were susceptible to monuron and in particular were susceptible to starvation while rats fed 26% casein were relatively resistant and showed early signs of recovery. The authors suggested that quantitative differences in the activities of enzymes involved in monuron detoxification were responsible for the increased susceptibility of protein-deficient rats. However, no attempt to measure monuron levels or enzyme activities were made and many other alterations in physiology occur after protein deprivation that could compromise the response of an animal to toxic substances.

116. Boyd, E. M., and Krupa, V. (1970) Protein-deficient diet and diuron toxicity. J. Agric. Food Chem. 18(6):1104-1107.

The acute toxicity of diuron was compared in rats fed protein deficient diets. For 28 days weanling Wistar rats were fed diets containing either 3.5% protein as casein, 26% protein as casein or 25% protein as mixed animal and plant proteins. Diuron (95% purity) was administered in cottonseed oil by gastric intubation. Animals were observed for 1 month, mortality was determined and gross and histopathologies were performed. The LD₅₀ was 1,017 mg/kg for rats on the mixed protein diet, 2,390 mg/kg for rats fed 26% casein and 437 mg/kg for rats on the low protein diet. The mean time to death was 23-28 hours for all groups and clinical symptoms of diuron toxicity were the same for all 3 groups. Death was caused by respiratory failure. Clinical signs indicated a depression of the central nervous system and cholinergic stimulation by diuron. Gross pathology included gastritis, enteritis, dehydrated cecum, congested brain and lungs, and yellowish kidney. Histological examination revealed gastrointestinal congestion, stress reactions of the adrenals, thymus and spleen and hepatic and renal lesions. Decreases occurred in organ weights except the adrenal and salivary glands, which were hydrated. Survivors recovered from toxic effects in 72 hours. The authors concluded that the toxicity of diuron was augmented 5-fold in rats fed low protein diets relative to those fed normal, mixed protein diet and these results were compared to the relative increase in toxicity of other pesticides in rats fed low protein diets reported in another publication.

117. Bradlaw, J. A., Garthoff, L. H., Graff, D. M., and Hurley, N. E. (1975) Detection of chlorinated dioxins: Induction of aryl hydrocarbon hydroxylase activity in rat hepatoma cell culture. Toxicol. Appl. Pharmacol. 33(1):166.

[Abstract, only.]

118. Bradlaw, J. A., Garthoff, L. H., Hurley, N. E., and Firestone, D. (1976) Aryl hydrocarbonhydroxylase activity of twenty-three halogenated dibenzo-p-dioxins. Toxicol. Appl. Pharmacol. 37(1):119.

[Abstract, only.]

119. Bradlaw, J. A., and Casterline, J. L. (1979) Induction of enzyme activity in cell culture: a rapid screen for detection of planar polychlorinated organic compounds. J. Assoc. Off. Anal. Chem. 62(4):904-916.

The use of TCDD as a standard for a bioassay based on enzyme induction is described. Rat H-4-11E hepatoma cells were homogenized and aryl hydrocarbon hydroxylase activity was determined in cell extracts. Benzo(a)pyrene was used as a substrate. The enzyme activity of TCDD-pretreated cells was compared to the activity of non-induced cells. In

this system, TCDD was a more potent inducer than 24 other halogenated dibenzo-p-dioxins, 11 dibenzofurans or 7 biphenyls. The concentration of TCDD that produced half-maximal induction was 0.38 nM. The potencies of various chemicals in this bioassay were compared to results reported elsewhere of toxic responses. The potencies in the enzyme induction bioassay correlated well with the relative potencies of the same chemicals in assays of other toxic responses. The authors concluded that the enzyme induction bioassay is a useful method for screening food extracts for polychlorinated planar substances.

120. Bradlaw, J. A., Garthoff, L. M., Hurley, N. E., and Firestone, D. (1980) Comparative induction of aryl hydrocarbon hydroxylase activity in vitro by analogues of dibenzo-p-dioxin. Fd. Cosmet. Toxicol. 18:627-635.

The potencies of various compounds on aryl hydrocarbon hydroxylase activity in hepatoma cell extracts is described. TCDD was the most potent compound tested and was selected as the standard for the method, when used as a screening technique. The methods and results reported in this article were described in a previous publication by Bradlaw and Casterline (1980).

121. Bradley, R. L., Shoemaker, J. P., and Hoffman, R. V., Jr. (1974) Treatment of experimental mammary adenocarcinoma with herbicides. Cancer Chemother. Rep. Pt. 1. 58(5):745-748.

The authors studied the chemotherapeutic potential of picloram on transplantable mammary adenocarcinomas in mice. Three separate studies were conducted. In the first experiment, male C3H mice (15 mice per group) received subcutaneous injections of picloram (10 mg/kg, 0.5 ml) or 0.5 ml saline. Picloram was dissolved in 95% ethanol diluted with sterile water, pH 7.3. Mice in both groups were injected with C3HBA tumor subcutaneously. In addition, each mouse received daily inoculations of picloram or saline for the duration of the experiment. Survival times and tumor sizes of controls and treated animals were analyzed by the student's t-test. Statistically significant decreases in tumor size were observed in picloram-treated animals on days 8 through 15 and on day 25. No significant differences were observed on days 26 through 36 or on survival times of the animals. In the second experiment, animals received 20 mg/kg subcutaneous doses of picloram. Eight male and 10 female C3H mice were used in each group. C3HBA tumors were injected in the same manner as above. Picloram was administered daily for 12 days. Then on alternate days starting with day 14, the animals were treated with 10 mg/kg doses until death or the end of the experiment. Control mice received saline injections. A statistically significant decrease (p less than 0.001) in tumor size was observed in picloram-treated animals compared to controls on days 34 through 53. Two of the 3 picloram-treated mice that were alive on day 82 no longer had tumors. Tumor sizes did not differ according to sex. A side-effect of picloram administration, i.e., edema, was noted in 9/18 treated animals about day 14. In a third experiment, the toxicity

of picloram was studied. Groups of 5 male and 5 female mice received up to 40 mg/kg picloram for up to 2 weeks. Dosing schedule was not reported. At 10 mg/kg picloram, mice became lethargic for 20-30 minutes; at 20 mg/kg lethargy lasted for 1 hour; at 30 mg/kg mice were lethargic for more than 1 hour, and 3/6 mice died on day 4. The LD₅₀ for picloram was calculated to be 30 mg/kg. No histologic differences were observed between control and treated mice and nontoxic effects were observed in tissues of normal mice receiving 10 or 20 mg/kg doses. In mice killed by picloram treatment, fatty metamorphosis of the liver was observed. Sex-related differences in picloram toxicity were not reported.

122. Brady, H. A. (1975) Picloram and dicamba persistence in forest environments. Proc. South. Weed Sci. Soc. 28:230-235.

[Not available.]

123. Brady, H. A. (1974) Soil moisture affects absorption of 2,4,5 T sprays. Pro. Soil Sci. Soc. of America. 27:206-210.

[Not available.]

124. Braman, R. S. (1975) Arsenic in the environment. In Arsenical Pesticides, ACS Symposium Series Vol. 7 [Washington, D.C.: American Chemical Society] pp. 108-123.

[Review article.]

125. Braman, R. S., and Foreback, C. (1973) Methylated forms of arsenic in the environment. Science 182(4118):1247-1249.

The quantities of cacodylic acid (dimethylarsenic acid), methylarsenic acid, arsenate and arsenite ions in environmental samples and human urine were determined. Water samples from ten lakes, bays, and rivers near Tampa, Florida, as well as seashells, bird eggshells, sandstone rock and urine samples from 3 men and 1 woman, between 27 and 42 years old, were analyzed for arsenic. The method of analysis, developed to detect arsenic in each of the 4 forms, involved an atomic absorption technique and had a lower detection level of about 1 nanogram. Water samples contained up to 3.6 ppb of arsenic, with less than 1% as methylated arsenic. The rock sample contained arsenic primarily as arsenate and arsenite ions, while the shells contained primarily methylated arsenic compounds. The urine samples contained an average of 22.5 ppb arsenic, with 66% as dimethylarsenic acid, 8% as methylarsenic acid, 8% as arsenate and 17% as arsenite. The authors concluded that environmental inorganic arsenic was reduced and methylated, eventually to dimethylarsine by bacteria, and that this biotransformation was confirmed by verifying the presence of some of the intermediates. Human biotransformation was proposed to be the result of

methylcobalamin-methionine reactions. Since the source of the arsenic that was eventually excreted into urine is not identified in this study, there is no way to differentiate whether the methylated arsenic was derived from inorganic arsenic or from exposure to methylated arsenic pesticides. The data can be used to illustrate that methylated arsenic compounds are not necessarily degraded to inorganic arsenic prior to excretion in human urine.

126. Brandt, M. R. (1971) Herbatox poisoning: a brief review and report of a new case. Ugeskrift for Laeger 133(11):500-503.

The author describes a case of intoxication by an herbicide preparation that contained 2,4-D. In a suicide attempt, a 47-year-old man ingested an estimated 300-600 mg/kg of 2,4-dichlorophenoxypropionic acid - 2,4-dichlorophenoxyacetic acid (2:1, w/w) as Herbatox. Within 5 hours, most muscles were rigid. Other symptoms that developed were vomiting, shallow respiration, foaming at the mouth, and coma. In 3 days, which included therapy involving artificial respiration, forced diuresis, and drug therapy (atropy and furasemid), the patient was fully awake, but lost his memory and ability to see colors for 5 days, and had pain and paresthesia of the left arm and leg. Five months after the incident, the only symptoms that remained were hypesthesia, algesia and mono-neuritis of the left leg. Laboratory tests revealed slight anemia, perturbances in the levels serum transaminases, and urine levels of 25 mg and 35 mg of dichlorophenoxypropionic acid and 2,4-D respectively per 100 ml of urine collected 1 day after the incident. The authors also reviewed literature on other instances of intoxication by Herbatox and concluded that this herbicide was more toxic than generally thought.

127. Braun, W. (1970) Chloracne. Therap. Umschau. 27(8):541-546.

[Review article.]

128. Bretag, A. H., and Caputo, C. (1978) The nature of the antimyotonic action of 2,4-D on the soleus muscle of the rat. Proc. Aust. Physiol. Pharmacol. Soc. 9(2):150 pp.

[Abstract, only.]

129. Brooker, M. P. (1976) The ecological effects of the use of dalapon and 2,4-D for drainage channel management. II Fauna. Arch. Hydrobiol. 78(4):507-525.

[Not available.]

130. Brown E. A. B., and Maling, H. M. (1975) The effects of paraquat and related herbicides on the acetylcholinesterase of rat lung. Fed. Proc. Fed. Am. Soc. Exp. Biol. 34(3):226.

[Abstract, only.]

131. Brown, J. W. (1962) Vegetational spray tests in South Vietnam. Defense Documentation Center, AD 176961. 119 pp.

[Background material.]

132. Brown, M. H. (1979) Love Canal and the poisoning of America. Atlantic 244(33):33-47.

[Editorial.]

133. Brown, V. K. (1968) Solubility and solvent effects as rate-determining factors in the acute percutaneous toxicities of pesticides. Soc. Chem. Ind. Monogr. 29:93-105.

[Review article.]

134. Bucher, N. L. R. (1946) Effects of 2,4-dichlorophenoxyacetic acid on experimental animals. Proc. Soc. Exper. Biol. Med. 63:204-205.

A brief description of the clinical symptoms of 2,4-D toxicity in mice and dogs is reported. Strain A mice were administered the sodium salt of 2,4-D in physiological saline subcutaneously, intraperitoneally or intravenously in single doses of 150-350 mg/kg or as 1 or 2 daily doses for 3 weeks to 3 months of 1/3 to 1/5 the LD₅₀ per day. Gross and histological necropsy examinations were performed and for chronically treated mice, growth rates, hematology analyses, transplanted sarcoma growth, and reproduction were assessed. The LD₅₀ was 280 mg/kg for subcutaneous treatment to mice. Myotonia which developed in mice injected (route not specified) with 2,4-D was described and included features that resembled clinical myotonia, including alleviation of muscle spasms by exercise, acerbation by rest and inducement by spasms by a local blow to the muscle. Hind limbs were more affected than forelimbs. Doses above 250 mg/kg 2,4-D produced diarrhea, inertia, coma, and sometimes death in mice. The liver, kidney and spleen of acutely treated mice were mottled and dilated blood vessels were observed in several organs. No tissue pathology, hematologic changes, tumor or body growth retardation, or reproductive problems were observed in chronically treated mice. Anorexia, vomiting and nasal and eye irritation were reported in treated dogs, but the dose, route or number of dogs treated were not reported. The descriptions of 2,4-D toxicity presented here do not include the route of administration, the doses (except related to myotonia), the numbers of animals studied or the proportions afflicted, nor methods or results for the studies of

reproductive effects or tumor growth. Therefore, this study does not contribute significantly to an understanding of 2,4-D toxicity.

135. Buratowski, J., Piasecki, M., and Warczynski, A. (1975) Effects of chemical toxic agents used in Vietnam on the visual system. Lek. Woj. 51(9):581-583.

[Foreign language.]

136. Bureau of Foods, Pesticides, and Product Safety FDA (1970) Toxicity of 2,4,5-T, 2,4-D and related comp'ds. March 13, '80, revised April 8, '80.

[Not available.]

137. Bureau of National Standards. (1981) Second DOW Sarcoma case found; company suggests smoking as a factor. Occupational Safety and Health Reporter. 10(4):1347.

[Review article.]

138. Burk, R. F., Lawrence, R. A., and Lane, J. M. (1980) Liver necrosis and lipid peroxidation in the rat as a result of paraquat and diquat administration. J. Clin. Invest. 65(5):1024-1031.

The toxicity of diquat in selenium-deficient rats was assessed. Male Holtzman rats maintained on control diets and vitamin E-deficient diets were administered 78 umol/kg diquat in saline, intraperitoneally. A group of rats fed a selenium-deficient diet were administered 19.5 umol/kg diquat. Ethane in expired air was determined by gas chromatography and used as a measure of lipid peroxidation. Mortality, renal and hepatic histology and serum enzyme activities were evaluated as measures of toxicity. Increases in ethane production were 3 and 28 times the control values for the vitamin E-deficient and selenium-deficient groups, respectively and death occurred for all animals in 24 and 2 hrs. for the 2 groups, respectively. Liver and kidney necrosis were observed at necropsy, while other organs were unaffected. Cod liver oil, which is rich in linoleic acid-related fatty acids, was used to replace corn oil in the selenium deficient diet and was found to cause a 3-fold increase in ethane production and no change in liver or kidney necrosis occurred. Neither liver necrosis nor increased ethane production occurred in control rats administered diquat. Selenium, administered as a single dose to rats on a selenium-deficient diet, had no effect on glutathione peroxidase activity in lung, plasma, liver or kidney tissues removed and assayed 10 hrs. after the injection. Both lipid peroxidation and mortality from diquat were reduced 6 and 10 hrs. after the selenium injection. Superoxide dismutase activity in several tissues was unaltered in rats fed the 1 selenium-deficient diet compared to those fed the control diet. The authors concluded that diquat

toxicity was increased in selenium-deficient rats and that the biochemical role of selenium in protection against lipid peroxidation remains unknown.

139. Burk, R. F., Lawrence, R. A., Lane, J. M., and Hamm, D. P. (1979) Lipid peroxidation and liver necrosis in selenium-deficient rats given diquat and paraquat. Gastroenterology 76(5)Pt. 2:1109.

[Abstract, only.]

140. Burton, J. A., Gardiner, T. H., and Schanker, L. S. (1974) Absorption of herbicides from the rat lung. Arch. Environ. Health 29(1):31-33.

Absorption of 2,4-D, 2,4,5-T and diquat by the lung of anesthetized rats were assessed. Male Charles River rats under sodium pentobarbital anesthesia were administered 0.1 ml of 1.0 -10 mM [14 C]-2,4-D, or [14 C]-2,4,5-T or 0.01-0.1 mM [14 C]-diquat by tracheal cannula. After 0.5-120 minutes, the trachea and lungs were removed, homogenized, digested, and counted to determine unabsorbed radioactivity. All compounds showed absorption (calculated from the unabsorbed radioactivity in the lungs) that followed first order kinetics. Half-times for absorption were 1.4, 1.7, and 51 minutes for 2,4-D, 2,4,5-T and diquat, respectively, and the corresponding rate constants were 30.1, 24.8, and 0.82 per hour. The amount of each compound absorbed was dependent on the concentration administered; the percentage of the dose absorbed in a given time was constant for various concentrations. Amitrole absorption was also studied. The authors concluded that the rates of absorption for the four compounds correlated with their lipid solubilities and not their molecular weights and the more soluble compounds probably entered the lung through membrane pores and through lipid regions of the membrane, while diquat probably only passed through pores.

141. Bus, J. S., Preache, M. M., Cagen, S. Z., Posner, H. S., Eliason, B. C., Sharp, C. W., and Gibson, J. E. (1975) Fetal toxicity and distribution of paraquat and diquat in mice and rats. Toxicol. Appl. Pharmacol. 33(3):450-460.

The fetotoxicity, maternal toxicity, and fetal distribution of diquat was studied in the rat. A dose of 15 mg/kg of [14 C]-diquat was given intravenously to pregnant rats on a single day of gestation from day 7 to 21. On day 22, the number of dead and resorbed fetuses was determined and compared to a vehicle control group that received saline only. Radioactivity in fetal tissues was examined at 3, 7, and 24 hours after a single intravenous dose of 15 mg/kg [14 C]-diquat was administered maternally on day 13, 16, or 21 of gestation. Fetal tissues were excised, dissolved in toluene and counted for radioactivity. The average percentage of dead plus resorbing fetuses was 57%, with peak fetotoxicity occurring from diquat administration on days 7-9 and on days 16-17. The incidence of maternal deaths averaged

20% with 8 of the 9 deaths occurring from treatment on or before day 13. Rat fetuses took up more radioactivity when [¹⁴C]-diquat was administered late in gestation. The levels of radioactivity in the whole fetus and in the fetal liver, kidney, and lung decreased at successive times after administration. Compared to the effects of [¹⁴C]-paraquat which were studied using the same dose and route as for diquat, [¹⁴C]-diquat caused a substantially higher degree of fetal toxicity and reached the rat fetus in much larger amounts than [¹⁴C]-paraquat. Neither compound was present in maternal plasma or fetal extracts in the form of metabolites. The authors concluded that the differential toxic effects of the 2 compounds were probably the result of differences in distribution of the compounds to the fetus.

142. Buselmaier, M. V., Rohrborn, G., and Propping, P. (1972) Pesticide mutagenicity investigations by the host mediated assay and the dominant lethal test in mice. Biol. Zentralbl. 91:311-325.

The authors tested 2,4,5-T in a host-mediated assay and a dominant lethal test in mice. Two bacterial strains were used as the indicator organisms on the host-mediated assay: Salmonella typhimurium G46 which reverts from histidine requiring to histidine independence upon exposure to a mutagen and Serratia marcescens a21, a leucine requiring strain which reverts to prototrophy after treatment with a mutagen. A plate incorporation assay in S. typhimurium was also performed to compare in vivo (host-mediated assay) and in vitro (plate assay) results. In the host mediated assay, NMRI mice (10-12 weeks old, 6 animals per group) received an intraperitoneal injection of bacteria immediately followed by a subcutaneous injection of 500 mg 2,4,5-T/kg body weight or 1000 mg 2,4,5-T butylester/kg body weight. Mice were killed 3 hours later and the bacteria was recovered from the peritoneal cavity. Results of the assay were analyzed by the Wilcoxon rank test. A result was positive at p 0.01. By this criteria, both 2,4,5-T and 2,4,5-T butylester were negative in the host-mediated assay using either S. typhimurium or S. marcescens as the indicator organism. Both compounds were also negative in the plate assay. In the dominant lethal assay, NMRI male mice (10 weeks old) received a single intraperitoneal injection of 100 mg/kg 2,4,5-T and were mated with untreated females of the same age. Each male was caged with three females for 1 week. The experiment continued for 6 weeks. Males were caged with new females each week to observe all stages of spermatogenesis. Females were killed 14 days after being placed with the males. Uteri were scored for numbers of dead implants. Experimental results were analyzed by the χ^2 method with p 0.0027 as the criteria for a positive result. 2,4,5-T did not induce dominant lethal mutations according to this criteria.

143. Buslovich, S. Yu., Aleksashina, Z. A., and Kolosovskaya, V. M. (1976) Embryotoxic effect of chloro-derivative phenoxyacid-herbicides. Zdravookhraneniye Belorussii (10):83-84.

The teratogenicity of 2,4-D sodium, amine, and diethylamine salts and butyl ester was studied in the rats. Pregnant albino rats were exposed

to half of the LD₅₀ dose of the 2,4-D compound and resorptions, and fetal size, weights and malformations were determined. One of 9 rats exposed to 2,4-D-butyl ester on day 9 of gestation had 8 live fetuses and 1 of 7 rats exposed on day 10 retained 2 fetuses. All other rats exposed to the butyl ester on days 4, 5, 6, 9, 10, 11, or 12 had 100% resorptions. Administration of 2,4-D diethylamine salt on days 5, 9, 10, or 13 increased the number of post-implantation deaths. Treatment with the sodium or amine salts of 2,4-D did not alter fetal viability. The weights and size of the fetuses exposed to 2,4-D butyl ester, 2,4-D diethylamine and, for the sodium and amine salts given on day 10 or 14 only, were reduced. Enlarged brain ventricles and hemoperitoneums were observed in some fetuses (the frequency or treatment groups with affected fetuses were not stated). The authors concluded that esters of 2,4-D with long side chains are more toxic than 2,4-D acids and should not be used near water that could result in human exposure and risk of embryotoxicity.

144. Buslovich, S. Yu., and Koldobskaya, F. D. (1972) Activity of hexokinase from the skeletal muscle of albino rats under the conditions of experimental myotonia. Voprosy meditsinskoi khimii. 18(4):403-406.

The effects of administering hydrocortisone or desoxycorticosterone (DOCA) or adrenalectomy on myotonia produced by 2,4-D was studied in the rat in vivo and in vitro. Female rats were administered 400 mg/kg 2,4-D diethylamine perorally and skeletal muscle hexokinase activity was determined from 15 minutes to 72 hours after 2,4-D treatment. Adrenalectomies were performed on some rats prior to 2,4-D treatment and other rats were administered 5-10 mg/kg DOCA and 5-10 mg/kg hydrocortisone (together) subcutaneously. 2,4-D was added to skeletal muscle homogenates and the effect of hydrocortisone succinate on hexokinase levels in vitro were determined. A myotonic response was observed in rats after 2,4-D treatment, and was accompanied by a decrease in hexokinase levels to a low of 11% of control levels after 1 hour. In adrenalectomized rats, no myotonic effect resulted from 2,4-D administration. Hexokinase levels, which were elevated after adrenalectomy, were decreased to almost normal levels by 2,4-D treatment. A combination of 10 mg/kg of hydrocortisone and DOCA reversed the effect of 2,4-D on hexokinase activity, but lower doses of the steroids or administration in the absence of 2,4-D produced a large decrease in hexokinase activity (to 30 to 60% of control levels). A small decrease (about 25%) in hexokinase activity was produced by 5×10^{-5} M 2,4-D in vitro and a substantial effect was observed in muscle homogenates from rats exposed to 2,4-D in vivo and studied in vitro. This last effect was reversed by addition of 100-200 ug of hydrocortisone succinate to the cultures. The authors concluded that hexokinase activity paralleled the development of myotonia and suggested that the change in activity was indicative of a disturbance of carbohydrate energy metabolism during myotonia.

145. Buslovich, S. Yu., Voinova, I. V., and Milchina, M. G. (1973) The distribution of chloro-derivative phenoxyacid herbicides in albino rats. Gigiena truda i professionalnyye zabolvaniya. 17(3):35-37.

The tissue distribution of 2,4-D and pharmacokinetics of blood clearance was studied in the rat. Groups of 10 rats were administered 555 mg/kg of 2,4-D sodium salt or 405 mg/kg of 2,4-D diethylamine salt by oral intubation and groups of 20 rats were given 50 daily oral doses of 91 or 23 mg/kg 2,4-D sodium salt or 28 or 111 mg/kg 2,4-D diethylamine salt. Tissue levels of 2,4-D were determined by degrading 2,4-D to 2,4-dichlorophenol with pyridine hydrochloride and analyzing the phenol spectrophotometrically. Peak blood levels were reached 3 hours after an acute dose and half-lives were calculated as 53 hours and 72 hours for the sodium and diethylamine salts, respectively, for the period from 3 to 72 hours after exposure and half-lives of about one-third these values were calculated for the period from 3-12 hours after exposure. The peak tissue levels were reached 3 days after 2,4-D was administered with the highest levels in the liver and lowest levels in the brain. Subsequently, the kidney retained the highest levels. After chronic exposure, the highest levels were observed for the liver and kidney and the lowest levels for the brain. No accumulation of 2,4-D was observed in any tissue. The authors concluded that the lack of tissue accumulation reflected the water-solubility and size of the dose of 2,4-D administered.

146. Buu-Hoi, N. P., Chanh, P. H., Sesque, G., Azum-Gelade, M. C., and Saint-Ruf, G. (1972a) Enzymatic functions as targets of the toxicity of "dioxin" (2,3,7,8-tetrachlorodibenzo-p-dioxin). Naturwissenschaften 59(4):173-174.

The effect of TCDD on the levels of 8 serum enzymes 10 days after TCDD administration was evaluated. Rats were administered 10 mg/kg of TCDD in olive oil intraperitoneally, after 10 days, blood was removed and assayed for glutamic oxaloacetic transaminase (S.G.O.T.), glutamic pyruvic transaminase (S.G.P.T.), aldolase, lactic dehydrogenase (LDH), hydroxybutyric dehydrogenase, alkaline phosphatase, arylesterase, and cholinesterase. No changes were observed in aldolase and alkaline phosphatase between levels for treated and control groups, while statistically significant increases in levels for TCDD-treated animals occurred for S.G.O.T., S.G.P.T., LDH, and hydroxybutyric dehydrogenase, and significant decreases occurred for arylesterase and cholinesterase. Some blood serum components were present at significantly higher concentrations in treated rats than controls. These components included urea, total lipids, and bilirubin. Glucose levels were lower in blood from treated rats than control rats. The authors concluded that the liver was the main target organ of TCDD toxicity, followed by the thymus and heart. Histopathologic findings were described in a separate article. (Buu-Hoi, et al, 1972). Physical appearance, mortality, body weights, and other parameters relevant to interpreting the clinical chemistry results were not reported. Furthermore, the dose of TCDD used in this study is accessive, relative to other reports which describe lethal effects of TCDD doses in the ug range.

147. Buu-Hoi, N. P. H., Chanh, P. H., Sesque, G., Azum-Gelade, M. C., and Saint-Ruf, G. (1972b) Organs as targets of dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin) intoxication. Naturwissenschaften 59(4):174-175.
- Histopathologic evidence of toxicity of TCDD to various organs of the rat is presented. Wistar rats weighing 210 g were given 10 mg/kg of TCDD in olive oil intraperitoneally. After 10 days the treated rats were examined for hematologic and histopathologic effects and were compared with vehicle controls. Treated rats lost 60-80 g, while control rats gained 60-70 g. Analysis of blood samples from treated rats revealed statistically significant increases over controls in hematocrits, leukocyte counts, and polynuclear neutrophils, while the proportion of lymphocytes decreased and the bone marrow did not change (the parameters measured for evaluating bone marrow were not reported). Severe hepatic lesions were described which were evident 6 days after treatment. Involution of the thymus, severe cardiac lesions and pulmonary alveolitis were also observed in treated rats. The authors mentioned that a dose of 1 mg/kg of TCDD produced the same qualitative changes as the 10 mg/kg dose. TCDD elicits toxicity in the ug range and the dose administered in the study is well beyond those used by most investigations to study various parameters of TCDD toxicity.
148. Buu-Hoi, N. P., Saint-Ruf, G., Bigot, P., et al. (1971) Preparation, properties and identification of dioxin (2,3,7,8-tetrachlorodibenzo-para-dioxin) in the pyrolysate of defoliants containing 2,4,5-T and its esters and in contaminated vegetation. C.R. Acad. Sci., Paris Ser. D. 273:708-7111. (French)
- [Background material.]
149. Byast, T. H., and Hance, R. J. (1975) Degradation of 2,4,5-T by South Vietnamese soils incubated in the laboratory. Bull. Environ. Contam. Toxicol. 14(1):71-76.

The authors describe the degradation of 2,4,5-T in four South Vietnamese soil samples under laboratory conditions. Two soil samples (1 and 2) were grey podzolic soils from an agricultural area, having pH values of 4.9 and 5.0, respectively. The other two samples (3 and 4) were saline alluvial soils from a mangrove swamp, pH values of 6.6 and 7.9, respectively. In the laboratory, samples 3 and 4 were air-dried to 15% moisture content from their original moisture of 35 and 47%, respectively. Samples 1 and 2 originally contained 15% moisture. Duplicate soil samples received acetone solutions of carboxy-labeled ^{14}C -2,4,5,-T butyl ester at 1 or 15 ppm. Controls received only acetone. After solvent evaporation the soil was transferred to respiration flasks. Samples 3 and 4 were returned to their original moisture content. Evolution of $^{14}\text{CO}_2$ and residual ^{14}C and 2,4,5-T were measured in each soil sample by scintillation counting. At both 1 and 15 ppm, $^{14}\text{CO}_2$ evolution was greatest in sample 2. The authors offered no explanation for this. At 1 ppm, soils evolved a maximum of 64-69%

$^{14}\text{CO}_2$ in 49 days. However, at 15 ppm a slow degradation was observed, 74-96% $^{14}\text{CO}_2$ in 168 days. Less than 4% of the 1 ppm application and only 1-16% of the 15 ppm application of 2,4,5-T could be extracted from the soil with or without hydrolysis. The authors speculated that the unextractable $^{14}\text{CO}_2$ had been incorporated into the soil organic matter. The authors did not state what relevance these data may have to the actual field situation.

150. Calderbank, A., and Slade, P. Diquat and paraquat. In Herbicides: Chemistry, degradation, and mode of action. Vol. 2 [eds., P. C. Kearney and D. D. Kaufman] (New York: Marcel Dekker Inc., 1976) pp. 501-540.

[Review article.]

151. Caldwell, R. S., Buchanan, D. V., Armstrong, D. A., Mallon, M. H., and Millemann, R. E. (1979) Toxicity of the herbicides 2,4-D, DEF, propanil and trifluralin to the Dungeness crab, Cancer magister. Arch. Environ. Contam. Toxicol. 8:383-396.

The authors describe the toxicity of 2,4-D free acid to various life stages of the Dungeness crab. Technical grade 2,4-D (98 percent active ingredient) was dissolved in acetone and added to seawater with a final acetone concentration of 100 ul/l. Control vessels contained only seawater or seawater with acetone. Chromatographic analysis to determine the final concentration of 2,4-D in seawater were not performed. The effects of 2,4-D on egg hatching and early development through the prezoal stage were measured in a 24 hour toxicity test. Duplicate beakers of 3,300, 10,000, 33,000, 100,000, or 330,000 ug/l 2,4-D each received 30 eggs. At the highest dose tested, 2,4-D inhibited egg hatching (1/58 eggs hatched). At 100,000 ug/l less than 20 percent of the hatching prezoae developed into the first zoeae stage. Motility of the first stage zoeae was inhibited at 100,000 ug/l. In 80 day long term toxicity assays, 2,4-D decreased survival of larvae statistically significant at the highest dose tested, 10,000 ug/l, but not at the lower doses, 1,000 ug/l. Exposure to both concentrations of 2,4-D increased the duration of larval stages. In juvenile crabs and in adults, no effects of 2,4-D were observed up to doses of 10,000 ug/l. The authors concluded that the larva was the stage most sensitive to 2,4-D. From their data, the maximum acceptable toxicant concentration (MATC) was below 1,000 ug/l for 2,4-D free acid.

152. California - Department of Food and Agriculture. (1978) Report on the aerial use of phenoxy herbicides. 75 pp.

[Background material.]

153. Cant, J. S. and Lewis, D. R. H. (1968) Ocular damage due to paraquat and diquat. Brit. Med. J. 2:224.

A case report of ocular damage after accidental contact of a mixture of diquat and paraquat to the eye is presented. A 36-year-old man accidentally splashed Preeglone Extra fluid (comprised of paraquat and diquat in the equal amounts and an unspecified surface-active agent) in one eye and on his eyelids as he was diluting the concentrate with water. He washed his eye with water. Irritation of the exposed eye occurred over the next 3 days, and after 1 week, 50% of the tarsal conjunctiva of the lower eyelid and a small amount of that of the upper eyelid were lost. The corneal epithelium was lost in some regions and

edematous in other regions. Mild uveitis was also present. Treatment with chloramphenicol and atropine was initiated (1 week after exposure) and adhesions between the denuded surfaces were repeatedly separated. In 11 days, the corneal epithelium was healed and the uveitis had subsided. The authors concluded that both dipyridilium compounds contributed equally to the effect and that the delayed nature of the response and difficulty of removal of the splashed chemicals when a surface-active agent is present create a significant hazard which should be guarded against by proper warning and proper handling.

154. Carere, A., Cardamone, G., Ortali, V., Bruzzone, M. L., and Di Giuseppe, G. (1976) Mutational studies with some pesticides in Streptomyces Coelicolor and Salmonella typhimurium. Mutat. Res. 38:136.

[Abstract, only.]

155. Carere, A., Ortali, V. A., Cardamone, G., Torracca, A. M., and Raschetti, R. (1978) Microbiological mutagenicity studies of pesticides in vitro. Mutat. Res. 57:277-286.

The authors tested dalapon, picloram, and 12 other pesticides for mutagenicity in Salmonella typhimurium and Streptomyces coelicolor. Four strains of S. typhimurium, TA1535, TA1536, TA1537, and TA1538, were used in the assay with and without rat liver S-9 metabolic activation. Upon exposure to a mutagen these strains revert from histidine requiring prototrophy. A streptomycin sensitive strain of S. coelicolor A3(2), hisA1, was used in a forward mutation assay. Mutagenic effects of a chemical are measured by induction of streptomycin resistance. Both assays were performed as a "spot test" i.e., absorbent paper saturated with solutions of the chemical to be tested were placed on agar which was seeded with bacteria. In the S. typhimurium assay, dalapon-Na (85% pure) was tested at 2000 ug/plate while picloram (99% pure) was tested as 200 ug/plate. Neither compound induced increased numbers of revertants in any of the 4 tester strains with or without metabolic activation. The experiment was repeated 3 times using triplicate plates for each chemical. Results of both positive and negative controls were reported. In the S. coelicolor forward mutation assay, both dalapon and picloram were tested at 200 ug/plate without metabolic activation. Picloram treated plates had approximately 40 times the number of resistant colonies compared to controls while dalapon treatment did not induce an increase in numbers of resistant colonies. Testing of these compounds was repeated three times and results reported were an average of triplicate platings. Both positive and negative controls were reported. However, no statistical analyses or criteria for a positive result were presented by the authors.

156. Carlson, M., Nolting, A. R., and Fullerton, R. W. (Attorneys for the Secretary of Agriculture of the U.S.) (1974) In re: 2,4,5-trichlorophenoxyacetic acid. Statement of position of the Secretary of Agriculture of the U.S. FIFRA Docket No. 295. U.S. Environmental Protection Agency; Before the Administrator. 26 pp.

[Not available.]

157. Carlstedt-Duke, J. M. B. (1979) Tissue distribution of the receptor for 2,3,7,8 tetrachlorodibenzo-p-dioxin in the rat. Cancer Res. 39(8):3172-3176.

The concentrations of TCDD cytosol receptor for various rat tissues are presented. Male Sprague-Dawley rats were killed and tissues were removed and homogenized. The cytosol fractions were prepared by centrifugation and incubated with [³H]-TCDD. After mild proteolysis (to increase resolution at a subsequent step in the purification), free TCDD was removed onto dextran-coated charcoal and the supernatant was analyzed for the receptor complex by isoelectric focusing in polyacrylamide gel. For each tissue, the radioactivity on the purified receptor was displaced by addition of 100 fold higher concentration of 2,3,7,8-tetrachlorodibenzofuran. TCDD receptor concentrations, reported in femtomol per mg protein were thymus, 25.2; lung, 20.8; liver, 13.9; kidney, 12.5; testis, 3.5; brain, 2.8; muscle, 0.3; adrenal and pancreas, none. The TCDD-binding species of ventral prostate cytosol was not affected by protease treatment and focused at a lower pH. The author concluded that TCDD-receptor distribution correlated with the sites of TCDD effects, especially of aryl hydrocarbon hydroxylase induction; prostate tissue was one exception, and the receptor characteristics of this tissue were different from those for all other tissues studied.

158. Carlstedt-Duke, J. M. B., Elfstrom, G., Hogberg, B., and Gustafson, T. (1979) Ontogeny of the rat hepatic receptor for 2,3,7,8 tetrachlorodibenzo-p-dioxin and its endocrine independence. Cancer Res. 39:4653-4656.

The level of hepatic cytosol receptor for TCDD was determined in rats at various ages. Livers were removed from male and female Sprague-Dawley newborn rats and aged 7, 21, 42 and 56 days old. [³H]-TCDD was added to liver cytosol and the receptor was isolated by isoelectric focusing in polyacrylamide gel. Cytosol receptor levels were also determined in adult rats after an orchietomy, ovariectomy, adrenalectomy or hypophysectomy was performed. Male and female rats of the same age had the same number of receptors. The lowest receptor concentrations were observed in 7-day-old and 56-day-old rats, with 13 fmol/mg protein. Twenty-one day old rats had the highest concentration, with 36 fmol/mg protein. Rats that underwent surgical removal of an endocrine gland or other organ had the same number of hepatic cytosol receptors as intact rats. The authors concluded that the peak TCDD-receptor concentration occurred at puberty, at the same time that maximum inductive responses were observed (by others).

159. Carrier, J. M. (1974) The effects of herbicides in South Vietnam. Part B. Working papers: The location of herbicide missions and Hickey's informants in South Vietnam: An appraisal. National Academy of Sciences - National Research Council. AD-779 028. 15 pp.

[Background material.]

160. Carter, C. D., Kimbough, R. D., Liddle, J. A., Cline, R. E., Zack, M. M., Barthel, W. F., Koehler, R. E., and Phillips, P. E. (1975) Tetrachlorodibenzodioxin: an accidental poisoning episode in horse arenas. Science 188(4189):738-740.

The consequences are described of an incident in which TCDD-contaminated industrial wastes were sprayed on three horse arenas and one road to control dust. Of the exposed humans (total number was not reported), one 6 year-old female developed cystitis and pyelonephritis, and three other children and one adult developed skin lesions, which resembled chloracne in two persons. Two adults developed arthralgia. At one farm, 48 of 85 horses exposed to the sprayed arena died 1 month to 2.5 years after the arena was sprayed, despite several attempts to remove the contaminated soil. Horses exposed to the other two arenas also died. Symptoms in horses included weight loss, skin lesions, intestinal colic, dark urine, hematuria, conjunctivitis, and joint stiffness. Birds, dogs, cats, and rodents in the area died within a month of the spraying, and 70 chickens exposed to the sprayed farm road died 2 weeks after it was sprayed. The presence of TCDD in the soil was confirmed by gas-liquid chromatography and by gas chromatography-mass spectrometry, and the toxicity of the soil was demonstrated in a dermal bioassay in rabbits. The concentration of TCDD in the arena soil was 32 (u)g/g and in a distillate residue suspected of being the source of TCDD in the salvage oil, 330 (u)g/g. The authors concluded that the incident described here resulted from improper disposal of the toxic chemical waste TCDD.

161. Case, A. A. (1976) Tetrachlorodibenzodioxin (TCDD) - clinical aspects of poisoning. Clin. Toxicol. 9(6):963-967.

[Review article.]

162. Case, A. A., and Coffmann, J. R. (1973) Waste oil: toxic for horses. Vet. Clin. North Am. 3(2):273-277.

The clinical symptoms of horses that became ill after exposure to waste oil was described. General descriptions of symptoms were reported, without indicating the frequency of the observed symptom or quantitating the severity of the lesions (for example, average weight loss or body weights or serum protein levels were not given). Horses were exposed to waste oil sprayed on three arenas in Missouri. No reports of toxicity were issued for exposed animals or people from 13 other arenas that were sprayed during the same period of time by the same

company. The toxic substance in the waste oil had not been identified at the time of the report. The affected horses were characterized by severe weight loss, listlessness, nasal discharge suggestive of respiring infection, ulcerative dermatitis, loss of hair including the mane and tail, inflammation of oral and nasal mucous membranes, decreased serum gamma globulin levels, prolonged bromsulphalein clearance time and decreased cholinesterase activities of red blood cells. Changes observed at necropsy included mucous membrane inflammation, atrophy of adipose tissue, generalizing edema, and severe biliary lesions including dilation of bile ducts filled with bile-stained mucous. The spleen and lymph nodes were reduced in size. Loss of body fat and of hair was observed in affected dogs, cats, and mice.

163. Centen, A. H. J., Strik, J. J. T. W. A., and Colombi, A. (in press 1979-80) "Coproporphyrinuria and chronic hepatic porphyria type A in people from Seveso (Italy) exposed to 2,3,7,8 - TCDD." In: The Diagnosis and Occurrence of Chronic Hepatic Porphyria in Man Caused by Chonis, ed. J.J.T.W.A. Strik and J. H. Koeman. (North-Holland: Amsterdam.)

[Not available.]

164. Chang, H., Rip, J. W., and Cherry, J. H. (1974) Effects of phenoxyacetic acids in rat liver tissues. J. Agr. Fd. Chem. 22(1):62-65.

The hepatic response of rats to subchronic administration of 2,4-D or 2,4,5-T was studied. Male Long-Evans rats were administered 2,4-D or 2,4,5-T (less than 0.05 ppm TCDD contaminant) in paired feeding experiments 28 to 49 days. The average doses of each herbicide were 130 mg/week for rats initially 4 weeks of age and 220 mg/week for rats initially 7 weeks of age. Liver weights and hepatic levels of carbohydrate, glycogen, protein, DNA and RNA polymerase activity were measured in liver preparations from treated and control rats. A few 2,4,5-T treated rats showed evidence of toxicity and weight loss. Two deaths occurred among the 2,4,5-T treated groups of 52 rats and no deaths occurred among the group of 19 rats treated with 2,4-D. Younger rats were more susceptible to the toxic effects of 2,4,5-T than the older rats. None of the results were analyzed for statistical significance. Increased liver weights and concomitant increases in hepatic glycogen, reducing sugar, RNA and protein content occurred in 2,4,5-T treated rats. Liver glycogen was increased in 2,4-D treated rats. Hepatic nuclear DNA isolated from 2,4,5-T treated rats decreased compared to controls, while cytosol DNA was not altered. A technical problem of recovery of DNA in the relevant fraction of sucrose gradients was offered to explain these differences. Analogous to effects reported elsewhere for plants, hepatic RNA polymerase activity was stimulated 20-30% by either herbicide. The magnitude of this response was much less than the stimulation observed in plants. The inability to establish the statistical significance of changes observed in this study preclude any conclusions from being drawn from the study.

165. Charles, J. M., Abou-Donia, M. B., and Menzel, D. B. (1978) Absorption of paraquat and diquat from the airways of the perfused rat lung. Toxicology 9(1-2):59-67.

The pharmacokinetics of transfer of diquat from the airways to the vasculature was determined in perfused rat lung. Lungs from female Sprague-Dawley rats were isolated, suspended in an artificial glass thorax, respired artificially, cannulated through the pulmonary artery and perfused. A dose of 1.86 nmol/lung of [¹⁴C]-diquat in isotonic sucrose was administered into the lung, and radioactivity in the effluent was monitored for the subsequent 30 min. Diquat introduced into the airways was cleared from the lung in a biphasic pattern with half-lives of 4 and 75 minutes. The initial rate of absorption of diquat introduced into the vasculature and taken up into lung tissue was calculated at 18.4 pmol/sec and efflux rate was 22.1 pmol/sec. The kinetics of paraquat uptake and removal were also reported. The authors concluded that low pulmonary absorption of diquat reflected its lipid insolubility and rapid efflux of diquat indicated that diquat was unlikely to be stored in the lung.

166. Chiou, C. T., Freed, V. H., Schmedding, D. W., and Kohnert, R. L. (1977) Partition coefficient and bioaccumulation of selected organic chemicals. Environ. Sci. and Technol. 11(5):475-478.

[Background material.]

167. Chou, C., Montgomery, M. L., and Yu, T. L. (1971) Methodology and analysis for residues of MCP and 2,4,5-T in wheat. Bull. Environ. Contamin. Toxicol. 6:576-580.

[Background material.]

168. Clark, D. G., and Hurst, E. W. (1970) The toxicity of diquat. Brit. J. Ind. Med. 27(1):51-55

The acute and chronic toxicities of diquat are described for several species. Diquat dichloride or dibromide (99% purity) was administered in water or physiological saline by stomach tube or subcutaneous injection for acute studies. Diquat was administered to Friesian cows, Alderley Park strain dogs, guinea pigs, rats and mice, albino rabbits, and Rhode Island hens. Male mice and females of all other species were used. The oral LD₅₀ values were: cows, 30 mg (of cation)/kg; dogs, 100-200 mg/kg; rabbits, 101 mg/kg; guinea pigs, 100 mg/kg; rat, 231 mg/kg, mouse, 125 mg/kg; and hen, 200-400 mg/kg. The subcutaneous LD₅₀ for both salts was 10-11 mg/kg in the rat. Death occurred 2-14 days after dosing and was preceded by lethargy, respiratory difficulty, weight loss, and after injection, pupillary dilatation. Histological changes were minimal. Dermal toxicity and irritation were studied in the rabbit. A dermal dose of 400 mg/kg of diquat produced no ill effects or irritations. Daily doses of 20 mg/kg only produced mild

erythema and thickening of the skin, and 20 daily applications of 40 mg/kg was lethal to 4 of 6 rabbits and produced weight loss and muscle weakness. Slight eye irritation occurred for 2 days after one drop of a 20% solution of diquat was injected into the conjunctival sac of one eye of a rabbit. Rats (50 per group) were fed diets of 0.100-0.1% diquat dichloride for 2 years and dogs (6 per group) were fed 1.75 mg/kg daily for 4 yrs., 0.4 and 0.8 mg/kg for 3 yrs., and 5 mg/kg for 2 yrs. Weight gain was decreased only in the highest dosage rat group and no deaths resulted. Cataracts occurred in all groups of rats except the lowest dosage group and in dogs in the two highest dosage groups. No other changes were detected by gross or histological examinations, or clinical blood and urine analyses. Rats fed diets of 0.05% diquat for a few months did not develop cataracts in the next 2 yrs. Protecting diquat-treated rats from light or giving supplemental ascorbic acid (200 mg/ml in drinking water) did not alter the development of cataracts. Nasal inflammation and bleeding from human exposure to diquat powder and nail damage and delayed healing of cuts on the hand from exposure to diquat herbicide concentrate were mentioned. The authors concluded that diquat produced moderate toxicity, and did not readily penetrate or irritate the skin.

169. Clark, D. E., and Palmer, J. S. (1971) Residual aspects of 2,4,5-T and an ester in sheep and cattle with observations on concomitant toxicological effects. J. Agric. Fd. Chem. 19(4):761-764.

The distribution of 2,4,5-T in sheep tissues after subacute exposure is described. Four daily doses of 250 mg/kg 2,4,5-T (100% purity) were administered in gelatin capsules to one Delaine ewe. After the exposure period, tissue was removed and analyzed for 2,4,5-T content by gas chromatography. Recoveries by this method were 95% for 2,4,5-T in urine samples, 93% in blood, 71% in lean tissue and 78% in fat. (Data were not corrected for losses from low recoveries.) Tissue levels were 34 ppm 2,4,5-T in omental fat, 40 ppm in muscle, 20 ppm in liver, 176 ppm in kidney and 60 ppm in renal fat. Toxic effects observed in this sheep included anorexia, dehydration, and muscle spasms and, at necropsy, hepatitis, rumen stasis and enteritis. The distribution, metabolism and excretion of 2,4,5-T propylene glycol ether esters in cows and sheep were also reported. The authors concluded that tissue levels of 2,4,5-T after 4 daily doses of 250 mg/kg each were very high, especially in the kidney, the site of elimination.

170. Clark, D. E., Palmer, J. S., Radeleff, R. D., Cruckshank, A. R., and Farr, F. M. (1975) Residues of chlorophenoxy acid herbicides and their phenolic metabolites in tissues of sheep and cattle. J. Agr. Fd. Chem. 23(3):573-8.

Distribution of 2,4-D and 2,4,5-T in tissues of cattle and sheep are described. 2,4-D was administered at 300, 1,000 or 2,000 ppm in feed (equivalent to 9, 30 and 60 mg/kg body weight daily) to cattle and at 2000 ppm to sheep for 28 days. 2,4,5-T (less than 0.5 ppm TCDD contamination) was administered as 2000 ppm in feed to sheep for 28

days. Muscle, fat, kidney and liver samples were removed 1 day after the last exposure from each treatment group and 1 week later from animals that received the 2,000 ppm doses of each compound. Tissue samples were analyzed for 2,4-dichlorophenol and 2,4,5-trichlorophenol in the 2,4-D and 2,4,5-T-treated groups, respectively, by 2 methods. Recovery was above 80% for both methods and 0.05 ppm was the lowest detectable level reported. Fat and muscle samples from all treatment groups had less than 0.05 ppm of the corresponding phenol. 2,4-dichlorophenol levels detected by one method were 0.08-0.16 ppm in liver and kidney from both species, and were 2-5 times higher by the second method, which included an alkaline and acid hydrolysis steps (which would cleave any conjugated phenols, releasing free phenols). Liver 2,4,5-trichlorophenol levels were .4 and 6.1 ppm by the 2 methods and for kidney were .8 and .9 ppm by both methods, in cattle. 2,4-D levels were 1 ppm in sheep liver and 9 ppm in sheep kidney 1 day after the 2,000 ppm dosage ended. All 2,4-D, 2,4-dichlorophenol, and 2,4,5-trichlorophenol levels were substantially lower in both species and all tissues 1 week after exposure ended than after 1 day, except for 2,4,5-trichlorophenol levels in the liver and kidney of sheep. All treatments produced temporary reductions in weight gain. Chlorophenol metabolites were not produced by tissue homogenates mixed with 2,4-D or 2,4,5-T or by the hydrolysis procedures used to analyze phenols. The authors suggested that hydrolysis was carried out by rumen microorganisms or by enzymatic processes within the animal. Silvex metabolism was also studied in these investigations. The authors concluded that high residues of the phenoxy herbicides were not likely to be encountered in the tissues of grazing animals from herbicides used for agricultural purposes.

171. Clark, D. E., Young, J. E., and Younger, R. L., Hunt, L. M., and McLaran, J. K. (1964) The fate of 2,4-dichlorophenoxyacetic acid in sheep. J. Agric. Food Chem. 12(1):43-45.

The tissue distribution and rate of elimination of by 2,4-D sheep was assessed. One yearling ewe was administered 4 mg/kg of [¹⁴C]-2,4-D in 95% ethanol in a gelatin capsule. Blood, urine and fecal samples were collected for the subsequent 4 days and tissues were removed at necropsy (on day 4). All samples were extracted with ethanol and analyzed for radioactivity. The level of detection of 2,4-D in tissue was 0.05 ppm by the radiometric method used. Radioactivity in extracts of blood reached the peak value 75 min. after dosing and was not detectable 24 hours after exposure. At 8.5 hours, 50% of the dose was recovered in the urine and in 28 hrs. 90% was recovered in the urine. By 70 hrs., 95.8% of the dose was excreted in the urine and 1.4% was recovered in the feces. Only 1 radioactive compound in the urine corresponding to 2,4-D standards was separated by paper chromatography and paper electrophoresis. The only tissues were detectable levels of 2,4-D were the thyroid with 0.56 ppm and the urinary bladder with 0.50 ppm; no detectable amounts were recovered from any edible tissues. The authors concluded that 2,4-D was excreted unchanged in urine by sheep with no detectable residual compound in edible tissue.

172. Cleland, M. (1980). Statement of Administrator of Veterans' Affairs before the Subcommittee on Medical Facilities and Benefits of the Veterans' Affairs Committee, House of Representatives. Feb. 25, 1980. 44 pp.

[Testimony.]

173. Cobb, L. M. and Grimshaw, P. (1979) Acute toxicity of oral diquat (1,1'-ethylene-2,2'-bipyridinium) in cynomolgus monkeys. Toxicol. Appl. Pharmacol. 51(2):277-282.

The acute oral toxicity of diquat was described in monkeys. Diquat (dichloride monohydrate) in water was administered by stomach tube to Macaca fascicularis monkeys. Single doses of 100, 300 or 400 mg/kg of diquat were given to two monkeys per dose and 200 mg/kg was administered to four monkeys. After 14 days, all survivors were killed and necropsies were performed by gross and histologic examination. Blood and urine samples collected before treatment and during the observation period were analyzed for biochemical and hematologic parameters. Tissue slices were stained for glycogen and fat. Within 4 days of treatment one monkey each that received 100 and 300 mg/kg doses and both monkeys given the highest dose died. No other death occurred during the observation period. Diarrhea and vomiting occurred in all monkeys. All monkeys that died and no survivors showed increase in polymorphonuclear leukocyte counts which persisted to day 4 after treatment. Other changes that were transient in survivors and persisted in fatal cases were increases in serum urea, plasma glucose, SGOT and SGPT levels and in urinary protein, glucose, and blood cells. Abnormal findings at necropsy included necrotic gastrointestinal epithelia, renal lesions, necrosis and exfoliation of the epithelium of the kidney tubules, and minimal hepatic necrosis of single hepatocytes and fat droplets. Vacuolation of the lens observed in some monkeys was not attributed to diquat treatment. Survivors were found to be normal at necropsy, except for one monkey that displayed mild hepatic necrosis. The authors attributed death to the gastrointestinal and renal lesions and estimated the LD₅₀ to be between 100-300 mg/kg diquat ion. The authors concluded that diquat toxicity in the monkey resembles toxicity observed after human ingestion.

174. Cocucci, S., DiGerolamo, F., Verderio, A., Covallaro, A., Colli, G., et al. (1979) Absorption and translocation of tetrachlorodibenzo-p-dioxin by plants from polluted soil. Experientia 35(4):482-484.

[Background material.]

175. Cohen, G. M., Bracken, W. M., Lyer, R. P., Berry, D. L., Selkirk, J. K., and Slaga, T. J. (1979) Anticarcinogenic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on benzo(a)pyrene and 7,12-dimethylbenz(a)-anthracene tumor initiation and its relationship to DNA binding. Canc. Res. 39:4027-4033.

The authors investigated the role of TCDD in the suppression of tumors induced by benzo(a)pyrene (BP) and 7,12-dimethyl benz(a)anthracene (DMBA) by studying its metabolic fate: specifically, covalent binding to epidermal DNA, RNA, and protein, and the hydrocarbon-deoxyribonucleoside adducts after treatment of mouse skin. Female Sencar mice aged 7 to 9 weeks were tested for tumorigenesis. All chemicals were applied in 0.2 ml of acetone. Groups of 30 were given a single application of either 100 nmol BP or 10 nmol DMBA, applied topically to their shaved backs. One week later they were given the first of twice-weekly doses of 2 ug 12-O-tetradecanoylphorbol-13-acetate. Additional groups were also pretreated with 1 ug TCDD 72 hours before BP or DMBA initiation. The mice were observed for carcinomas and papillomas, and development was recorded weekly. Carcinomas and papillomas were removed at random for histologic verification. For mice pretreated with TCDD, there was a significant decrease in the incidence of DMBA- or BP-induced papillomas, and in the number of papillomas per mouse. For DMBA, TCDD pretreatment decreased the percent of mice with papillomas at 15 weeks from 100 to 10 percent, and the actual number of papillomas per mouse from 9.1 to 0.1. For BP, TCDD pretreatment reduced the percentage of mice with papillomas at 15 weeks from 85 to 25 percent, and the number of papillomas from 3.8 to 0.3. To study in vivo macromolecular binding, female CD-1 mice were used to test one compound as well as the Sencar. Treatment was similar to the tumor experiments, except that the initiating compounds used were [³H]BP (approximately 250 uCi/animal; 100 and 200 nmol of the compound for Sencar and CD-1, respectively) and [³H]DMBA (10 uCi/animal, and 10 nmol of the compound for Sencar). Certain animals were pretreated topically 72 hours before initiation with 1 ug TCDD. In some of the pretreated mice, an epoxide hydratase inhibitor 1,2-epoxy-3,3,3-trichloropropane (TCPO) was administered topically 10-15 minutes before initiation with [³H]BP. Three or 24 hours after the initiator was applied, the mice were killed. Using heat treatment, the epidermal material was removed from the animal, and DNA, RNA, and protein were extracted, and the amount of covalently bound radioactivity was determined. The DNA samples had an A₂₆₀:A₂₈₀ ratio of 1.89 ± 0.01, and an A₂₆₀:A₂₃₀ ratio of 2.34 ± 0.05 (mean ± S.E. of 16 determinations). In animals pretreated with TCDD, the binding of [³H]DMBA to both DNA and RNA was markedly decreased at both 3 and 24 hours after treatment. The effects of TCDD on binding of [³H]DMBA correlate with the decrease in the incidence of tumors, as previously reported. On the other hand, with TCDD pretreatment there was an increase in the binding of [³H]BP in the Sencar mice. In CD-1 mice, there was a marked increase in binding to DNA and protein, and a smaller increase in binding to RNA. Thus, it appeared to the authors that the decrease in tumorigenesis in TCDD-pretreated mice was associated with an increase in [³H]BP binding to DNA. The application of TCPO 10-15 minutes before initiation of pretreated mice caused an increase in [³H]BP binding to DNA, RNA, and

protein which was notably higher than when only [³H]BP was given, but which was similar to the effects of [³H]BP initiation with only TCDD pretreatment. Using DNase I, phosphodiesterase, and alkaline phosphatase, the authors also enzymatically hydrolyzed the extracted DNA to deoxyribonucleosides and separated the enzymatic hydrolysates. Fraction volumes were measured for radioactivity. A Sephadex LH-20 color chromatography elution profile 24 hours after [³H]BP treatment showed that the four deoxyribonucleosides eluted between 100 and 200 ml; some radioactive material eluted in this region, and between 30 and 90 ml. A radioactive hydrocarbon-deoxyribonucleoside adduct, apparently BP-7,8-dihydrodiol-9,10-epoxide, bound predominantly to the exocyclic NH₂ group of guanine (and to a lesser extent to the exocyclic NH₂ group of adenine) eluted at between 516 and 574 ml. In mice pretreated with TCDD alone or in combination with TCPO, there was no hydrocarbon-deoxyribonucleoside adduct corresponding to this; most radioactivity eluted before 84 ml. There was a correlation noted in the pretreated mice between significant tumor inhibition and the absence of the diol-epoxide-guanosine adduct from DNA. To trace the metabolism by epidermal homogenates of [³H]BP, Sencar mice were pretreated with either 1 ug TCDD or 100 nmol BP 24 or 72 hours, respectively, before sacrifice. They were killed by cervical dislocation, and their shaved backs were depilated with a cream that was washed off with water after 7 minutes. The scraped-off epidermis (4 mice/ml) was removed and suspended in a solution of 0.05 M Tris-0.25 M sucrose buffer₃ (pH 7.5) and then homogenized. This solution was then incubated with [³H]BP at 37° for 30 minutes, after which the reaction was stopped with 1 volume acetone. Ethyl acetate extracts were dried with sodium sulfate, concentrated by vacuum rotary evaporation, separated by high-pressure liquid chromatography, and eluted with a 30-70 percent methanol-water gradient. Metabolites which eluted included 9,10-dihydro-9,10-dihydroxybenzo(a)pyrene (9,10-diol), 8-dihydrodiol, quinones, 9-hydroxybenzo(a)pyrene, and 3-hydroxybenzo(a)pyrene. Just before 9,10-diol a major band of radioactivity eluted. Metabolites formed to a greater extent with TCDD pretreatment than with BP pretreatment. Untreated CD-7 mice tested in the same way showed only minimal metabolism in epidermal homogenates. The major dihydrodiol formed in all cases was 7,8-dihydrodiol, a precursor of the highly reactive mutagenic and carcinogenic (+)-7 beta, 8 alpha-dihydroxy-9 alpha, 10 alpha-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene. In homogenates from TCDD pretreated mice, the ratio of monohydroxybenzo(a)pyrenes to 7,8-dihydrodiol was much greater than in BP pretreated mice.

176. Cohn, W. E. (1977) Validity of animal testing. Chem. Eng. News 55(35):5.

[Editorial.]

177. Colburn, W. A. (1978) A model for the dose-dependent pharmacokinetics of chlorophenoxy acid herbicides in the rat: The effect of enterohepatic recycling. J. Pharmaco. Biopharmac. 6(5):417-426.

A two-compartment kinetic model of the distribution and elimination of 2,4,5-T is presented and used to analyze previously published pharmacokinetic data for these 2 herbicides. The concentrations of herbicide in the 2 compartments, representing the gut (compartment 2) and the remainder of the organism (compartment 1) are dependent upon 4 first-order rate processes. These processes include biliary excretion (k_{12}), fecal excretion (k_{20}), intestinal reabsorption (k_{21}) and renal excretion (V_{max}/k_m). At high plasma concentrations, renal excretion mechanisms become saturable and the kinetics of this process then become zero-order with a rate constant equal to V_{max} . Another model which was the same as the 2-compartment model except that a third compartment was inserted to represent the kidney. This compartment was described by 2 first-order rate constants, V_{max}/k_m to represent transfer between the plasma and kidney, and k_{30} , urinary excretion. Values for the rate constants from data on 2,4,5-T in the rat from another investigator was used to simulate the time course of plasma and urinary clearance of 2,4,5-T. For a low dose of 5 mg/kg 2,4,5-T, the simulated data matched the actual data for urinary and fecal excretion and kidney to plasma ratios. At a higher dose of 50 mg/kg, the model did not simulate the initial, non-linear portion of the plasma or urine curves. The model was modified to simulate kinetics after bile duct cannulation by eliminating the k_{21} component and the non-linear portion of the clearance curves were thereby reduced. The pharmacokinetics of 2,4,5-trichlorophenoxy propionic acid was also studied. The author concluded that the non-linear portion of the pharmacokinetic profiles for urine and plasma were dependent upon enterohepatic recycling of 2,4,5-T as well as saturation of renal mechanisms at high doses. The author also concluded that both the biological half-life and the toxicity of 2,4,5-T would be reduced by inhibiting biliary recycling.

178. Colby, C. (1978) Miscarriages suffered: Monkeys show effects of dioxin. Congressional Record - Senate. 124(118):S12271.

[Testimony.]

179. Collins, C. V. (1967) Herbicide operations in Southeast Asia, July 1961-June 1967. DTEC 67-0020. Pacific Air Force, APO San Francisco DTIC No. AD 779796. 76 pp.

[Background material.]

180. Collins, T. F. X., Williams, C. H., and Gray, G. C. (1971) Teratogenic studies with 2,4,5-T and 2,4-D in the hamster. Bull. Environ. Contam. Toxicol. 6(6):559-567.

The teratogenic effects of 7 commercial preparations of 2,4,5-T with various levels of TCDD contamination and 3 commercial preparations of 2,4-D were studied in the hamster. The test compounds were administered orally on days 6 to 10 of gestation. On day 14 of gestation, corpora lutea and fetuses were counted. Fetuses were stained with alizarin red and fixed in Bouin's fluid and observed for skeletal and visceral malformations, respectively. 2,4-D, which was tested at doses of 20-100 mg/kg, produced no dose-related alterations in fetal viability, fetal weights or frequency of malformations. Administration of 2,4,5-T preparations without detectable TCDD contamination at daily doses of 20-100 mg/kg, produced dose-related increase in fetal mortality and incidences of malformations and decreases in fetal weights. These effects were augmented in animals that received 2,4,5-T with 0.1, 0.5, 2.9 or 45 ppm TCDD. Exposure to the highest 2 levels of TCDD contamination, however, resulted in edematous fetuses which masked the decrease in fetal weights that resulted from the exposure. The malformations in 2,4,5-T treated fetuses were related to cranial development, but included only 2 cases of cleft palate. Exposed fetuses were observed with hemorrhagic gastrointestinal tracts, which were attributed to direct toxicity on the fetal organ rather than an effect on development.

181. Committee on Veterans' Affairs. (1979) Herbicide "Agent Orange" - Hearing before the Subcommittee on Medical Facilities and Benefits of the Committee on Veterans' Affairs, House of Representatives, 95th Congress - Second Session, Oct. 11, 1978. Washington, DC.: U.S. Government Printing Office, 62 pp.

[Testimony.]

182. Commoner, B. (1978) Toxicologic time bomb. Hosp. Prac. 13(6):56 and 59.

[Editorial.]

183. Commoner, B. (1977) Seveso: The tragedy lingers on. Clin. Toxicol. 11(4):479-482.

[Editorial.]

184. Commoner, B., and Scott, R. E. (1976) US Air Force studies on the stability and ecological effects of TCDD (dioxin): An evaluation relative to the accidental dissemination of TCDD at Seveso, Italy. St. Louis, Mo.: Center for the Biology of Natural Systems, Washington University, 51 pp.
- [Review article.]
185. Commoner, B., and Scott, R. E. Accidental contamination of soil with dioxin in Missouri: effects and countermeasures. (no ref.) 23 pp.
- [Not available.]
186. Comptroller General of the United States. (1979) U.S. ground troops in South Vietnam were in areas sprayed with Herbicide Orange. U.S., Washington, DC., No. FPCD-80-23. 12 pp.
- [Background material.]
187. Comptroller General of the United States. (1979) Health effects of exposure to Herbicide Orange in South Vietnam should be resolved. U.S., Washington, DC., No. CED-79-22. 38 pp.
- [Background material.]
188. Conaway, C. C., and Matsumura, F. (1977) Alteration of cellular utilization of thymidine by TCDD (2,3,7,8 tetrachlorodibenzo-p-dioxin). Bull. Environ. Contam. Toxicol. 13(1):52-56.

Alterations in thymidine incorporation by hepatic slices from TCDD-treated rats are described. Male Sprague-Dawley rats were administered 5 ug/kg TCDD in acetone-corn oil (1:9) by stomach tube. Controls received vehicle only. Ten days later, the liver was excised, and liver slices were incubated in medium with ³H-thymidine or ³H-uridine for 16 min. and then incubated in nonradioactive medium for 1 hr. Tissue was then homogenized and subfractionated by centrifugation. Radioactivity of each subcellular fraction was determined. The nuclei of treated cells contained 24% of total cellular radioactivity, compared to 12% for controls. No significant changes occurred in any other fraction or for ³H-uridine incorporation. Incorporation of ³H-thymidine by isolated nuclei from TCDD treated rats was higher than for controls and 78% of the nuclear radioactivity was associated with the chromatin material after the TCDD treatment, compared to 51% for controls. The authors concluded that TCDD disrupted nuclear utilization of thymidine and suggested that TCDD might disrupt DNA synthesis as its general mechanism of action in living cells.

189. Conning, D. M., Fletcher, K., and Swan, A. A. B. (1969) Paraquat and related bipyridyls. Br. Med. Bull. 25(3):245-249.
- [Review article.]
190. Cook, R. R., Townsend, J. C., Ott, M. G. (1980) Mortality experience of employees exposed to tetrachlorodibenzo-p-dioxin (TCDD). J. Occup. Med. 22:47-50.
191. Cooper, P. (1977) Dioxin: A new biological probe? Fd. Cosm. Toxicol. 15(5):481-483.
- [Review article.]
192. Corthay, J., Medilanski, P., and Benakis, A. (1977) Induction of hepatic microsomal enzymes by diuron, phenobenzuron, and metabolites in rats. Ecotoxicol. Environ. Safety 1(2):197-202.
- The effect of subacute administration of diuron to rats on microsomal liver enzyme activity is presented. Male Wistar rats (4 per group) were administered feed with 691 ppm diuron for 3, 7 and 14 days. Rats were weighed weekly. One day after the exposure period ended, rats were killed and hepatic microsomes were isolated and assayed for protein, cytochrome P-450, aminopyrine N-demethylation and aniline para-hydroxylation activities. Weight gain for treated and nontreated rats were comparable. The only parameters that were altered in treated rats were the microsomal protein content and cytochrome P-450 content which increased transiently at 7 days to 25-50% above control values. Other compounds were also studied in these experiments. The authors concluded that the high dose of diuron tested, which would probably be encountered only from occupational accidents showed weak effects on parameters related to microsomal enzyme induction.
193. Coulston, F. (1970) Working paper: 2,4,5-T. Institute of Experimental Pathology and Toxicology. Albany Medical College, Albany, N.Y. 26 pp.
- [Review article.]
194. Courtney, K. D. (1979) Postpartum CPK and LDH cardia and serum isozymes after 2,4,5-T, carbaryl or aniline treatment. Toxicol. Appl. Pharmacol. 48(1):A139.
- [Abstract, only.]

195. Courtney, K. D. (1977) Prenatal effects of herbicides: Evaluation by the prenatal development index. Arch. Environ. Contam. Toxicol. 6:33-46.

The teratogenicity fetotoxicity and embryolethality were evaluated in rats administered various esters of 2,4,5-T and 2,4-D by various routes in various vehicles. About 45 protocols were used for these experiments, in which various esters of 2,4-D, 2,4,5-T (with less than .05 ppm TCDD contaminant), and their structural analogs were administered during the second trimester to pregnant CD-1 mice. The compounds were administered by oral gavage or subcutaneous injection in a corn oil-acetone mixture (90% oil), dimethyl sulfoxide (DMSO), or 15% sucrose. On day 18 of gestation, fetuses were weighed, stained with Bouin's solution, and examined for malformations. Maternal weight gains and liver weight-to-body weight ratios were also recorded. The results for experimental groups were compared with corresponding vehicle control groups. Results were expressed in terms of a prenatal development index. 2,4,5-T, administered in doses from 0.45 to 1 mM/kg in different vehicles by different routes did not produce a statistically significant change in maternal weight but caused a consistent increase in maternal liver weight to body weight ratio. Three fetal effects were elicited by different dosages of 2,4,5-T. Cleft palate was observed at the lowest dosages and at higher doses was observed concomitant with increased fetal mortality. In addition to these effects, the highest dosages produced fetotoxicity which was detected as a decrease in fetal weight. The magnitude of the response elicited by the same dose of 2,4,5-T administered in various vehicles reflected the degree of solubility of the administered dose and corresponding bio-availability. The esters of 2,4,5-T produced the same types of maternal and fetal effects as 2,4,5-T, although cleft palate was produced at the same doses that produced fetal toxicity and mortality. 2,4-D and its esters elicited vehicle-dependent changes in maternal weight gain, and increased maternal liver weight to body weight ratios. Fetal weights were decreased at low dosages of 2,4-D which did not cause fetal mortality or cleft palate. In general, the structural analogs did not alter maternal liver to body weight ratios or fetal parameters, except Silvex which adversely altered all 3 fetal parameters although at doses higher than the active doses of 2,4,5-T. Agent Orange, administered orally at 1 mM/kg on days 12-15 resulted in an increased maternal liver to body weight ratio, decreased fetal weight and cleft palate (which was absent in the control group). The authors suggested that 2,4-D and 2,4,5-T act by different mechanisms because their combination in Agent Orange did not produce an additive effect, but rather had a potency intermediate between 2,4-D and the more toxic compound, 2,4,5-T.

196. Courtney, K. D. (1976) Mouse teratology studies with chlorodibenzo-p-dioxins. Bull. Environ. Contam. Toxicol. 16(6):674-681.

The teratologic effects of TCDD were studied in mice. On days 7 through 16 of gestation, pregnant CD-1 mice were administered doses of

25-400 ug/kg/day of TCDD in 5% anisole in corn oil by gastric intubation or 25-200 ug/kg/day of TCDD in DMSO subcutaneously. On day 18, fetuses were removed, weighed, and fixed in Bouin's solution for pathologic examination. Adult female CD-1 mice administered 10 ug/kg TCDD in anisole-corn oil orally for 14 days, showed no lethal effects or weight loss. Doses of 200 and 400 ug/kg/day to pregnant mice produced marked edema, vaginal bleeding and spontaneous abortions. At 100 ug/kg/day, TCDD adversely affected survival and weights of fetuses and maternal weight gain. At 25-50 ug/kg/day, TCDD produced an increase in the maternal ratio of liver to body weight. Subcutaneous doses of 25 ug/kg TCDD incidences of 82% for cleft palate, 53% for renal malformation and 11% for clubfoot. Control incidences were zero for cleft palate, 1% for kidney malformations and 4% for clubfoot. Oral doses of 50 ug/kg TCDD produced 19% cleft palate, 72% kidney malformations and 7% clubfoot. Other chlorodibenzo-p-dioxins were studied in these experiments. The authors concluded that TCDD was the most fetotoxic and teratogenic of the compounds they studied and that the bioavailability of TCDD given orally is less than that of subcutaneously administered TCDD.

197. Courtney, K. D. (1970) In Pesticides Symposia, Hales & Assoc., Florida. pp. 277-283.

[Not available.]

198. Courtney, K. D., Ebron, M. T., and Tucker, A. W. (1977) Distribution of 2,4,5-trichlorophenoxyacetic acid in the mouse fetus. Toxicol. Letters 1(2):103-108.

The distribution of radioactivity in pregnant mice administered [¹⁴C]-2,4,5-T was reported. Pregnant CD-1 mice were administered 100 mg/kg of [¹⁴C]-2,4,5-T orally on day 13 or daily from day 10 through 13 of gestation. From 0.5 to 72 hrs. after the last dose, mice were killed and maternal tissues and blood, placentas, and fetuses were analyzed for radioactivity. The location of radioactivity in fetal tissues was visualized by autoradiography. All tissues showed peak levels of radioactivity 8 hours after a single dose was given. At 48 hours only traces of radioactivity remained in maternal tissues. After 4 doses, maternal blood levels were 10 times higher than after the single dose, skeletal muscle had the same concentration and other organs in general as well as the fetuses had 2-3 fold higher levels. The autoradiographs revealed that the liver, eye, and ventricles contained most of the fetal radioactivity 30 minutes after a single dose. By 2 hours, radioactivity was distributed among most of the internal organs and by 8 hours, radioactivity was increased in the liver and brain. At later times, radioactivity was reduced. 2,4,5-T was deposited on the skin and could have come from the amniotic fluid. No radioactivity was associated with the palates at any time. Urine samples analyzed spectrophotometrically contained only 2,4,5-T. Half of the dose was excreted in 24 hr. Although the method used to identify urinary metabolites was not described sufficiently to convincingly demonstrate that

likely metabolites of 2,4,5-T could be separated, the results clearly demonstrate that 2,4,5-T administered to pregnant mice reaches the fetus and seems to be eliminated from both organisms at similar rates.

199. Courtney, K. D., Gaylor, D. W., Hogan, M. D., Falk, H. L., Bates, R. R., and Mitchell, I. (1970) Teratogenic evaluation of 2,4,5-T. Science 168:864-866.

The teratogenic effects of 2,4,5-T contaminated with 30 ppm TCDD were evaluated on 2 strains of mice and on the rat. The herbicide was administered subcutaneously in dimethylsulfoxide or orally in an aqueous honey solution. The test preparation was administered daily to C57BL/6 mice from days 6 to 14 or days 9 to 17 of gestation, to AKR mice from days 6 to 15 and to rats from days 10 to 15. C57BL/6 mice were killed on day 18 of gestation, AKR mice on day 19 and rats on day 20. Both mothers and fetuses were examined for gross histopathological effects and one-third of each mouse litter was stained with alizarin red and examined for skeletal anomalies. Daily doses of 113 mg/kg or 46 mg/kg administered by either route to both strains of mice or to rats produced substantial increases in the proportion of litters with at least 1 malformed fetus (up to 100%) and in the proportion of abnormal fetuses per litter (30-80%) compared to untreated controls or controls administered vehicle only. Cleft palate and cystic kidney accounted for almost all fetal abnormalities. Oral administration of 113 mg/kg 2,4,5-T caused a significant increase in fetal mortality per litter of rats or either strain of mice. Lower doses of 2,4,5-T did not produce teratotoxic or fetotoxic effects in mice, although rats were susceptible to these effects from doses of 10 mg/kg 2,4,5-T. The ratio of liver weight to body weight was elevated significantly in fetuses and in mothers of C57BL/6 mice treated on days 9 to 17 with 113 mg/kg 2,4,5-T. Although 2,4,5-T treatment of AKR strain mice did not induce cystic kidney malformations in fetuses, hybrid litters of C57BL/6 females mated with AKR males were susceptible to this defect from 2,4,5-T exposure. A dose-related increase in the incidence of hemorrhagic gastrointestinal tract in rat fetuses from 2,4,5-T exposure was attributed to direct toxicity on the fetal organ rather than a developmental defect.

200. Courtney, K. D., and Moore, J. A. (1971) Teratology studies with 2,4,5-trichlorophenoxyacetic acid and 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol. Appl. Pharmacol. 20:396-403.

The teratogenic effects of 2,4,5-T preparations of known purities and of TCDD was evaluated in 3 mouse strains and in the rat. Two inbred strains of mice, DBA/2J and C57Bl/6J and CD-1, a random-bred mouse strain were tested, as well as CD strain rats. Pregnant rats were administered TCDD and 2,4,5-T in dimethylsulfoxide (DMSO) subcutaneously on days 6 through 15 of gestation and fetuses were removed and examined on day 17 (CD-1) or day 18. Pregnant rats were administered 2,4,5-T in a 15% sucrose solution by gastric intubation and TCDD in DMSO from days 6 through 15. Rat fetuses were examined on day 20 of

gestation. Vehicle controls but no untreated controls were included. Neither an analytical grade nor a technical grade of 2,4,5-T nor TCDD were fetocidal in mice or rats at doses below those which produced maternal toxicity. A dose-related decrease in fetal weight was observed in CD-1 mice administered 2,4,5-T which was considered a toxic effect on the fetus. This effect was not seen in the rat or with TCDD treated animals. An increased maternal liver to body weight ratio and decreased maternal weight gain occurred in some groups of rats and some mouse strains from 2,4,5-T or TCDD administration. TCDD and 2,4,5-T each produced cleft palate in all mouse strains but not in the rat. Administration of both agents together did not result in a potentiation of the effect in the mouse. Renal anomalies, including unilocular cystinephrotic kidney and hydronephrosis, were observed in CD-1 mice treated with 2,4,5-T and in all strains of mice and in rats treated with TCDD with no potentiation observed when both agents were administered. C57Bl/6J showed the highest sensitivity to TCDD-induced kidney anomalies. In a postnatal study, rats delivered from 2,4,5-T-treated mothers showed the same weight gains and mortality to 21 days of age and timing of eye-opening as those delivered from vehicle controls. The authors suggested that differences in teratologic responses of the various strains to each agent may reflect differences in distribution or metabolism of the compounds.

201. Courtney, K. D., Putnam, J. P., and Andrews, J. E. (1978) Metabolic studies with TCDD (dioxin) treated rats. Arch. Environ. Contam. Toxicol. 7(4):385-396.

Food and water consumption and changes in body weight were monitored in rats given a single dose of TCDD and fed modified diets. Female Wistar rats were administered 100 ug/kg TCDD (99%+ pure) in 5% anisole in corn oil by oral intubation. Control rats received the vehicle only. Supplemental diets were administered by oral intubation given in 5 ml portions 3-7 times per day. Diets were supplemented daily with either 15 ml of water, 15 or 35 ml of an oral electrolyte solution, or 15 ml of a nutrient supplement dissolved in electrolyte solution. The typical pattern of weight loss in treated rats were biphasic, with an abrupt loss during the first 7-10 days followed by an abrupt gain during the next 4-5 days and then a sustained gradual weight loss. Concomitant changes in food and water consumption occurred. The TCDD-treated rats administered supplemental diets showed the same mortality and the same weight changes which occurred at the same times as TCDD-treated rats fed ad libitum. The rats on supplemental diets voluntarily consumed almost as much water as non-supplemented rats. In general, the TCDD-treated rats that survived had smaller weight losses, gained more weight during the 4-day period between the two phases of weight loss, and had normal organ weights, all of which contrasted with those of rats that did not survive. Brain, adrenal, and kidney weights did not change in any TCDD treatment group. Urinalysis revealed high protein content during the first week after TCDD treatment which returned to normal in rats that survived. A transient orange color of the urine observed 2 weeks after TCDD treatment was not accompanied by fluorescence under UV light. Other control rats

deprived of water or food for 4 days lost as much weight as TCDD-treated rats and became irritable and restless. They regained the weight after 4 days on a normal diet, in contrast to TCDD-treated rats which were listless and achieved their initial body weight in 20-30 days. No changes in oxygen uptake by hepatic mitochondria were observed 7 or 14 days after TCDD treatment. The authors concluded that some of the observed changes resulted secondary to the primary effect of cessation of food and water consumption or to non-utilization of nutrients, but the present studies could not distinguish between primary and secondary effects.

202. Coutselinis, A., Kentarchou, R., and Boukis, D. (1977) Concentration levels of 2,4-D and 2,4,5-T in forensic material. Forensic Science 10:203-204.

Human tissue levels of 2,4-D and 2,4,5-T are presented for a fatal case of poisoning. Blood, liver, spleen and kidney samples were removed at autopsy from a woman who consumed an unknown amount of Tributon 60 (containing a 3:2 mixture of 2,4-D; 2,4,5-T) 16 hours before death occurred. These samples were analyzed for 2,4-D and 2,4,5-T by thin-layer chromatography. The levels of 2,4-D were 82.6 mg/100 ml blood, and 2.1, 1.2 and 8.2 mg/100g of liver, spleen and kidney, respectively. 2,4,5-T levels were 18.2 mg/100 ml blood and 0.5, 0.5, and 2.2 mg/100g of liver, spleen and kidney, respectively. The authors concluded that a large amount of herbicide was ingested but did not have enough information to estimate the exact amount.

203. Crabtree, H. C., Lock, E. A., and Rose, M. S. (1977) Effects of diquat on the gastrointestinal tract of rats. Toxicol. Appl. Pharmacol. 41(3):585-595.

The effects of oral diquat administration on redistribution of water from the blood to the gastrointestinal tract of rats is described. Male Alderley Park rats were administered 900 $\mu\text{mol/kg}$ of diquat in saline by stomach tube or 90 $\mu\text{mol/kg}$ in saline, subcutaneously. Controls received saline vehicle only. Body weights were determined at the time of dosing and 24 hr. later. Mortality was recorded for 8 weeks. Water content was determined for the gastrointestinal (GI) tract by clamping both ends and weighing the tissue and its contents before appreciable evaporation occurred. Hematocrits were determined and tissue water was estimated as the amount of weight loss that occurred during drying to a constant weight. Within 24 hrs. of oral diquat dosing, water intake was reduced to 19% of controls. After 24 hr., signs of toxicity, including subdued activity, piloerection, and characteristic green feces were evident. Within 3 days, half of the total deaths occurred and these animals, unlike the 3-day failed to gain weight. After subcutaneous dosing, water intake and signs of toxicity resembled those after oral dosing but the symptoms appeared within 4-6 hr. The earliest deaths occurred after 5 days and some rats died as late as 8 weeks after dosing. All rats that died after 14 days (but no survivors) had severely extended terminal ileums and cecums. Water

content of the GI tract was maximum 1 day after oral dosing and the magnitude was dose-related, with twice the water content of control after 900 umol/kg was administered. The same effect occurred after subcutaneous dosing but did not appear until 3 days after dosing and was maximum at 8 days (the last day water content was measured). This elevation did not occur in the survivors who also did not have distended abdomens. Significant blood, muscle and liver dehydration occurred 24 hr. after oral dosing. Hemoconcentration after subcutaneous dosing was transient and peaked 17 hr. after injection. The authors concluded that deaths after oral diquat administration could be attributed to rapid fluid loss to the GI tract and later deaths and deaths after subcutaneous dosing appeared to have unrelated and unidentified causes. Information on volumes of urine excreted by rats in this experiment would help in understanding the mechanisms of water balance that are crucial to this study and concurrent histology of tissues from the GI tract would also help to elucidate the source of fluid shifts.

204. Crabtree, H. C., and Rose, M. S. (1978) Effect of diquat dichloride on gastric emptying and fluid accumulation in the rat stomach. Toxicol. Appl. Pharmacol. 45(1):259-260.

[Abstract, only.]

205. Crabtree, H. C., and Rose, M. S. (1976) Early effects of diquat on plasma corticosteroid concentrations in rats. Biochem. Pharmacol. 25(22):2465-2468.

The effect of diquat administration on corticosteroid production in vivo and in vitro was studied in the rat. Male Sprague-Dawley rats were administered 250 ug/kg dexamethasone (subcutaneously) and 2 hrs. later were anesthetized with phenobarbital (150-200 mg/kg, subcutaneously). Two hrs. after the anesthetic was given, diquat dichloride monohydrate in saline was administered, an oral dose of 900 u moles/kg or an intraperitoneal or subcutaneous dose of 10-260 u moles/kg. From 1 to 4 hrs. later, the animals were killed by decapitation and blood was removed and analyzed fluoremetrically for corticosteroids and by radioimmunoassay for adrenocorticotrophic hormone (ACTH). In vitro incubation of rat adrenals was performed in the presence of diquat for 2 hrs., with ACTH added during the last 30 min. Adrenal tissue was then homogenized and analyzed for corticosteroid content. Plasma corticosteroid levels (in non-dexamethasone-pretreated rats) were increased maximally within 30 min. of a subcutaneous or intraperitoneal dose of 90 u moles/kg diquat to four times above levels of vehicle controls. This effect was sustained for 4 hrs. and occurred after oral administration, with a delay in the onset of the effect. The effect was maximum after 4 hrs. with doses of 50 u moles/kg of diquat and greater. The effect was blocked by pretreatment with dexamethasone and the ACTH levels were significantly elevated in diquat treated rats after 1 hr., compared to ACTH levels in rats administered dexamethasone

and diquat. ACTH administration in vivo did not alter the plasma corticosteroid levels in diquat treated or control rats. In vitro, diquat at 25-50uM inhibited corticosteroid synthesis significantly in the presence of ACTH but not in its absence. The authors concluded that diquat caused increased adrenal steroid synthesis by stimulating release of ACTH from the pituitary.

206. Craig, D. A. (1975) Use of herbicides in Southeast Asia. Historical report. San Antonio Air Logistics Center, Directorate of Energy Management, Kelly AFB, Texas. 58 pp.

[Background material.]

207. Creso, E., De Marino, V., Donatelli, L., and Pagnini, G. (1978) Effetti neuropsicofarmacologic: della TCDD. Boll. Soc. It. Biol. Sper. LIV:1592-1596.

[Foreign language.]

208. Croft, W., Yang, K. H., and Peterson, R. E. (1977) Pathological effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the common bile duct of rats. Fed. Proc. Fed. Am. Soc. Exp. Biol. 36(3):1060.

[Abstract, only.]

209. Crosby, D. G. (1976) Nonbiological degradation of herbicides in the soil. In Herbicides: physiology, biochemistry, ecology. Vol. 2. ed., L. J. Audus, (New York: Academic Press) pp 65-97.

[Review article.]

210. Crosby, D. G., Moilanen, K. W., and Wong, A. S. (1973) Environmental generation and degradation of dibenzodioxins and dibenzofurans. Environ. Health Perspec. 5:259-265.

The authors studied the degradation and generation of TCDD and other dioxins in a laboratory simulating a field situation. Photochemical generation of TCDD was measured by ultraviolet irradiation of 2,4,5-T, 2,4,5-trichlorophenol, or sodium 2,4,5-trichlorophenate, in a "sunlight simulator" equipped with F40BL fluorescent lamps. The authors did not report the details of the experiment. No TCDD was detected as a photo-generation product of any of the three chemicals. The authors explained their failure to detect TCDD by its extreme instability in sunlight. TCDD (5 mg/ml) in purified methanol, was nearly 60% degraded by 6 hours irradiation in the "sunlight simulator." The applicability of this system to a field situation was not known by the authors.

211. Crosby, D. G., and Tutass, H. O. (1966) Photodecomposition of 2,4-dichlorophenoxyacetic acid. J. Agric. Food Chem. 14:596-601.

[Background material.]

212. Crosby, D. G., and Wong, A. S. (1973) Photodecomposition of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in water. J. Agric. Food Chem. 21(6):1052-1054.

[Background material.]

213. Crosby, D. G., and Wong, A. S. (1977) Environmental degradation of TCDD. Science 195(4284):1337-1338.

The authors studied the photodecomposition of TCDD in a model environmental system. Residual TCDD was measured by gas-liquid chromatography. Thin layers of Agent Orange containing 15 ppm TCDD on glass petri dishes were exposed to summer sunlight. Identical plates were shielded from light and served as dark controls. After 6 hours of exposure to sunlight, less than half of the applied TCDD was present on plates. Agent Orange was also applied in droplets (size unspecified) over excised leaves of a rubber plant and on the surface of loam soil and exposed to sunlight. Less than 50% of the TCDD was present on leaves 2 hours after irradiation, while 80% of the TCDD was present on the soil 6 hours after irradiation. Dark controls remained unaffected. From these and previous experiments, the authors concluded that TCDD photodecomposition requires 1) dissolution in a light transmitting film, 2) the presence of an organic hydrogen donor, such as solvent or pesticide, and 3) ultraviolet light. All three conditions would have been met during Agent Orange spraying in southeast Asia.

214. Crosby, D. G., Wong, A. S., Plimmer, J. R., and Woolson, F. A. (1971) Photodecomposition of chlorinated dibenzo-p-dioxins. Science 173(3998):748-749.

The authors studied the photodecomposition of TCDD. When TCDD was diluted in methanol (5 mg/l) and irradiated by summer sunlight or a "sunlight simulator", photodecomposition occurred in 24-36 hours. Photolysis products included 2,3,7-trichlorodibenzo-p-dioxin and a dichloro homolog identified by mass spectrometry. However, when TCDD was added as a spot (2.4 ppm in ethanol) on glass plates coated with sandy soil or silty clay loam soil, irradiation with an ultraviolet lamp for 96 hours produced negligible decomposition. The same result was achieved when the soil was moistened with water. Similarly, when TCDD was applied as a dry film to glass dishes or in a suspension of water, quantitative recovery of TCDD was observed after 14 days irradiation. This study suggests that TCDD is photodecomposed in the presence of organic hydrogen donors such as organic solvents, but not on surfaces of soil and water.

215. Crossen, P. E., Morgan, W. F., Horan, J. J., and Stewart, J. (1978) Cytogenetic studies of pesticide and herbicide sprayers. New Zeal. Med. J. 88(619):192-195.

The authors studied chromosomal damage in 57 pesticide and herbicide sprayers by the sister chromatid exchange (SCE) test. The test group was composed of healthy males from a wide variety of occupations that involved spraying pesticides and herbicides. Exposure to specific pesticides was not reported. A control group of 15 males with no known history of exposure was included in the study. Peripheral blood samples were obtained from each test subject and lymphocytes cultured from the samples for 72 hours followed by 4 hour incubation with colchicine. Twenty well spread metaphases were examined for each subject. Scoring of sister chromatid exchanges was done using coded samples. No positive controls were reported by the authors. Only a one time sampling was done by the investigators. When the means of sister chromatid exchanges of control and exposed subjects were compared no significant differences in the two groups were observed. However, 5 sprayers had an SCE rate 3 standard deviations outside the mean of the controls. The data were then analyzed according to length of exposure and use of protective clothing. Only the group of sprayers with greater than 1 year exposure and no use of protective clothing showed an elevated SCE rate. The authors concluded that these data suggest a possible genetic hazard from pesticide and herbicide spraying. However, because of the small number of subjects, the unknown nature of exposures, and the one time sampling, no real conclusion can be made about this study.

216. Crow, K. D. (1980) Direct testimony before the U.S. Environmental Protection Agency, FIFRA Docket No. 415 et al., Nov. 14.

[Testimony.]

217. Crow, K. D. (1979) Lipid profiles in dioxin-exposed workers. Lancet 1:982.

[Editorial.]

218. Crow, K. D. (1978a) Chloracne - an up to date assessment. Ann. Occup. Hyg. 21:297-298.

[Review article.]

219. Crow, K. D. (1978b) Chloracne: the clinical disease. New Scientist pp. 78-80.
[Review article.]
220. Crow, K. D. (1977) Effects of dioxin exposure. [letter to the editor] Lancet 2(8028):82-83.
[Editorial.]
221. Crow, K. D. (1970) Chloracne. Transactions of the St. Johns Hospital Dermatological Society 56:79-99.
[Review article.]
222. Cunningham, H. M., and Williams, D. T. (1972a) Effect of tetrachlorodibenzo-p-dioxin on growth rate and the synthesis of lipids and proteins in rats. Bull. Env. Contam. Toxicol. (U.S.) 7:45-51.

The effects of dioxin treatment on hepatic protein and lipid synthesis was studied in the rat. Single doses of 0.1-10.0 ug/kg of TCDD in corn oil were given to weanling male Wistar rats by oral intubation. From 1-7 days later, ¹⁴C-leucine and ³H-sodium acetate were injected intraperitoneally and 1 hour later liver, adipose, and muscle tissues were removed and homogenized. Lipids were extracted by a method described elsewhere and protein in the aqueous phase was precipitated with trichloroacetic acid, solubilized and counted for radioactivity by liquid scintillation methods along with radioactivity incorporation into lipid. Thin-layer chromatography was used to fractionate various lipid fractions. At 10 ug/kg, TCDD caused a statistically significant decrease in weight gain after 3 days and in radioactivity (per mg liver) associated with total lipids, triglycerides, phospholipids, and epididymal lipids while liver protein synthesis increased. The effects on synthesis and body weights were not consistently reproduced in subsequent experiments presented in this report. The effect on hepatic total lipid incorporation was greater 7 days after TCDD treatment than from 1-3 days after treatment. Both the liver weight and the total lipid content of the liver increased with TCDD treatment, so that the total incorporation of radioactivity into lipids (DPM per liver) did not change, but the specific activity of the liver lipids (DPM per mg lipid) decreased substantially. The authors concluded that TCDD restricted the transport of lipids out of the liver. The adequacy of biochemical techniques in separating incorporated from unincorporated counts, in separating and recovering quantitatively the protein and lipid fractions, and difficulties in reproducing effects in each experiment limit the interpretation of these data. Changes in specific activity of the isotope, effects of protein and lipid degradation during the 1 hour pulse period, and large variability in data preventing a two-three fold changes in incorporation from being statistically significant difference further weaken this study.

223. Cupello, J. M., Young, A. L., and Smith, J. C. H. (1977) A method for simulating subsurface disposal of herbicides. Weed Sci. 25(4):368-372.

The authors describe a laboratory model to simulate field disposal practices of phenoxy herbicides using subsurface injection and to investigate the effects of massive quantities phenoxy herbicides to undercut and uncut sorghum. Special growth boxes were designed for use in the experiment so that root systems of the plants could be reached. Four treatment variables were used: 1) under cut control, 2) undercut treated, 3) uncut control, and 4) uncut treated. Three treated replicates and two control replicates were used in the study. Herbicide treatment was 20.1 ml of a 50:50 mixture of the butyl esters of 2,4-D and 2,4,5-T (equivalent to 2,240 kg/ha) and occurred on day 3 for plants with uncut root systems and on day 22 for plants with cut root systems. Due to variations within the model system undercut boxes could not be compared to uncut ones. Treatment of both cut and intact root systems with herbicides caused a statistically significant reduction in plant growth. The authors concluded that this was a reliable model for assessment of herbicidal effects in the laboratory.

224. Cutting, R. K., Phuoc, T. H., Ballo, J. M., Benenson, M. W., and Evans, C. H. (1970) Congenital malformations, hydatidiform moles and stillbirths in the Republic of Vietnam 1960-1969. (Washington, DC.: Department of Defense. U.S. Government Printing Office No. 903.233.) 29 pp.

To determine the incidence of stillbirths, hydatidiform moles, and congenital malformations, data were collected from 22 hospitals throughout the Republic of Vietnam. The primary data source for all but 4 hospitals was the Daily Summary Ledger, prepared by the chief midwives. The following criteria were used to record stillbirths, malformations and moles: 1) stillbirths were determined by fetal weights of 750 grams or more with a gestation period of at least 24 weeks; 2) malformations were recorded for both live births and stillbirths and, 3) hydatidiform moles were counted only if delivered. The following is a breakdown of the 488,852 birth events from 1960-1969: 470,200 live births; 15,812 stillbirths; 2,840 hydatidiform moles; and 2,355 congenital malformations, of which 80% were anencephaly, cleft lip/palate, clubfoot, and hydrocephaly. Data were sorted into two five-year time periods, 1960-1965 a period of eight spraying and, 1966-1969 a heavy spray period, to identify incidence trends associated with herbicide usage. Data show the following: for the period 1960-1965: mole rate - 6.6 per thousand, stillbirth - 36.1 per thousand, malformation - 5.5 per thousand; for the period 1966-1969: mole rate - 5.6 per thousand, stillbirth - 32.0 per thousand, malformation - 4.5 per thousand. The following biases limit the use of the findings: 1) the majority of the data were obtained from population centers and large hospitals; 2) private clinics and hospitals were not surveyed; 3) data were collected only on ethnic Vietnamese where a large segment of the population were Chinese; 4) data on home delivery and morbidity characteristics were not collected; 5) gaps in data appeared due to destroyed records. Authors concluded that "meaningful correlation of any province's annual abnormal birth events to quantitative herbicide data were precluded by inconsistent sampling of birth data."

225. Dalderup, L. M. (1974) Safety measures for taking down buildings contaminated with toxic material. J. Soc. Geneesk. 52:582-623.

Results are described of clinical laboratory tests of workers who were involved in dismantling a factory in Amsterdam, the Netherlands, that was contaminated with TCDD. Twelve healthy workers participated in the project and each worker wore a suit with its own oxygen supply while working in the contaminated building. Physical measurements that were performed included lung function tests, X-ray photographs of the thorax, and photographs of the exposed skin areas. Nine different enzyme levels and hematologic parameters were monitored, and urinalysis was performed. Each worker was given a physical examination and his medical history was studied for conditions or medications that would complicate interpretation of the results. Apart from 1 case of slight glucosuria which was noted prior to exposure, no serious abnormalities were detected by any of the tests. Two additional men entered the contaminated area, without protective head gear. Both men had abnormal liver function tests at least once in the 11 week period after exposure (the type of test or magnitude of the deviation from normal was not stated). These workers were not part of the demolition team, so no pre-exposure values for liver function tests were available for these men. The authors concluded that the air-supplied suits used in this decontamination operation provided adequate protection of the workers from the adverse effects of TCDD.

226. Dalgaard-Mikkelsen, S. V., and Poulsen, E. (1962) Toxicology of herbicides. Pharmacol. Rev. 14:225-250.

[Review article.]

227. Daniel, J. W., and Gage, J. C. (1966) Absorption and excretion of diquat and paraquat in rats. Brit. J. Industr. Med. 23:133-136.

The excretion of diquat in urine, feces and bile was determined in the rat. Male Wistar rats were administered 14-85 mg/kg [¹⁴C]-diquat dibromide or dichloride in aqueous solution by gastric intubation or subcutaneous injection. Urine, feces, and bile (from cannulated bile ducts collected for 24 hr. from restrained rats) were collected and analyzed for radioactivity and for diquat and metabolites by a colorimetric procedure after separation by ion exchange chromatography. Recovery of the administered radioactivity was reported to be 90-101%. From 84 to 97% of an oral dose of either diquat salt was excreted in the feces during the 3 days following exposure and 4 to 11% appeared in the urine. From 0.5 to 5.4% of the oral dose of diquat dibromide was excreted as urinary metabolites, 68-81% as fecal metabolites, and 1.1-4.8% was excreted in the bile. After diquat was injected, 88-98% of the dose of dibromide salt was excreted in the urine and up to 2% in feces, and 81-90% of the dose of dichloride salt was recovered in the urine in 2 days. After fecal homogenates were incubated for 24 hr. at 37°C, 52% of the diquat content was degraded, compared to 8% when the microbial content was heat-denatured prior to incubation. Paraquat

excretion was also studied. The authors concluded that diquat was poorly absorbed from the gut and that biotransformation resulted only from intestinal microbial degradation and reabsorption of metabolites, as no metabolites were detected in the urine after subcutaneous dosage.

228. Danon, J. M., Karpati, G., Carpenter, S. (1978) Subacute skeletal myopathy induced by 2,4-dichlorophenoxyacetate in rats and guinea pigs. Muscle and Nerve (1) Mar/Apr:89-102.

The histochemical and histopathological changes associated with myopathy after 2,4-D administration are described. Sprague-Dawley rats and Hartley guinea pigs were administered 200-250 mg/kg/day of 2,4-D in three separate doses per day, by intraperitoneal injection. After 2-5 days of treatment, biceps muscles were removed and prepared for histochemical analyses, phase microscopy, or electron microscopy. During the day the treated animals remained myotonic and approximately 20% of the animals died and were not studied further. A total of 14 rat biceps and 12 guinea pig biceps were studied. Histochemical changes in muscles from treated rats and guinea pigs included extensive Z-disc streaming in type 2 fibers, masses of proliferating sarcotubular profiles, neutral lipid droplets in muscle fibers, and depletion of type 28 fiber glycogen and amylophosphorylase. Increases in the numbers of necrotic and of regenerating fibers were seen in muscles after 3 days of treatment. Regenerating fibers were also seen 2 and 7 days after treatment was terminated. Fat droplets, sarcomere smudging, necrotic fibers, and vacuoles were also identified in both species by phase microscopy. Electron microscopy revealed, in addition, proliferation and dilatation of the sarcoplasmic reticulum, proliferation of myofilaments in cytoplasm and nuclei, loss of myofilament organization, and rounded mitochondria in both species. The authors concluded that in both species 2,4-D produced proliferative effects, possibly related to auxin activity, and degenerative effects that may have occurred secondarily, after a significant amount of cellular energy was diverted to the proliferative activities.

229. Danow, R. A., Truchelut, G. B., and Bartlett, C. M. (1966) OCONUS defoliation test program. US Army Biological Center, Fort Detrick, Frederick, MD. Technical Report No. 79.

[Background material.]

230. Davidson, J. H. (1980) Update of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Down to Earth 36(2):19-22.

[Background material.]

231. Davis, D. E. (1979a) Dioxin and the Vietnam veteran. The Bulletin. pp. 58-59.

[Editorial.]

232. Davis, D. E. (1979b) Herbicides in peace and war. BioSci. 29(2):84, 91-94.

[Editorial.]

233. Davis, G. G. (1974) Fluometuron and 2,4,5-T residues in soil, sediment, runoff water and percolation water. Dissertation Abstracts International: Section B 34(11):5285B-5286B.

[Abstract, only.]

234. Davring, L. (1975) Effects of a 2,4,5-T ester on early oogenesis, fertility, and development in Drosophila melanogaster. Hereditas 80:255-262.

The authors studied the effects of a commercial preparation of 2,4,5-T butoxyethyl ester (500 g/l spraying fluid) on oogenesis, fertility, and development in female Drosophila melanogaster. The 2,4,5-T used in this study contained less than 0.1 ppm TCDD as a contaminant. Twenty-four-hours-old Canton-S strain adult flies were fed on wheat agar medium containing 0, 50, 100, 250, or 500 ppm 2,4,5-T for 4 days. Eight replicate cultures containing 10 males and 10 females were set up for each dose level. Development of eggs from exposed females was studied. Decreased fertility (adults/eggs laid) was observed as low as 50 ppm 2,4,5-T and was dose dependent. However, fertility of controls in this experiment was unusually low. The author's explanation for this phenomenon was that flies were grown on wheat agar medium instead of the usual corn agar medium. The number of eggs laid by treated flies also decreased with increasing doses of 2,4,5-T. The authors speculated that the decrease in oviposition may reflect disturbances in oogenesis or an aversion to laying eggs in the treated medium or both. Chromosome disturbances during oogenesis were also studied by examining defective egg follicles. Technical routine handling of ovaries from control flies resulted in a loss of approximately 30% of the egg follicles. The authors did not report an estimate on the loss of egg follicles from treated flies. Egg follicles were collected from freshly eclosed (hatched) females and females that were more than 24 hours old at the beginning of treatment. Flies were grown on medium containing either 1 or 250 ppm 2,4,5-T for 5 days. On the 6th day, the flies were killed and ovaries fixed on slides for microscopic evaluation. The percentage of defective egg follicles increased with 2,4,5-T treatment and was dose dependent. Freshly eclosed females treated with 2,4,5-T had more defective egg follicles than the older females. In addition, about 95% of the first metaphase chromosomes in stage 14 oocytes became a single compact chromosome plate, a normal event. However, in stage 14 oocytes from 2,4,5-T treated females first metaphase chromosomes were distributed in two or three groups in about 26% of the oocytes examined. No effects of 2,4,5-T on the male were presented in this study.

235. Davring, L., and Hultgren, K. (1977) Cytogenetic effects on in vivo bone-marrow cells of Mus musculus induced by a commercial 2,4,-T ester product. Hereditas 85:123-134.

The authors studied the cytogenetic effects induced by 2,4,5-T on mouse bone marrow cells. The effects of 2,4,5-T butoxyethyl ester and its formulation components was also studied. For this experiment the high concentration of 2,4,5-T that could be obtained in water was tested. Male mice 15 weeks old 5 per group were given a single intraperitoneal injection of 2,4,5-T or 5 daily injections. Mice receiving only 1 injection were killed 6, 24, and 48 hours after treatment, while those receiving 5 injections were killed 6 hours after the last injection. Two strains of mice were used: (Swiss x CBA) x Swiss, called strain 9, and (DBA x CBA) x DBA, called strain 13. In other experiments, strain 9 mice were more resistant to the effects of chemical exposure than strain 13 mice. Both negative and positive controls were included in the study. Fifty metaphases were scored for chromosome aberrations. Results were analyzed by analysis of variance, students' t- and rank-test. In strain 9 mice receiving a single injection of 2.5 mg 2,4,5-T/kg body weight of metaphases with aberrations was 1.2% at 6 hours, 0% at 24 hours, and 1.5% at 48 hours. Control mice had 0.7% metaphases with aberrations. Similarly strain 13 mice injected once had 0.4% metaphases with aberrations at 6 hours, 0.8% at 24 hours, and 1.0% at 48 hours. Negative controls had no aberrations. In mice receiving multiple injections, strain 9 had 2% metaphases with aberrations, strain 13 had 2.4% metaphases with aberrations and negative controls had 1.2%. Mice receiving 2.4 mg 2,4,5-T ester in complete herbicide had 2.8% cells with chromosome aberrations at 6 hours, 5.2% at 24 hours and 4.2% at 48 hours, which is an increase in chromosome aberrations compared to controls. Furthermore, mice which received injections of the solvent and emulsifier also had increased chromosome damage. Similar results were obtained using strain 13. In mice receiving multiple injections, 2,4,5-T ester commercial preparation as well as the solvent and the emulsion produced increased chromosome aberrations.

236. Debate continues over Dow's dioxin theory. (1979) China and Eng. News Sept 24, 1979:27-28.

[Editorial.]

237. DeBruin, A. (1976) "Chapter 9: Biological Exposure Tests," In Biochemical Toxicology of Environmental Agents. (New York: Elsevier/N. Holland Inc.)

[Review article.]

238. Dedrick, R. L. (1971) Extrapolation of teratogenic effect. Chem. Eng. News 49(20):5-6.

[Editorial.]

239. Defoliant, cancer: Studies show link. (1980) Science News 117:230.

[Editorial.]

240. de Larrard, J., and Barbaste, M. (1969) Intoxication suicidaire mortelle agro-chimique a l'hormone desherbante 2,4-D. Arch. Mal. Prof. Med. Trav. 30:434.

[Abstract, only.]

241. Dencker, L. (1976) Chapter IV: The herbicide 2,4,5-T. Tissue localization of some teratogens at early and late gestation related to fetal effects. Acta Pharmacol. Toxicol. (Suppl) 39(1):59-72.

The uptake of radioactivity by C57BL mouse fetuses and hamster fetuses was determined after pregnant animals were administered [¹⁴C]-2,4,5-T. Mice were administered 24 ug (5uCi) of [¹⁴C]-2,4,5-T in 50% alcohol intravenously and hamsters received twice this dose. Autoradiography was performed on tissues removed 4-24 hours after the dose was given. The concentrations of radioactivity per gram of fetal tissue and per ml of maternal serum were determined. Mice and hamsters dosed between day 8 and 10 and autopsied within 12 hours of treatment, contained radioactivity which was localized in the gut. Autoradiographs of mice treated after day 10 showed radioactivity distributed in all tissues except the gut, with increased amounts of activity from treatment later in pregnancy. No radioactivity was observed in fetal hamster tissue from treatment on day 10 or 11. Radioactivity in mice treated on day 16 was highest in the kidney and visceral yolk sac, followed in descending order by the blood, liver, and muscular tissues. The brain was free of radioactivity. Comparing results of treatments from days 11-16, the concentration of radioactivity was always higher than in the chorio-allantoic placenta and the concentration in the amniotic cavity was always low. The ratio of the concentration of radioactivity between fetal tissue and maternal serum increased 20-fold from day 12 to day 18 which was an estimated 500 fold increase in 2,4,5-T transported to the fetus when correlated with the fetal growth weight at this time. The author postulated that the dramatic increase in uptake of 2,4,5-T by fetuses after day 11 of gestation may reflect an increased permeability of the chorioallantoic placenta which functionally replaces the yolk sac placenta at this time in development. The authors did not comment on the inability of 2,4,5-T to be transported to the fetal hamster. The latest treatment was given on day 12 and if this trend is sustained through day 16, no teratologic effects would be predicted in this species.

242. De Reuck, J., De Coster, W., Willens, J., and vander Eecken, H. (1979) Influence of 2,4-dichlorophenoxyacetate and of dantrolene sodium on the target phenomenon in tenotomized rat gastronemius muscle. Acta Neuropathol. 46:167-168.

The effect of 2,4-D on the formation of target fibers in tenotomized rat muscle with and without neurotomy was assessed. Female Wistar rats were subjected to unilateral tenotomy (10 rats) or bilateral tenotomy (10 rats) of the Achilles tendon. The sciatic nerve was sectioned (on the side opposite the unilateral tenotomy) in all rats. Starting the day after surgery, half of the rats in each group were administered 90 mg/kg 2,4-D subcutaneously daily for 12 days. The remaining rats were administered only vehicle (triethanolamine buffer). One rat from each group was killed after 1, 2, 5, 7, and 12 days. The gastrocnemius muscles were excised and examined histopathologically and histochemically for succinate and lactate dehydrogenase, cholinesterase, and nonspecific esterase activities. In control tenotomized muscles, type I target fibers developed by day 5 and increased in numbers at later times; type II target fibers first appeared on day 12. Target fibers were preceded by contraction bands. Neither the fibers nor the bands were present in intact muscles or muscles that underwent tenotomy and neurotomy. Treatment with 2,4-D (or dantrolene, which was also described) did not affect the number or time course of target fiber formation in any group. The authors concluded that 2,4-D increased resting membranes resistance in association with a reduced chloride conductance to produce muscle toxicity.

243. Desi, I., and Sos, J. (1962a) Central nervous injury by a chemical herbicide. Acta. Medica. Acta. Sci. Hung. 18:429-433.

The authors investigated the effects of acute and sub-acute administration of 2,4-D on the electroencephalographic activity and conditioned reflex responses of 24 rats, 4 cats, and 2 dogs. 2,4-D, at a dose of 200 mg/kg, was administered intraperitoneally only once in the acute experiments and once daily until death in the sub-acute experiments. The animals usually died on the 6th day of 2,4-D treatment. Electrodes were inserted into the cortex and reticular formation of the brains of the experimental animals and EEG readings were recorded before and after 2,4-D administration. Response to a previously learned and reinforced conditioned reflex (sound and electric shock) was measured in animals treated with 2,4-D for 5 consecutive days. In acute experiments, EEG recordings revealed an inhibition of desynchronization within 15 minutes of administration of 2,4-D. The inhibition lasted about 60 minutes then returned to normal. In the sub-acute experiments, EEG measurements revealed a gradual decrease in the frequency of spontaneous electrical activity. In addition, the duration of desynchronization decreased as the 2,4-D treatment continued. By the third day of treatment, desynchronization was reduced 81% while on the fourth day it had further decreased to 91% of the normal duration. By the fifth day no desynchronization was evident. Response to a previously learned conditioned reflex also decreased steadily with continued 2,4-D treatment. All response to the conditioned reflex was lost by the fifth day of treatment. Histologic

examination of the spinal cord showed demyelination of the pyramidal tract. The authors concluded that humans working with 2,4-D herbicides should use caution and be specifically examined for symptoms of neurologic disorders. The data presented in this report is identical to data presented in another paper by Desi et al (VA 1429/Q). It appears that both papers discuss the same set of experiments. Therefore, the shortcomings of the experimental procedures in the study have already been discussed in the annotation of VA 1429/Q.

244. Desi, I., and Sos, J. (1962b) The effect of 2,4-dichlorophenoxyacetic acid and triorthocresyl phosphate on the function of the upper part of the central nervous system (Bioelectric activity and conditioned reflexes). Gig. Sanit. (12):38-46.

The effect of 2,4-D on the EEG activity in the brain of adult rats, cats and dogs was investigated. Acute and subacute experiments were conducted. Eight rats were given a single subcutaneous injection of 2,4-D at a dose of 200 mg/kg. These animals were wired for EEG examination. Another set of 8 rats, along with 4 cats and 2 dogs, received a daily dose of 200 mg/kg of 2,4-D for several consecutive days. EEG examinations were also conducted on these animals. A third set of 8 rats received the same consecutive daily dose of 2,4-D but were tested for retention of a previously learned conditioned-reflex response. When the experiments were concluded, usually after 6 days when the animals died, brain and spinal cord tissues were examined histologically. The EEG pattern in the 8 rats that received a single dose of 2,4-D became abnormal within 24 hours of the administration. A reduction or a complete absence of the desynchronization pattern was noted in these animals. In animals that received consecutive daily doses of 2,4-D, electrical activity in the brain decreased steadily as the experiment progressed. A decrease in wave frequency and a drastic reduction in the duration of desynchronization were the main abnormalities. By the fifth day of 2,4-D administration, the desynchronization period had disappeared completely. In the rats that had learned a conditioned response, consecutive doses of 2,4-D caused a deterioration of the conditioned-reflex function. The number of correct responses diminished by 21% after 24 hours, by 58% after 2 days, by 63% after 3 days and 79% by the end of the fourth day. The conditioned response disappeared completely by the fifth day of the experiment. Histologic examination of brain tissue revealed no pathological change, but the pyramidal tract of the spinal cord exhibited signs of demyelination. The effect of triorthocresylphosphate on the EEG activity of rats, cats, and dogs was also investigated and compared with the effects produced by 2,4-D. The authors concluded that 2,4-D inhibited the electrical activity of the brain and the site of action was in the reticular formation and cerebral cortex. They stated that EEG examination of humans working with 2,4-D could be used to detect early symptoms of intoxication. Control animals were not used in these experiments, so the effect of anesthesia, surgery and brain tissue manipulation on the EEG results is not known. EEG abnormalities in the heavily intoxicated animals were stated to be the result of 2,4-D administration, but the authors did not consider that the extreme debilitation of the animals may have effected the EEG patterns as well.

245. Desi, I., Sos, J., and Nikolits, I. (1962a) New evidence concerning the nervous site of action of a chemical herbicide causing professional intoxication. Acta. Physiol. 22:73-80.

The effect of 2,4-D on the electroencephalographic activity in thyroidectomized and thyroxine-substituted cats was investigated. Fifteen adult cats, 11 experimental and 4 controls, were used. Following attachment to the EEG electrodes, the cats were subjected to thyroidectomies. The experimental cats were given 100 ug/kg of thyroxine daily while the controls received no treatment. After 10 days of observation, the experimental cats were given a daily dose of 100 mg/kg of purified 2,4-D. The electrical activity of the cerebral cortex and reticular formation was recorded daily. The duration of desynchronization following stimulation of the reticular formation and the cardiac rate were also measured daily. EEG activity in the control cats decreased 24 hours after thyroidectomy and remained at a low level. EEG activity in the thyroxine-substituted experimental cats remained normal after thyroidectomy, but became abnormal 24 hours following administration of 2,4-D. EEG activity in the experimental cats included decreased frequency combined with increased amplitude of the EEG wave patterns. After 5 days of treatment with 2,4-D, the EEG tracing displayed exclusively big, slow waves indicative of brain dysfunction. Desynchronization time in the brain of experimental cats was drastically reduced following administration of 2,4-D. By 24 hours after administration, the time of desynchronization decreased by 50% and by 4 days later, little or no desynchronization response was evident. Treatment with 2,4-D also decreased the cardiac rate by 25% after the first day and 36% after the fifth day. The authors concluded that 2,4-D reduced central nervous excitability directly and was not due to alterations in thyroid function. In particular, they stated that 2,4-D probably damaged the reticular formation and that functional disorders in different organs were secondary to the central nervous effect.

246. Desi, I., Sos, J., Olasz, J., Sule, F., and Markus, V. (1962b) Nervous system effects of a chemical herbicide. Arch. Environ. Health 4:101-108.

Acute neurotoxicity of 2,4-D in rats, cats, and dogs was studied. In one set of experiments, a single dose of 200 mg/kg of 2,4-D was administered intraperitoneally. For the other set of experiments, this dose was repeated daily and continued until the death of the animal. Electroencephalograms of treated and control animals were recorded. A single dose of 200 mg/kg of 2,4-D in rats caused a decrease in electrical activity and time of desynchronization in the brain. Desynchronization in these animals was completely abolished 25 minutes after administration of 2,4-D. This effect was reversible, however, since normal EEG patterns were regained by 50 minutes after treatment. Rats, cats, and dogs given consecutive daily doses of 2,4-D also displayed a decrease in brain electrical activity and desynchronization that steadily worsened with each day of treatment. By the fifth day of treatment, EEG examination revealed no desynchronization activity in

these animals. Rats that had been conditioned to respond to a loud noise lost all continued learning by the fifth day of continuous dosage of 2,4-D. Histologic examination of brain tissue revealed no observable alterations, but demyelination of nerve fibers of the pyramidal tract in the spinal cord was noted. The authors concluded that the reversible desynchronization observed in treated animals was caused by 2,4-D effecting cerebral cells via the bloodstream. They concluded that intraperitoneal administration of 2,4-D primarily damaged cells in the reticular formation, which in turn induced electrical dysfunctions in the cerebral cortex. This study is of limited value since the authors did not describe the preparation of the 2,4-D compound or their use of control animals. In addition, it is unclear whether identical EEG irregularities were observed in all species of animals that were examined (rats, dogs, cats). Biological effects noted in the dogs could particularly have been correlated to effects observed in humans exposed to 2,4-D chemicals.

247. DeSilva, D. P., and Michel, H. B. (1974) Effects of mangrove defoliation on the estuarine ecology and fisheries of South Vietnam. National Academy of Sciences - National Research Council. NTIS Publication No. AD779014.

[Background material.]

248. Diaz-Colon, J. D., and Bovey, R. W. (1976) Selected Bibliography of the Phenoxy Herbicides, Vol. I: Fate in the Environment. U.S. Department of Agriculture, 61 pp.

[Bibliography.]

249. Diaz-Colon, J. D., and Bovey, R. W. (1977) Selected Bibliography of the Phenoxy Herbicides, Vol. III: Toxicological Studies in Animals. U.S. Department of Agriculture, 105 pp.

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250. Diaz-Colon, J. D., and Bovey, R. W. (1978a) Selected Bibliography of Phenoxy Herbicides, Vol. V: Interrelations with Microorganisms. U.S. Department of Agriculture, 88 pp.

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251. Diaz-Colon, J. D., and Bovey, R. W. (1978b) Selected Bibliography of the Phenoxy Herbicides, Vol. VII: Military Uses. U.S. Department of Agriculture, 26 pp.

[Bibliography.]

252. Diaz-Colon, J. D., and Bovey, R. W. (1980) Selected Bibliography of the Phenoxy Herbicides, Vol. IX: Toxicological and Physiological Effects of 2,4-D. U.S. Department of Agriculture, 92 pp.
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253. Diaz-Colon, J. D., Bovey, R. W., and Young, A. L. (1979) Selected bibliography of recent literature on the substituted dibenzo-p-dioxins. Texas Agricultural Experiment Station, Texas A & M University System. 50 p.
- [Bibliography.]
254. Dietz, Jr., E. A., and Moore, L. O. (1978) Monomethylarsonic acid, cacodylic acid, and their sodium salts. In Analytical Methods for Pesticides and Plant Growth Regulators, Zweig, G., ed. Volume X. [New York: Academic Press.] pp. 385-401.
- [Review article.]
255. Dilling, W. L. (1977) Interphase transfer processes II. Evaporation rates of chloromethanes, ethanes, ethylenes, propenes, and propylaxes from dilute aqueous solutions. Comparisons with theoretical predictions. Environ. Sci. and Technol. 11(4):405-409.
- [Background material.]
256. Di Giovanni, J., Juchau, M. R., Berry, D. L., and Slaga, T. J. (1979) 2,3,7,8-tetrachlorodibenzo-p-dioxin: potent anticarcinogenic activity in CD-1 mice. Biochem. Biophys. Res. Commun. 86:577-584.

The effects of TCDD-pretreatment on tumor induction by benzo(a)pyrene (BP) and 7,12-dimethylbenz(a)anthracene (DMBA) were studied. Groups of 30 female CD-1 mice 7-9 weeks old received topical applications to the shaved skin of the back of .01, .1, or 2 ug TCDD/kg in .2 ml acetone. After varying intervals of time (5 minutes or 1, 3, 5, or 10 days) they were given tumor-initiating doses of DMBA. Tumor promotion with 10 ug 12.0-tetradecanoylphorbol-13-acetate (TPA) applied topically began one week after initiation and continued twice weekly for 20 or 24 weeks. Mice were examined for the presence of papillomas. TCDD was found to inhibit significantly the initiation of tumors by DMBA; mice treated with 1 ug TCDD 5 days before DMBA initiation showed only 6 percent as many tumors as mice receiving no TCDD. Tumor incidence increased with decreasing pretreatment time. The effect of TCDD pretreatment on BP tumor initiation was also examined in female CD-1 mice. TCDD (1 ug) was applied to shaved skin 1, 3, or 5 days prior to BP initiation. TCDD inhibited papilloma formation in mice although the effect was not as strong as that observed in DMBA induced mice. The group of mice receiving TCDD 5 days before BP initiation had 35% as many tumors as

mice receiving no TCDD. As with DMBA, the inhibition effect of TCDD decreased with decreasing pretreatment time. The authors were aware that TCDD pretreatment is known to raise aryl hydrocarbon hydroxylase, (AHH) activity in mice sensitive to tumor induction. They then investigated the effect of TCDD pretreatment on metabolism of DMBA. In a test similar to the tumor induction study, mice were given a topical application of 1 ug TCDD or .2 ml acetone only, 3 days prior to sacrifice. Metabolite profiles on DMBA from epidermal homogenates showed a greatly increased rate of formation of hydroxylated DMBA products in TCDD-pretreated mice, and proportionately greater increases in polyhydroxylated metabolite. The authors noted both qualitative and quantitative changes in the metabolism of DMBA under conditions similar to the tumor experiments. To study further the inhibitory effects of TCDD pretreatment, the authors traced the covalent binding of [³H] DMBA in mouse epidermal DNA, RNA, and protein. Topical application of 1 ug TCDD was followed 3 days later by administration of [³H] DMBA. Mice were killed 3 and 24 hours later; it was found that TCDD pretreatment reduced covalent binding of DMBA to epidermal DNA by 60-70 percent, and the RNA by 45-55 percent, but that there was no effect on binding to proteins. The authors suggest that TCDD-pretreatment increases the rate of inactivation of the DMBA molecule relative to the rate of AHH activation in mouse skin.

257. DiGiovanni, J., Viaje, A., Berry, D. L., Slaga, T. J., and Juchau, M. R. (1977) Tumor initiating ability of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and Aroclor 1254 in a two-stage system of mouse skin carcinogenesis. Bull. Environ. Contam. Toxicol. 18(5):552-556.

The authors investigated the ability of TCDD to initiate tumors in the two-stage system of mouse skin carcinogenesis. Female Charles River CD-1 mice, 7-9 weeks old, were shaved 2 days prior to treatment; only mice in the resting phase of the hair cycle were chosen for the experiment. Groups of 30 preshaved mice received either 2 ug/TCDD (98.6 % purity) in 0.2 ml acetone mouse, or TCDD at the same dose 5 minutes prior to initiation with dimethylbenz(a)anthracene (DMBA) (2.56 ug/mouse), a known tumor initiator. No untreated group was included. The dose of TCDD used killed nearly one-third of the animals at 32 weeks, the time of termination of the experiment. One week after initiation with TCDD or TCDD and DMBA, mice received twice-weekly application of 5 ug TPA in 0.2 ml acetone for 32 weeks. Incidences of papillomas and carcinomas were observed and recorded weekly. No statistical analyses of results were presented. When TCDD was administered alone, it showed weak tumor initiating ability (0.1 papillomas/mouse, 14 % survivors with papillomas) after 32 weeks. Concurrent application of TCDD with DMBA resulted in a small increase in tumor initiating ability compared to DMBA alone (2.2 papillomas/mouse TCDD and DMBA, 1.9 papillomas/mouse DMBA alone). However, since no statistical evaluation of results and no control data were presented, it is not possible to assess the significance of this study.

258. The dioxin curse. (1977) Atlas World Press Review, pp. 13-15.
[Editorial.]
259. Dioxin: The 10-year battle that began with Agent Orange. (1979)
Nature 278:108-109.
[Editorial.]
260. A discussion on herbicides. Statement on 2,4,5-T and TCDD. (1976)
[Journal interview] J. For. 73(7):410-412.
[Background material.]
261. Dispute resolution conference on 2,4,5-T. (1980) Vet. Hum. Toxicol.
22(1):40-42.
[Review article.]
262. Dmitriyev, V. I. (1974) Harmful effects of chemical substances used by
the U.S. Army in Indochina. Voenno-Med Zh. 1:88-90.
[Review article.]
263. Dougherty, R. C., and Piotrowska, K. (1976) Screening by negative
chemical ionization mass spectrometry for environmental contamination
with toxic residues: Application to human urines. Proc. Natl. Acad.
Sci. USA 73(6):1777-1781.

The frequency of detecting 2,4,5-T in urine samples collected from healthy college students and in food samples is reported. Urine samples were collected from 11 men on a college swimming team, 21 male football players and 25 dorm residents. Urine samples were analyzed by negative chemical ionization mass spectrometry and in some cases the chemical identification of 2,4,5-T was confirmed by gas chromatography-mass spectrometry. Seminal fluid samples from 7 sexually active men and fingernail scrapings for 2 people were collected and analyzed for polychlorophenoxy acid by mass spectrometry. Other products, including 4 paper products, 8 beverages, 10 food products and liquid detergent were also analyzed by mass spectrometry. 2,4,5-T was detected in 9%, 24% and 36% of the samples from swimmers, football players, and dorm residents, respectively, and in beef fat. No other samples had detectable levels of 2,4,5-T. The authors concluded that low levels (in the range of parts per billion) of 2,4,5-T in biological and food samples were within the range of detection of the method used.

264. Dougherty, W. J., Coulston, F., and Golberg, L. (1976) The evaluation of the teratogenic effects of 2,4,5-trichlorophenoxyacetic acid in the Rhesus monkey. Environ. Qual. Saf. 5:89-96.

The teratogenicity of 2,4,5-T was evaluated in the monkey. Pregnant Rhesus monkeys (10 per group) were administered 0.05, 1, or 10 mg/kg 2,4,5-T (with 0.05 ppm TCDD contaminant) in gelatin capsule by oral intubation on days 22 through 38 of gestation. The control group received the vehicle only. At birth, the offspring were weighed, examined, and returned to the mother. Two infants were scheduled to be killed and examined after birth, at 6 months and at 1 year. The stillborn rate was 13.3% for all groups combined and no statistically significant changes were observed for any treatment group compared to the control group for numbers of stillborns, abortions, live births, gestation length, or initial body weight or growth rate. No teratogenicity was observed in any offspring examined, although all infants had not been killed and examined at the time of the report. Although clinical chemistry, histology, and skeletal X-ray examinations were scheduled for all infants, none of the results were included in the report. The authors concluded that 2,4,5-T was non-teratogenic in the monkey. A premature conclusion from an incomplete study seems inappropriate, since apparently half of the infants had not been examined when the report was prepared.

265. Dougherty, W. J., Coulston, F., and Golberg, L. (1973) Non-teratogenicity 2,4,5-trichlorophenoxyacetic acid in monkeys (Macaca mullatta). Toxicol. Appl. Pharmacol. 25:442.

[Abstract, only.]

266. Dougherty, W. J., Herbst, M., and Coulston, F. (1975) The nonteratogenicity of 2,4,5-trichlorophenoxyacetic acid in the Rhesus monkey (Macaca mulatta). Bull. Environ. Contam. Toxicol. 13(4):477-482.

The reproductive effects of 2,4,5-T were examined in the Rhesus monkey. Forty pregnant monkeys were divided into 4 equal groups which were administered 0.05, 1.0 or 10.0 mg/kg of 2,4,5-T (with 0.05 ppm TCDD contaminant) in gelatin capsules or, for the control group, empty gelatin capsules by stomach tube on day 22 to 38 of pregnancy. No signs of maternal toxicity were observed in any treatment group. Nine of the 10 control monkeys delivered live offspring and the tenth fetus aborted on day 68 of gestation. One premature infant born on day 143 of gestation died 21 days later. The low treatment group produced 10 live infants with one death at 37 days of age. The other 2 groups each had 8 live births. One abortion and one stillborn on day 119 occurred in the middle dose group and 2 abortions (prior to day 50) occurred in the high dose group. The lengths, weights, clinical chemistry values, and hematology for all infants were within normal values for the duration of the study (1 year of age). No malformations were observed in the aborted fetuses, stillborns, or infants (which were sacrificed

at birth, 6 months, or 1 year). The highest dose used in this study was 80% of the dose that produced toxicity in adult Rhesus monkeys. Despite the small number of infants per group, the data give no trends toward adverse fetal effects and suggests that the monkey is not susceptible to the effects seen in other species when 2,4,5-T was administered from day 22-38 of gestation.

267. Dow Chemical Co. (1978) Chlorinated dibenzo-p-dioxins - Bibliography. 39 pp.

[Bibliography.]

268. The DOW Chemical Company. (1974) 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T). FIFRA Docket No. 295. EPA before the Administrator. Dow prehearing memo No. 2. 208 p.

[Background material.]

269. Dragsnes, L., and Helgeland, K. (1974) Effects of the herbicide 2,4,5-T on the incorporation of ^{14}C -thymidine, ^3H -uridine and ^3H -L-leucine into L929 cells. Acta Pharmacol. Toxicol. 35(2):103-112.

The uptake and incorporation of radioactive precursors of RNA, DNA, and protein by L929 cells was studied in vitro in the presence of 2,4,5-T. Mouse L929 fibroblast cells were cultured in the presence of ^{14}C -thymidine, ^3H -uridine, or ^3H -L-leucine for 20 minutes. The cell layer was then treated with cold 10% trichloroacetic acid and the acid soluble supernatant and insoluble fractions were counted for radioactivity. Radioactive components in acid-soluble extracts were separated by thin-layer chromatography. 2,4,5-T was added at 2.25 mM to the medium (a 100% pure preparation and a stock 2,4,5-T preparation with an estimated 2-3 ppm dioxin were mentioned but not specified for individual experiments). The effect of 2,4,5-T on ^3H -leucine uptake (acid-soluble counts) was variable over a 10 hour exposure period, while protein synthesis (acid-insoluble counts) was strongly inhibited to a maximum of 23% of control incorporation. Uptake and incorporation of ^3H -uridine and ^{14}C -thymidine were inhibited by 2,4,5-T. Above 0.75 mM, 2,4,5-T produced a dose-dependent increase in ^3H -leucine uptake and a concomitant decrease in incorporation uptake and incorporation of ^3H -uridine and ^{14}C -thymidine were inhibited by doses above 0.5 mM of 2,4,5-T. The rate of ^3H -uridine uptake as a function of uridine concentration was biphasic. The more rapid rate at low concentrations of uridine appeared more sensitive to inhibition by 2,4,5-T than the slower phase at high uridine concentrations. The increased uridine uptake was not associated with increased uptake of any specific phosphosorylated uridine nucleotide pool. The authors concluded that 2,4,5-T produced the observed effects by producing alterations in plasma membrane permeability.

270. Dragsnes, L., Helgeland, K., and Jonsen, J. (1975) Effects of the herbicide 2,4,5 trichlorophenoxyacetic acid on growth and morphology of L929 cells. Acta Pharmacol. Toxicol. 36(2):97-102.

The effects of 2,4-D and 2,4,5-T added to culture medium of mouse fibroblasts on cell growth rate and cell morphology are described. 2,4,5-T (with an estimated 2-3 ppm dioxin contamination) in culture medium at a final concentration of 0.25 to 2.25 mM was added to L929 strain mouse fibroblast cells in monolayer culture. Medium with 2.25 mM 2,4,5-T was replaced with control medium for some cultures after 3 or 6 days. After 1-14 days of culture, cells were trypsinized and counted. Cell morphology was evaluated by phase microscopy. 2,4,5-T produced a dose-dependent inhibition of cell growth over 6 days of culture. The effect was reversible, with an increase in cell number observed 1 day after treatment medium had been replaced by control medium, and with complete recovery approached 9 days after control medium was introduced. Accumulation of cytoplasmic particles occurred with treatment. The number and time of appearance of particles were dependent on 2,4,5-T dose, and they disappeared after 2,4,5-T was removed. At high 2,4,5-T concentrations, cell rounding and detachment were evident. The authors concluded that the effect of 2,4,5-T on L929 cells resembled the 2,4-D effect reported by other investigators with 2,4,5-T showing more potency than 2,4-D in producing this effect.

271. Drill, V. A., and Hiratzka, T. (1953) Toxicity of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid. Arch. Ind. Hyg. Occ. Med. 7:61-67.

The acute and subchronic oral toxicities of 2,4-D and 2,4,5-T were examined in dogs. Both compounds (98.5-98.9% purity) were administered in capsules once for the acute studies (to a total of 22 days) and daily for 5 days per week over 13 weeks to 26 dogs for the subchronic studies. Body weights and hematology were monitored and autopsies were performed at the end of the 90 day subchronic treatment period or 14 days after a single acute dose. The lung, heart, liver, kidney, adrenals, spleen, thyroid, and ovary or testes were examined histologically, as well. The LD₅₀ was about 100 mg/kg for both compounds and death was delayed until 2-9 days after the dose was administered. Both compounds caused anorexia. 2,4,5-T treatment did not cause marked effects in treated animals even after a fatal dose was given. Myotonia, in some dogs limited to mild ataxia and stiffness of the hind limbs, was observed in 2,4-D treated dogs. Hind limbs were affected first and sometimes solely. Sneezing, rubbing of the eyes, and diarrhea were occasionally observed after 2,4,-D treatment. Dogs treated with either compound were observed with pneumonia or with redness in the small intestine which histologically was associated with inflamed and necrotic areas. Other pathologic findings were hepatic necrosis and renal tubular degeneration in one to two dogs that received high doses of 2,4,5-T. Hepatic congestion was commonly observed in dogs that received fatal doses of either compound. All dogs survived that were given 2-10 mg/kg of either dose subchronically and none survived that were given 20 mg/kg of either compound once

daily. The dogs that survived were free of symptoms. Body weights were decreased only from fatal doses and the effect was first seen 7-12 days prior to death. Other symptoms observed prior to death in dogs given either compound were weakness, stiffness in the hind legs, difficulty in swallowing, and bleeding from the gums. The only alteration in blood counts was a terminal fall in the percentage of lymphocytes in three dogs that died. Redness of areas of the gastrointestinal tract were noted after treatment with 2,4-D and with 2,4,5-T, and slight increases in heart and kidney weights were observed after fatal doses of either compound. No treatment-related microscopic changes were observed. The authors suggested that the delayed death resulting from subchronic dosing of 2,4-D indicated a cumulative effect of 2,4-D.

272. Dudley, A. W., and Thapar, N. T. (1972) Fatal human ingestion of 2,4-D a common herbicide. Arch. Path. 94:270-275.

The pathology of acute 2,4-D poisoning in man is described. A 76-year-old man with senile dementia ingested an unknown quantity of pure 2,4-D in kerosene. He was found in a comatose condition and never recovered consciousness before his death, 6 days later. High concentrations of 2,4-D were found in the tissues removed at autopsy. Cardiac dilations, pulmonary edema, and passive congestion of the kidneys were observed at autopsy and led the authors to conclude that death resulted from cardiac arrest caused by auricular fibrillation. Other pathological findings included widespread plaques of acute demyelination in the brain, peripheral neuropathy, gastroenteritis, and hepatic necrosis, with normal endocrine and musculoskeletal systems.

273. Dugois, M. M. P., Amblard, P., Aimard, M., and Deshors, G. (1968) Acne chlorique collective et accidentelle d'un type nouveau. Bull. Soc. Fr. Derm. Syph. 75:260-261.

The skin lesions that occurred in workers following an industrial explosion are described. The explosion occurred in a factory that manufactured 2,4,5-trichlorophenol, from excessive pressure in the reactor pipelines. Twenty-one of the workers that were in the vicinity of the explosion site within 3 days of accident and that developed chloracne were examined. Details of the accident and total number of exposed workers were not reported. The authors indicated that the majority were exposed for only a few hours, but did not explain what decontamination steps were taken. The characteristics of the lesions were described, without indicating how common these characteristics were among the affected individuals. Edema and burning occurred during the 2 days after exposure, and regressed after several days. Folliculitis developed on the face and extremities during the next 2 weeks, followed by classic chloracne lesions and keratosis which persisted for the next year. The only other symptom described was painful paroxysmic pressure over the hypochondrium, which the authors assumed originated in the liver. The authors concluded that the skin lesions resulted after the absorbed toxic substance was released from body stores and eliminated through the skin. They did not know the complete identity of the toxic substance.

274. Dugois, P., Marechal, J., and Colomb, L. (1958) Chloracne due to 2,4,5-trichlorophenol. Arach. Mal. Prof. 19:626-627.

Generalizations are presented related to 17 cases of chloracne in workers at a 2,4,5-trichlorophenol plant. Histologically, lesions were characterized as follicular keratin cysts and atrophied sebaceous glands. Inflammatory reactions and secondary formation of blackheads occurred during the course of the disease. Clinically, mild forms in which only the face and arms were involved were distinguished from moderate cases that involved half of the body and severe cases that persisted for many months. Moderate and severe cases were not completely curable because elimination of the keratinized material from the cyst left atrophic scars. A strong chlorine odor was reported to be associated with the cysts. General disorders observed in the patients included asthenia, anorexia, weight loss, headache, and digestive and hepatic disorders. Incidences of these symptoms or further descriptions of the patients or their health was not provided. The chemical substance in the manufacturing process which was responsible for chloracne could not be identified; (the methods used were not reported). The authors concluded that no therapy was effective for the chloracne and modifications in the manufacturing process successfully prevented new outbreaks of chloracne.

275. Dunachie, J.F., and Fletcher, W.W. (1967) Effect of some herbicides on the hatching rate of hen's eggs. Nature 215:1406-1407.

In a brief report, the effects of diquat, dalapon and 2,4-D on hatching rates are presented. Hen's eggs (25 per group) were injected with each herbicide by a previously published method that was not described in this report. Diquat was dissolved in water and dalapon and 2,4-D were administered in acetone. Three doses of dalapon and 2,4-D tested: 200, 100, and 10 ppm, corresponding to 10, 5, and 0.5 mg/egg, respectively, diquat was tested at 100, 10, and 5 ppm. The percentages of eggs that hatched were: 100% for all 3 doses of dalapon; 50%, 70%, and 80-90% for 200, 100, and 10 ppm of 2,4-D, respectively; and 10%, 10%, and 60% for 100, 10, and 5 ppm diquat, respectively. None of the compounds produced any deformities, although methods used for detecting deformities were not described. The authors used for detecting deformities were not described. The authors concluded that their results for herbicides contrasted with results for organophosphorus compounds, which produced malformations.

276. Durham, W. F., and Williams, C. H. (1972) Mutagenic, teratogenic and carcinogenic properties of pesticides. Annu. Rev. Entomol. 17:123-148.

[Review article.]

277. Dussart, L. (1946) Acne chlorique, dermatose professionnelle. Arch. belges Derm. 3:218.

[Not available.]

278. Dux, E., Toth, I., Dux, L., and Joo, F. (1978) The localization of calcium by X-ray microanalysis in myopathic muscle fibers. Histochemistry 56(3-4):239-244.

The location of calcium in muscles from rats treated subacutely with 2,4-D was described on the electron microscopic level. Adult Wistar rats were administered 50 mg/kg 2,4-D intraperitoneally, daily for 3 weeks. The soleus muscles were then removed, immersed in the presence of ammonium oxalate to precipitate calcium, and prepared for electron microscopy. Calcium identification was verified by energy-dispersive X-ray microanalysis. Calcium precipitates were localized in the vesicles of the sarcoplasmic reticulum of control rat muscle. In muscle from 2,4-D treated rats, calcium was not observed in the vesicles, but rather, just beyond the vesicles and in the myofibrils at the Z line of the A-I junction. The authors concluded that 2,4-D blocked reuptake of calcium by the sarcoplasmic reticulum and the higher levels of calcium outside the vesicles could cause long-lasting activation of the contractile system.

279. Dwyer, J. H., and Smith, R. (1980) Statement before the President's Task Force--the Interagency Work Group to Study the Possible Long Term Effects of Phenoxy Herbicides and Contaminants (the IAG), Sept. 22., 1980. 15 pp.

[Testimony.]

280. Eberstein, A., and Goodgold, J. (1979) Experimental myotonia induced in denervated muscles by 2,4-D. Muscle and Nerve. 2:364-368.

The authors investigated the effect of 2,4-D administration on contraction responses in muscles of rats that were denervated for 10 days or longer. The right hind limb of each of thirty male Wister rats was surgically denervated by excision of a 0.5 centimeter segment of the sciatic nerve. The denervated rats were divided into three groups: 5 rats remained untreated; 19 rats were injected with 2,4-D one hour prior to anesthesia and subsequent contraction experiments; and 6 rats received 2,4-D 30 to 40 minutes after anesthesia. Animals received a single intraperitoneal injection of 2,4-D at a dose of 225 mg/kg. The purity of the 2,4-D compound was not stated. Electromyographic measurements of the anterior tibialis muscle were made before and after administration of 2,4-D. The contractual properties of the denervated extensor digitorum longus muscle were tested by attaching the proximal tendon of the muscle to the strain gage of a myograph. Denervated muscles of the untreated rats were tested in the same manner. Administration of 2,4-D produced a statistically significant increase in the relaxation time of muscle denervated 15 days prior to the administration. This prolongation did not occur until 50 minutes after administration; prolongation was not evident at the 20-minute interval. Tetanic prolongation reached its maximum 100 minutes after treatment with 2,4-D. Electromyographic examination of the denervated muscles showed fibrillation activity prior to 2,4-D treatment. After 2,4-D administration, no appreciable change was noted. No unusual electrical discharges and no repetitive firing were evident. The authors concluded that 2,4-D produced a prolonged relaxation time in muscles by slowing the rate of calcium uptake and producing a higher calcium concentration in the sarcoplasmic reticulum of the muscle. The absence of repetitive firing in denervated muscle treated with 2,4-D was tentatively thought to be due to increase of the threshold for action potentials caused by prolonged denervation. No comparisons were made regarding how these findings related to 2,4-D induced myotonia in humans. In addition, the authors did not fully discuss the role of peripheral nerve and neuromuscular involvement in 2,4-D induced myotonia.

281. Ebron, M., and Courtney, K. D. (1976) Difference in 2,4,5-T distribution in fetal mice and guinea pigs. Toxicol. Appl. Pharmacol. 37:144-145.

[Abstract, only.]

282. Eckardt, R. E., and Scala, R. A. (1978) Toxicology: assessing the hazard. Occ. Medicine 20(7):490-493.

[Not available.]

283. Edson, E. F. (1960) Applied toxicology of pesticides. Pharm. J. 185:361-367.

[Review article.]

284. Effects of herbicides in Vietnam and their relation to herbicide use in the United States. (1975) Council for Agricultural Science and Technology, Report No. 46, 14 pp.

[Review article.]

285. El-Dib, M. A., Aly, O. A. (1976) Persistence of some phenylamide pesticides in the aquatic environment-III. Biological degradation. Water Res. 10(12):1055-1059.

The authors report on the biological degradation of Monuron in Nile River water, which contained a mixed microbial population derived from natural water and sewage. Monuron was added to Nile River water at 10 mg/liter. Water samples were removed over a period of 4 months and analyzed for Monuron and its metabolites by thin layer chromatography. Monuron remained undegraded by the microbial population of the water.

286. Ellgehausen, H., Guth, J. A., and Esser, H. O. (1980) Factors determining the bioaccumulation potential of pesticides in the individual compartments of aquatic food chains. Ecotoxicol. Environ. Saf. 4:134-157.

[Review article.]

287. Elo, H. A., and Ylitalo, P. (1979) Distribution of 2-methyl-4-chlorophenoxyacetic acid and 2,4-dichlorophenoxyacetic acid in male rats; evidence for the involvement of the central nervous system in their toxicity. Toxicol. Appl. Pharmacol. 51(3):439-446.

The tissue distribution of [^{14}C]-2,4-D was studied in rats after pretreatment with 2,4-D. Male Sprague-Dawley rats (4) were administered 250 mg/kg of 2,4-D sodium salt subcutaneously and after 3 hr., [^{14}C]-2,4-D was administered intravenously. Cerebrospinal fluid (CSF) was collected for 1 hr. (starting 30 min. after the isotope injection) and plasma and tissue samples were removed at the end of the CSF collection period and counted for radioactivity. Control rats (5) were administered saline instead of the 2,4-D pretreatment dose. The largest increases in tissue radioactivity for 2,4-D pretreated rats over controls was seen for the brain, and CSF followed by the liver and then the muscle, heart, testes and lung. The plasma and kidney levels were decreased. (All tissue levels were expressed as a percentage of the tissue to plasma ^{14}C ratio). The distribution of 2-methyl-4-chloro-phenoxyacetic acid was also studied. The authors concluded that

cerebral [¹⁴C]-2,4-D concentrations were increased substantially in 2,4-D pretreated rats than in saline pretreated rats, but was unable to attribute this effect to increased 2,4-D influx, decreased efflux, or both.

288. Elo, H., and Ylitolo, P. (1977) Substantial increase in the levels of chlorophenoxyacetic acids in the CNS of rats as a result of severe intoxication. Acta Pharmacol. Toxicol. 41:289-294.

Levels of 2,4-D in various rat tissues were determined. Male Sprague-Dawley rats were administered 250 mg/kg 2,4-D sodium salt subcutaneously, followed in 3 hours by an intravenous dose of [¹⁴C]-2,4-D. 1.5 hours later, radioactivity was determined in the brain, cerebro-spinal fluid, liver, kidney, muscle and plasma. 2-methyl-4 chlorophenoxyacetic acid was also studied and details of the protocol and results for 2,4-D were reported to be similar to those for MCPA, but were not specified. Compared with rats that received only the radioactive dose of 2,4-D, in pretreated rats the brain and cerebrospinal fluid contained higher 2,4-D levels (relative to plasma levels). Muscle, liver and kidney levels showed slight or no increase after pretreatment, compared to non-pretreated rats. The authors concluded that 2,4-D treatment may have impaired function of the blood-brain barrier.

289. Elovaara, E., Savolainen, H., Parkki, M. G., Aitio, A., and Vainio, H. (1977) Neurochemical effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Wistar and Gunn rats. Res. Commun. Chem. Pathol. Pharmacol. 18(3):487-494.

[Background material.]

290. Emerson, J. L., Thompson, D. J., Gerbig, C. G., and Robinson, V. B. (1970) Teratogenic study of 2,4,5-trichlorophenoxyacetic acid in the rat. Toxicol. Appl. Pharmacol. 17:317.

[Abstract, only.]

291. Emerson, J. L., Thompson, D. J., Strebing, R. J., Gerbig, C. G., and Robinson, V. B. (1971) Teratogenic studies on 2,4,5-trichlorophenoxyacetic acid in the rat and rabbit. Fd. Cosmet. Toxicol. 9:395-404.

The teratogenic effects of 2,4,5-T were examined in the rat and in the rabbit. Pregnant Sprague-Dawley rats were administered from 1 to 24 mg/kg of 2,4,5-T (with 0.5 ppm dioxin contaminant) in a suspension of hydroxy propylmethyl cellulose by gavage on days 6 through 15 of gestation. Control rats were administered the vehicle, only. Pregnant New Zealand white rabbits received from 10 to 40 mg/kg 2,4,5-T in gelatin capsules on days 6 through 18 of pregnancy. Control rabbits received empty capsules. On day 20 of gestation, rats were killed and corpora lutea and resorptions were counted. Fetuses were weighed, examined for

gross malformations, and then stained with Bouin's fluid and examined for visceral malformations or stained with Alizarin red and examined for skeletal abnormalities or examined histologically. Rabbits were killed on day 29 of gestation and the same types of fetal examinations were performed as those for the rat study except that the rabbit fetuses were incubated for 24 hours to evaluate survival rate prior to sacrifice for evaluation of malformations. Although the group of rats treated with 1 mg/kg 2,4,5-T had significantly fewer implantations and a smaller mean litter size, the decreases were small and the higher doses did not produce this effect. No increases in the incidences of corpora lutea or resorptions or in the mean weight of offspring occurred in any group. No major abnormalities were observed by gross or histologic examination and no increases in skeletal deviations in the experimental groups compared to controls were observed in the rat fetuses. Hydronephrosis was observed in both the control and high dosage groups. No significant changes in incidences of implantations, corpora lutea, resorptions, litter size, or fetal weights were observed except for an increase in litter size at the highest dose and a decrease in corpora lutea per dam in the middle dosage group. Neonatal mortality resulted primarily from respiratory insufficiency. The rates for the control, 10, 20, and 40 mg/kg treatment groups were 7, 26, 11, and 16%, respectively. The authors concluded that the observed neonatal mortality, which was caused by a failure of the mares to dialate, was caused by inappropriate incubation conditions rather than by 2,4,5-T. They concluded that 2,4,5-T is not teratogenic in the strains of rat or rabbit studies.

292. Environmental cancers: Humans as the experimental model. (1976) Environ. Sci. and Tech. 10(13):1190-1195.
[Editorial.]
293. Environmental Sciences Laboratory. (1980) Bibliographic references: Dioxins. 48 pp.
[Bibliography.]
294. EPA suspends the major uses of two herbicides. (1979) Environ. Sci. and Tech. 13(6):640-641.
[Editorial.]
295. Epstein, S. S. (1973) Teratological hazards due to phenoxy herbicides and dioxin contaminants. Environmental Science Research Series. 2:708-729.
[Review article.]

296. Eriksson, M., Hardell, L., Berg, N. O., Moller, T., and Axelson, O. (1979) Case-control study on malignant mesenchymal tumors of the soft tissue and exposure to chemical substances. Lakartidningen 76:3872-3875.

A case-control study of a male study population of 110 cases of which 38 were deceased and 219 controls was conducted to determine the relationship between the incidence of mesenchymal soft-tissue tumors and exposure to chemical substances. Controls were matched to cases by age and municipality. Exposure was determined through blind telephone and mail questionnaire survey techniques. Data collected included occupational history, exposure conditions in work environment, smoking, etc. To avoid bias special attention to phenoxy acids or chlorophenols were avoided. Specific data on occupational exposures were obtained from persons reporting agricultural, forestry or horticultural employment. Chi-square values and relative risk for matched case-control pairs were calculated using the methods developed by Miettinen (1969) and (1970). Confidence intervals were constructed utilizing the method proposed by Mittlinen (1976). In one analysis, persons exposed to phenoxy acids alone had relative risk of 6.8 and a dose-effect relationship was indicated although insignificant. The authors concluded that exposure to phenoxy acids probably constitutes a risk factor with respect to occurrence of malignant mesenchymol soft tissue tumors and that the risk is associated not only with those phenoxy acids which, like some chlorophenols, may contain polychlorinated dibenzodioxins and dibenzofurans, but with other phenoxy acids as well.

297. Erne, K. (1975) Phenoxy herbicide residues in Swedish fish and wildlife. Environ. Qual. Saf. 3(suppl.) Pesticides Issue:192-195.

The author assayed fish and wildlife samples for phenoxy herbicide residues by gas chromatography (detection limit 0.05 ppm). Three hundred samples of apparently healthy freshwater fish were removed from 120 localities in Swedish watersheds. Extent of herbicide application in these areas was not reported. Analysis of the samples showed that 30% of the samples contained 2,4-D or MCPA at levels up to 0.8 ppm. Of 50 samples of other aquatic ecosystem organisms (crustaceans, birds, mammals), none contained detectable phenoxy acid residues. Of 250 samples of wildlife (deer, hare, birds) found dead, 25% contained 2,4-D and/or 2,4,5-T. Residue levels in liver and kidneys, those organs that would contain the highest concentration of herbicide, did not exceed 6 ppm and most fell below 1 ppm. In healthy wildlife shot in herbicide-treated forests, phenoxy acid residues were found in 32% of the samples. Liver and kidney levels did not exceed 4.5 ppm and most were below 1 ppm. In addition, the author reported on feeding studies in hares and reindeer. Mountain hares (sex, age, and number unspecified) were fed leaf and bark vegetation from sprayed forest areas containing 2-4 ppm 2,4-D and 2,4,5-T (ratio of 1) for 8 weeks. No signs of toxicity were observed in any of the animals. Pregnant reindeer fed about 40 ppm 2,4-D and 2,4,5-T (ratio unspecified) during the last 4-6 weeks of gestation exhibited no signs of toxicity. In addition, no

abortions or fetal abnormalities occurred. Tissue levels of 2,4-D and 2,4,5-T were 1.1 and 1.6 ppm in the liver and up to 8.9 and 13.3 ppm in the kidneys, respectively. The ratio of 2,4,5-T to 2,4-D increased from 1.0 in the feed to 2.0 in reindeer plasma, suggesting a slight accumulation.

298. Erne, K. (1966a) Studies on the animal metabolism of phenoxyacetic herbicides. Acta Vet. Scand. 7:264-271.

The formation of 2,4-D conjugates was studied in the rat and pig and protein binding was studied in horse plasma. Pigs (numbers not given) were orally administered 3 or 23 doses of 50 mg/kg 2,4-D or were fed a diet supplemented with 500 ppm 2,4-D for 5 months. Urine samples collected at the end of the exposure period were hydrolyzed with acid and with alkali to determine to proportion of urinary 2,4-D present as acid-labile and alkali-labile conjugates. The identification of conjugates was confirmed by thin-layer chromatography. Plasma from the pig fed the 500 ppm 2,4-D diet was analyzed for acid- and alkali-labile conjugates. Blood from one rat administered a single oral dose of 100 mg/kg 2,4-D butyl ester and 1 pig administered 3 oral doses of 50 mg/kg 2,4-D butyl ester and blood, urine, and liver tissue from 1 pig given 23 oral doses of 50 mg/kg 2,4-D butyl ester were extracted and analyzed for free and esterified 2,4-D content by thin-layer chromatography. Binding of 500 ug of 2,4-D equilibrated with 5 ml of 50% horse plasma was determined by the elution pattern of the mixture on Sephadex G-25. Less than 20% of the 2,4-D in any urine sample was conjugated and none of the plasma 2,4-D was conjugated. Urine, plasma and tissue from animals given 2,4-D butyl ester contained 2,4-D acid and only traces of the ester. 2,4-D in the presence of horse plasma eluted with partition ratio (K_d) of 0.95 when the column was equilibrated and eluted with 50% of horse serum. Two peaks with K_d values of 0 and 2.2 were obtained for 2,4-D when buffer without horse serum was used in the equilibration and elution buffers. The authors concluded that urinary 2,4-D was conjugated to only a minimal extent and 2,4-D butyl ester was metabolized to the acid rapidly. A weak protein interaction was evidenced by the presence of 2,4-D in the protein peak ($K_d = 0$). The methods used to determine protein-binding and conjugation have been superseded by more sensitive methods.

299. Erne, K. (1966b) Distribution and elimination of chlorinated phenoxyacetic acids in animals. Acta Vet. Scand. 7:240-256.

The tissue distribution and elimination of 2,4-D, 2,4-D butyl ester, and 2,4,5-T were studied in the cow, pig, rat and chicken. An aqueous emulsion of 2,4-D butyl ester in petroleum solvent and aqueous solutions of the other test herbicides were administered to albino rats, pigs, calves (6-8 weeks old), male chicks (1 week old) and White Leghorn chickens orally by stomach tube or, for long term exposure in water or feed. At selected intervals, animals were killed, and tissues were examined for histopathological changes and were analyzed for phenoxyacid content. Plasma levels of 2,4-D were determined in calves

(2), pigs (5-10 per group), rats (35 per group) and chickens (5-10 per group) given 50-200 mg/kg 2,4-D and in calves (2) and pigs (4) given 100 mg/kg 2,4-D butyl ester (amine or sodium-potassium salt). Plasma levels of 2,4,5-T were determined in rats (10) and pigs (2) given 100 mg/kg 2,4,5-T. Half-lives for all the compounds were similar within a species, with values of 8-10 hr. for calves, 10-12 hrs. for pigs, about 3 hr. for rats and 8 hr. for chickens. Oral doses of 50 mg/kg/day of 2,4-D and 2,4-D butyl ester to pigs (4-5 per group) or 300 mg/kg/day to 2 hens resulted in 2,4-D plasma levels that gradually decreased over 23 doses and urine levels that increased (exception for 1 pig per group, in which plasma levels rose and intoxication developed). After 2,4-D acid or butyl ester was combined with blood in vitro, almost all of the 2,4-D was recovered in the plasma and not in the cells. Tissue levels after a single dose in all species were highest in excretory organs and low in the brain. Tissue half-lives were 5-10 hrs. in the rat and 10-30 hrs. in the other species. No sex differences were seen for the patterns of distribution and elimination. Clinical symptoms for all species fed diets or water with 500-1000 ppm for 2 mo. to 2 yrs. were anorexia and reduced weight gain. No evidence of tissue accumulation of 2,4-D was observed. A pregnant pig fed a diet with 500 ppm 2,4-D throughout gestation delivered 15 piglets, of which 10 died within 24 hrs. of birth. The authors concluded that both phenoxy acids were absorbed well, distributed rapidly to tissues and excreted rapidly in the urine by all species. In the pig indirect evidence suggested placental transfer of 2,4-D.

300. Erne, K., and Sperber, I. (1974) Renal tubular transfer of phenoxy-acetic acids in the chicken. Acta Pharmacol. Toxicol. 35(3):233-241.

The rates of renal tubular excretion of 2,4-D and 2,4,5-T were studied in the chicken. 2,4-D or 2,4,5-T was infused over 3 min. into a leg vein of White Leghorn or broiler chickens. Urine was collected from each urethra separately over the subsequent hour and analyzed for phenoxy acid by thin-layer chromatography and gas chromatography. Phenol red excretion was determined as well for each kidney and urine was analyzed spectrophotometrically for phenol red. The difference in renal excretion by the separate kidneys referred to as the apparent excretion fraction (EF) was expressed as a percentage of the dose. Only chickens with EF values below 10% for phenol red were used. A dose of 46 umol of 2,4-D per chicken (about 1.5 kg body weight) yielded EF values of 8.9 to 15.7% and recoveries of 18-41% of the dose in the collected urine. A dose of 90 umol of 2,4-D gave EF values of 4.6-5.4% with 23-36% recovery. A dose of 38-39 umol of 2,4,5-T produced EF values of 1.6-7.2% and doses of 55-58 umol given with 200 ug phenol red produced EF values of 0.5-1.2%. When phenol red (200 ug) infusion was initiated 30 min. after the test compound was introduced, the EF values for 2,4-D and 2,4,5-T were reduced by 90-100%. Other chlorinated phenoxy acid compounds were studied in this experimental system, as well. The authors concluded that all compounds they studied except 2,4,5-T underwent tubular excretion because they were associated with EF values above 10%. The transport mechanism was saturable at high doses and was shared by phenol red. The authors also suggested that

affinity of the phenoxy acids to the transport mechanism was related to its degree of chlorination.

301. Espir, M. L. E., Hall, J. W., Shirreffs, J. G., and Stevens, D. L. (1970) Impotence in farm workers using toxic chemicals. Br. Med. J. 1:423-425.

Four out of a team of five males engaged in spreading agricultural chemicals reported difficulty in achieving and maintaining penile erections. Three men were married and had children. This type of information was not provided for the fourth man. The men, aged 35 to 46, were all treated with methyltestosterone and advised to discontinue working with chemicals. For three subjects, the symptom was alleviated within 3 months of the termination of exposure and for the fourth subject within 1 year. No other health problems were identified in the workers. The authors concluded that the chemicals used on the farm were responsible for producing impotence, but the active chemical(s) could not be identified. Among the chemicals the men used were fertilizers and pesticides, including substituted phenoxy compounds (dichloroprop, 2,4-D, and M.C.P.A.).

302. Esposito, M. P., Tiernan, T. O., and Dryden, F. E. (1980) Dioxins. U.S. Environmental Protection Agency, Industrial Environmental Research Laboratory, Office of Research and Development. US-EPA, Cincinnati, Ohio. 351 pp.

[Review article.]

303. Evans, J. O., and Duseja, D. R. (1973) Herbicide contamination of surface runoff waters. U.S. Environmental Protection Agency, Office of Research and Development, No. EPA-R2-73-266. 99 pp.

[Not available.]

304. Ewing, A. (1980) Few long term effects from dioxin - Monsanto. Chem. News 6(1):3.

[Not available.]

305. Eyzaguirre, C., Folk, B. P., Zierler, K. L., Lilienthal, J. L. (1948) Experimental myotonia and repetitive phenomena: the veratrinic effects of 2,4-dichlorophenoxyacetate (2,4-D) in the rat. Am. J. Physiol. 155:69-77.

The authors studied the effects of 2,4-D administration on muscle tension and electrical activity of muscle and nerve in the rat. The triceps surae muscle of Whelan and Sherman rats was exposed and attached to the strain gage of a myograph. Muscle tension was recorded

under isometric conditions after 100-250 mg/kg 2,4-D was administered to the rat intraperitoneally. Action potentials and electrical activity of the muscle and sciatic nerve were recorded as well. Various other compounds were administered and their effects on the response of muscle and nerve to 2,4-D were recorded. 2,4-D produced myotonia muscles of conscious, pentobarbital-anesthetized, and curare-treated rats and in denervated muscle. Repetitive spikes of changes in electrical potential were generated in 2,4-D treated muscle by a single shock applied to the nerve or muscle. The myotonic response decreased with repeated stimulation but recovered completely after 10 minutes of rest. Muscle with 2,4-D induced myotonia showed elevated sensitivity to mechanical stimuli. The myotonic effect of 2,4-D was potentiated in rats administered potassium intraperitoneally and was diminished after quinine, or alpha-tocopheryl phosphate, magnesium, or calcium was injected. 2,4-D administration potentiated the electrical response of muscle to intra-arterially injected acetylcholine. The authors concluded that the myotonic effect of 2,4-D resembled the effect produced by many other compounds as well as the myotonic effect of 2,4-D observed in man and in the goat.

306. The facts about Agent Orange: A time for reason. (1980) The Congressional Record, March 31, H 2394-H 2397.

[Editorial.]

307. Fahrig, R. (1974) Comparative mutagenicity studies with pesticides. IARC Sci. Pub. 10:161-179.

[Review article.]

308. Faith, R. E., and Luster, M. I. (1979) Investigations on the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on parameters of various immune functions. Ann. N.Y. Acad. of Sci. 320:564-571.

Humoral and cell-mediated immunity were evaluated in rats after perinatal exposure to TCDD. Female Fischer rats were bred to male Wistar rats and were administered 5 ug/kg TCDD (99+% purity) on day 18 of gestation and on postnatal days 0, 7 and 14 or only on postnatal days 0, 7, and 14. Offspring thymus and spleen lymphocytes were cultured with phytohemagglutinin (PHA) or concanavalin A (Con A) and proliferative responses were measured as an increase in the level of [³H]-thymidine incorporation into DNA. The procedure used to measure delayed hypersensitivity response to tuberculin challenge was not described in this report. Antibody titers were determined by passive hemagglutination method in serum of rats that were immunized twice with bovine gamma globulin. Lymphocytes were labeled with ⁵¹Cr and injected into recipients. The concentration of radioactivity in the recipient thymus, liver, lymph nodes and spleen were used as a measure of homing patterns. Thymus and body weights of rats treated postnatally with TCDD had recovered to normal by 128 days of age, while rats treated pre- and postnatally took longer to recover. Lymphocyte responses to mitogens and delayed hypersensitivity responses were diminished in both treatment groups and these effects, observed in cell from 25-128 day old rats were absent by 270 days of age. TCDD treatment had no effect on antibody titres. Alterations in homing patterns were observed in treated recipients injected with untreated donor cells and in untreated recipients injected with treated donor cells. The direction of the effect varied with the tissue and treatment regimen. The authors concluded that TCDD was equally immunosuppressive in Fisher/Wistar rats which represent a strain that elicits good immunologic responses, as in Fisher rats, which are poor immunologic responders (and were studied in experiments reported elsewhere).

309. Faith, R. E., Luster, M. I., and Moore, J. A. (1978) Chemical separation of helper cell functions and delayed hypersensitivity responses. Cellular Immunol. 40:275-284.

The effects of TCDD on cell-mediated and on humoral activity in the rat are described. The data were presented in a previous publication, see Faith and Moore, 1977.

310. Faith, R. E., and Moore, J. A. (1977) Impairment of thymus-dependent immune functions by exposure of the developing immune system to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). J. Toxicol. Environ. Health. 3:451-465.

The effects of perinatal TCDD treatment of rats on parameters of humoral and cell-mediated immune functions are described. Fischer-344 rats were administered 5 ug/kg TCDD (99+% purity) in acetone-corn oil by gavage, on day 18 of gestation and on postnatal days 0, 7, and 14 or only on postnatal days 0, 7, and 14. Spleen and thymic lymphocytes were isolated from the offspring and cultured with phytohemagglutinin (DHA) or concanavalin A (Con A) mitogen and proliferation response was measured as the level of [³H]-thymidine incorporation into DNA. Delayed hypersensitivity was measured in oxazolone-sensitized rats by injecting [³H]-thymidine, followed in 24 hours with a dermal application of oxazolone to 1 ear. Radioactivity in plugs from each ear 24 hours later was determined and their rates was used as an index of reactivity. Antibody titers were measured in rats administered 2 bovine gamma globulin injections; Passive hemagglutination was used to measure antibody titers, by a microtiter technique. Selected tissues are examined by light microscopy. Thymic, and body weights were depressed for 39 days after postnatal exposure and for 145 days after pre- and postnatal exposure. TCDD-treated groups showed reduced responsiveness of spleen and thymus lymphocytes to PHA and to Con A; and reduced delayed hypersensitivity responses; the reduction was usually larger after pre- and postnatal treatment than postnatal treatment only, although the effect was observed in both groups at 59 days of age (the last age tested). TCDD treatment did not reduce human antibody response to bovine gamma globulin. The authors concluded that TCDD-treated rats resembled rats treated with corticosteroids, allogenic lymphocytes or thymectomy and that TCDD-induced immune suppression was long lasting, with only certain T-cell subpopulations affected. The rats that were treated pre- and postnatally received 1 more dose than the rats treated only postnatally; this difference in total dosage, could account for the differences with the authors attribute to an increased sensitivity of fetal rats.

311. Fanelli, R. (1977) TCDD determination in human samples (cerebrospinal fluid and mesenteric fat). 28th Technical Report of the Mario Negri Institute of Pharmacological Research to the Seveso Authority. Milan, Italy. 28 pp.

[Not available.]

312. Fanelli, R., Castelli, M. G., Martelli, G. P., Nosedà, A., and Garattini, S. (1980) Presence of 2,3,7,8- tetrachlorodibenzo-p-dioxin in wildlife living near Seveso, Italy: A preliminary study. Bull. Environm. Contam. Toxicol. 24:460-462.

The authors presented preliminary results of a study of TCDD residue levels in wildlife in the contaminated areas around Seveso, Italy.

Where a chemical plant exploded all animals except hares were collected from areas known to have considerable environmental contamination. Hares were collected in various places inside and around the contaminated area. Animal tissues were analyzed by gas chromatography and low resolution mass fragmentography. Detection limits were not reported. All 14 field mice sampled had TCDD residues, ranging from 0.07-49 ppb (mean 4.5 ppb). Most of these animals came from an area where TCDD in the top 7 cm of soil ranged from 0.01-12 ppb, suggesting that rodents living on polluted soils accumulate whole body TCDD concentrations up to the same order of magnitude of the soil. Three of 5 hares sampled were positive for TCDD contamination and had an average liver concentration of TCDD of 7.7 ppb (2.7-13 ppb range). One toad sampled had a whole body TCDD concentration of 0.2 ppb, while one snake had 2.7 ppb liver TCDD accumulation and 16 ppb TCDD in adipose tissue. Of 2 samplings (5 g each) of earthworms, 1 sample contained 12 ppb TCDD. No levels of TCDD in animals outside the contaminated areas were reported. The results from these few animals suggest an accumulation of TCDD in wildlife, but no conclusions can be drawn without more information.

313. Fang, S. C., Fallin, E., Montgomery, M. L., and Freed, V. H. (1973) The metabolism and distribution of 2,4,5-trichlorophenoxyacetic acid in female rats. Toxicol. Appl. Pharmacol. 24:555-563.

The pharmacokinetics of 2,4,5-T and tissue distribution were studied in pregnant and non-pregnant rats. Female Wistar rats (4 pregnant and 13 non-pregnant) were administered 0.17, 4.3, or 41 mg/kg [^{14}C]-2,4,5-T in corn oil by stomach tube. Urine and feces collected for 3 days following treatment were analyzed for radioactivity by liquid scintillation counting and $^{14}\text{CO}_2$ in expired air was monitored continuously over the 3-day period with an electrometer. Tissue accumulation of 2,4,5-T was determined in a group of 9 non-pregnant rats given 1 mg of [^{14}C]-2,4,5-T per rat 1-72 hours prior to tissue removal and analysis for radioactivity and in a group of 6 pregnant rats given 0.043 mg [^{14}C]-2,4,5-T per rat 4-24 hours prior to tissue removal and analysis. Radioactive tissue and urinary metabolites were identified by thin layer and paper chromatographics. No $^{14}\text{CO}_2$ was detected in expired air. The rate of elimination of 2,4,5-T was the same for pregnant and non-pregnant rats and for non-pregnant rats in all three dosage groups. After 1 and 7 days, 75% and 85%, respectively, of the dose was excreted in the urine and 8% and 11%, respectively, in the feces. Only 10% of the urinary radioactivity was in the form of metabolites, including two benzene-extractable compounds and one water-soluble compound. Maximum tissue levels were reached 6-12 hours after exposure, with the highest levels in the kidney. About 90% of the tissue radioactivity was in the form of 2,4,5-T acid. Maternal distribution of radioactivity in pregnant rats resembled the distribution in non-pregnant rats. The placental level was higher than all other tissues, except the kidney. Fetal transfer of radioactivity was higher at more advanced stages of pregnancy than earlier stages. The average half-life for 2,4,5-T in adult rat tissues was 3.4 hours and for newborns exposed to 2,4,5-T in milk was 97 hours. Tissue levels of 2,4,5-T for neonates exposed to milk containing 2,4,5-T were substantially higher than levels for

neonates that resulted from placental transfer. The authors concluded that 2,4,5-T was eliminated rapidly from the adult rat and at a much slower rate from the newborn rat.

314. Fara, G. M. (1977a) Seveso: Studies on teratogenic and other chronic effects of chemical pollutants following an accident in a chemical plant. Teratology 16:365.

[Abstract, only.]

315. Fara, G. M. (1977b) Quaderno di Documentazioni 28:5.

[Not available.]

316. Fedorova, L. M., and Belova, R. S. (1974) Incorporation of 2,4-dichlorophenoxyacetic acid into the organs of animals: paths and dynamics of its excretion. Gig. Sanit. 2:105-107.

The distribution and excretion of 2,4-D was studied in the rat. A single dose of 0.05 mg/kg 2,4-D in water was administered to rats by gastric intubation. After 1-35 days, rats (3 per group) were killed and tissues, as well as urine and feces collected during the post-exposure period, were analyzed for radioactivity. Female rats were administered 0.04-0.06 mg/kg 2,4-D immediately after they gave birth and several offspring were killed daily for 7 days and their stomach and intestines were analyzed for radioactivity, to estimate 2,4-D transfer in milk. Transplacental transfer was measured administering 0.04 mg/kg TCDD on day 19 of gestation and on the next day analyzing radioactivity in the placenta, uterus, fetuses and amniotic fluid. Some rats were administered 100 mg/kg 2,4-D, urine and feces of males were collected for 25 days, and the stomach and intestines of offspring of treated mothers were removed from 1-7 days after maternal treatment. These urine, fecal and (indirect) milk samples were analyzed for 2,4-dichlorophenol by thin-layer chromatography. After 1 hr., 28-37% of the administered dose of 2,4-D was in the digestive tract; 7.7% and 6.3% of the dose remained in rats 26 and 35 days, respectively, after treatment. Over half of the administered dose is excreted in urine on the second day and 58-70% is excreted in urine on the first and second days. From 2.5-6.1% is excreted in the feces in 2 days. 2,4-D was excreted in milk, with peak levels excreted 2-3 days after treatment. 2,4-D distribution in the placenta, uterus, fetuses and amniotic fluid was uniform and each accounted for 2.7-4.9% of the dose, (all 4 tissues contained about 17% of the dose). 2,4-Dichlorophenol was not detected in any of the analyzed samples. The authors concluded that about 17% of 2,4-D administered crossed the placenta and 1% was transferred in milk to offspring; these levels were considered by the authors to be important factors in establishing threshold doses for embryo- or gonadotoxic effects of 2,4-D. The indirect methods used to measure levels of 2,4-D in milk is unlikely to produce accurate results, since fetal absorption rates from the intestine are not considered. Placental

transfer was calculated inaccurately, as uterine and placental contents do not necessarily reach the fetus.

317. Fee, D. C., Hughes, B. M., Taylor, M. L., Tierman, T. O., and Hill, C. E. (1975) Analytical methodology for herbicides orange. Volume II: Determination of origin of USAF stocks. Aerospace Research Laboratories. Technical report ARL TR 75-0110. 30 p.

[Background material.]

318. Feldmann, R. J., and Maibach, H. I. (1974) Percutaneous penetration of some pesticides and herbicides in man. Toxicol. Appl. Pharmacol. 28:126-132.

The extent of absorption of 2,4-D and diquat applied to the forearm of volunteers was examined. The extent of urinary excretion of ^{14}C -radioactivity from ^{14}C -labeled pesticides was determined after each compound was administered intravenously to 6 subjects. All of the dose of 2,4-D was excreted into the urine within 5 days, with a half-life for excretion of 13 hours. Only 61.2% of intravenously administered ^{14}C -diquat was excreted in the urine in 5 days, and the half-life was 4 hours. Each ^{14}C -labeled compound was applied to the forearm and the volunteers were asked not to wash the forearm for 24 hours. Absorption was calculated from the amount of radioactivity excreted in 5 days and, for diquat, this value was corrected for the proportion of the dose found to be excreted in urine after intravenous injection. Only 5.8% of the dermally administered dose of 2,4-D and 0.3% of the dose of diquat was absorbed. Absorption of 10 other herbicides was also measured with these methods. Diquat was the least absorbed compound. The authors suggested that these rates of absorption will be influenced by the degree of sweating and condition of the skin.

319. Fetisov, M. I. (1966) Occupational hygiene in the application of herbicides of the 2,4-D group. Gig. Sanit. 31:383-386.

Acute toxicity in rats and mice, and the health conditions of occupationally exposed workers for 2,4-D and 2,4-D butyl ester were briefly summarized. For 2,4-D the LD_{50} for the mouse and rat were 300 and 1000 mg/kg, respectively, and for 2,4-D butyl ester were 380 and 920 mg/kg, respectively. (The route of administration or other details of the protocols used were not provided). After one-fifth the LD_{50} was administered repeatedly, the cumulation coefficients were determined to be 2.6 for 2,4-D and 2.1 for 2,4-D butyl ester. Subacute dermal application of 2,4-D produced marked local irritation without systemic toxicity. For mice tail immersion into 50% 2,4-D butyl ester for 4 hours daily for 3-5 days produced 70% mortality. The threshold concentration for inhalation exposure (4 hr. daily for 4 mo.) was 0.01 mg/l for 2,4-D. The concentration of 2,4-D and its butyl ester exceeded 10 mg/m³ in 55% of the locations surveyed in two chemical factories. A

study of the disease incidence and physiological functions was undertaken in a group of 105 herbicide factory workers and in 45 aviation workers involved in herbicide spraying. Common complaints of aviators were fatigue, headaches, poor appetite, abdominal pain and taste sense impairment. Olfactory and taste impairments were confirmed by a test protocol. No details of protocols or results were provided and the health of the herbicide factory workers was not described. The authors concluded by recommending 2 mg/m³ as the maximum permissible concentration of 2,4-D in the work zone of agricultural workers.

320. Field, B., and Kerr, C. (1979) Herbicide use and incidence of neural-tube defects. Lancet. 1(8130):1341-1342.

[Abstract, only.]

321. Firestone, D. The 2,3,7,8-tetrachlorodibenzo-para-dioxin problem: a review. In Chlorinated Phenoxy Acids and their Dioxins; ed. C. Ramel, (Ecol. Bull. No. 27, Stockholm: Swedish Natural Science Research Council, 1978) p. 39-52.

[Review article.]

322. Firestone, D. (1973) Etiology of chick edema disease. Environ. Health Perspec. 5:59-66.

[Review article.]

323. Flamm, B. R., and Cravens, J. H. (1971) Effects of War Damage on the Forest Resources of South Vietnam. J. For. 69(11):784-789.

[Editorial.]

324. Flick, D. F., Firestone, D., and Higginbotham, G. R. (1972) Studies of the chick edema disease 9. Response of chicks fed or singly administered synthetic edema-producing compounds. Poult. Sci. 51:2026-2034.

The toxic effects of various dioxin preparations were evaluated in the chicken. A mixture of 58% TCDD, 42% 2,3,7-trichlorodibenzo-p-dioxin and traces of di- and pentachlorodibenzo-p-dioxins was fed to White Leghorn chickens for 21 days. Feeding was initiated at 1 day of age at dietary levels of 0.01, 0.1 and 10 ppm dioxins in feed. These doses corresponded to total dioxin intakes of 1.9 17.1 and 51.9 ug. All eight chickens fed the highest dose died, seven deaths occurred within the first 7 days and the remaining chickens that died on the 17th day showed a severe edema. Four of five and three of five chickens fed 0.01 ppm and 0.10 ppm diets, respectively, died. These chickens showed severe decreases in body weight gain. Tissue changes among the three treatment groups included enlarged adrenals, and pale liver, pancreas

and kidney. Other dioxins mixtures were evaluated for toxic effects in the chicken. The authors concluded that the tri- and tetra-chloro derivatives of dibenzo-p-dioxin were the most lethal compounds tested and produced much tissue damage and moderate edema while the hexa- and heptachloro derivatives were more potent in producing edema and tissue damage.

325. Flint, G. W., Alexander, J. J., Funderhurk, O. P. (1968) Vapor pressures of low volatile esters of 2,4-D. Weed Sci. 16:541-544.

[Background material.]

326. Florsheim, W. H., and Velcoff, S. M. (1962) Some effects of 2,4-dichlorophenoxyacetic acid on thyroid function in the rat: effects on iodine accumulation. Endocrinology 71(1):1-6.

Alterations in thyroid function after 2,4-D administration to rats is described. Male Sprague-Dawley rats were administered daily subcutaneous doses of 2,4-D in aqueous solution. Body weights, organ weights, and thyroid function tests were carried out (length of treatment or time from the end of treatment to sacrifice were not reported). Statistically significant effects of ^{131}I daily doses of 80 mg/kg 2,4-D on thyroid function included increased ^{131}I uptake over 24 hours and decreased serum protein-bound iodine levels, while weights of the thyroid, pituitary, adrenals, testes, and whole body, thyroid cell height and thyroid ^{131}I half-life, renal histology, protein excretion, and serum electrolyte patterns were unchanged. Thyroidal ^{131}I uptake was not altered by 2,4-D in hypophysectomized rats or in rats fed an iodine depleted diet. Goiter development or the ratio of thyroid to serum radioiodine concentrations in rats fed a goitrogenic diet for 2 weeks were not affected by 2,4-D administration. Other parameters that were not altered by 2,4-D exposure were pituitary and serum thyroid-stimulating hormone levels, and the proportion of thyroxine among total iodinated thyroid amino acids. The authors concluded that 2,4-D produced an increase in ^{131}I uptake by a direct effect on the peripheral iodide pool and not an alteration in pituitary thyrotrophic hormone production.

327. Foissac-Gegoux, P., LeLievre, A., Basin, B., and Warot, P. (1962) Polyneuritis after use of a herbicide: 2,4-D acid. Lille. Med. 7:1049-1051.

A case report is presented of polyneuritis which developed in a man after he sprayed 2,4-D. Two solutions, containing 235 g/l and 410 g/l of 2,4-D had been applied over several days from a sprayer, towed by a tractor. The tractor was open and heavy winds resulted in herbicide being blown into the cab, exposing the operator. The day following the last day of the spraying operation, the 52-year-old male worker experienced pain on the right side of the face that developed into paresthia and anesthesia and on the next day developed hypoesthesia of the right

leg. Two weeks later, symptoms of the right leg improved while anesthesia and weakness of the left leg developed and the facial symptoms remained. The patient presented at a clinic after an additional 2 weeks, at which time Achilles reflex and all but deep sensations were found to be absent. No disorders of the arms or of other organ systems were detected. Polyneuritis and disorders of the right trigeminal nerve were treated with vitamin B and in 6 weeks the patient was discharged. The condition of his face and left leg had improved and responses to electrodiagnostic stimulation were normal. Neurological impairment of the right muscle still remained and the Achilles reflex was still absent, but atrophy did not develop. The authors concluded that the cause of polyneuritis was exposure to 2,4-D.

328. Folmar, L. C. (1976) Overt avoidance reaction of rainbow trout fry to nine herbicides. Bull. Environ. Contam. Toxicol. 15(5):509-514.

The author describes the avoidance reaction of rainbow trout to water containing dalapon, 2,4-D dimethylamine salt, and diquat. Fish (10 per trial) were placed in the holding area of a Y-shaped avoidance maze. Tests at each concentration were repeated five times. Avoidance of herbicides was evaluated statistically by the chi-square test on the assumption that if a fish could not discriminate between treated and untreated water they would enter each section of the maze with equal frequency. Herbicides were tested at 0.1, 1.0, or 10.0 mg/l. These concentrations were within the range of expected water concentrations of each herbicide under recommended application rates. Fish avoided concentrations of 1 and 10 mg/l dalapon and 2,4-D. According to the author, avoidance reactions occurred well below the 96 hr LC₅₀ values for these herbicides. No avoidance was observed in fish exposed to diquat. The author concluded that the fish would probably not be killed by and would not avoid low levels of dalapon and 2,4-D.

329. Forth, W. (1977) 2,3,7,8-tetrachlorodibenzo-1,4-dioxin (TCDD): the Seveso accident. Dentsches Arzteblatt. 44(3):2617-2628.

[Review article.]

330. Fowler, B. A., Hook, G. E. R., Lucier, G. W. (1977) Tetrachloro-dibenzo-p-dioxin induction of renal microsomal enzyme systems: Ultrastructural effects on pars recta (S₃) proximal tubule cells of the rat kidney. J. Pharmacol. Exp. Therap. 203(3):712-721.

Induction of 2 microsomal enzymes by TCDD was localized to specific regions and cells of the rat kidney and was correlated with ultrastructural changes in these tissues. Male Charles River rats were administered a single oral dose of 25 ug/kg TCDD (98% purity) in acetone-corn oil (1:6) 18 hours to 16 days before they were killed. Kidney tissue was prepared for electron microscopy and renal microsomes were isolated and assayed for benzpyrene hydroxylase activity by a

fluorometric method and for glucuronyl transferase by a spectrophotometric method. Other kidneys were separated into the outer strip of the medulla (rich in S₃ proximal tubular cells), the inner medulla and the cortex and each region was assayed for both enzymes. The only overt sign of TCDD toxicity was a mild decrease in growth rate of treated rats. Both enzyme activities were elevated 18 hours after TCDD treatment and reached plateau levels within 3 days. Benzpyrene activity was induced 40 fold at day 7 and glucuronyl transferase was induced 4-fold. Ultrastructural changes in kidneys of treated rats were limited to a massive increase in the smooth endoplasmic reticulum of the terminal straight (S₃) segments of the proximal tubule (parsrecta). No changes in microsomal protein levels or tissue weight occurred in any region of the kidney after TCDD treatment while the activities of both enzymes were induced substantially in all 3 regions. The highest specific activities for the enzymes were associated with the 105,000 xg pellet which contained both smooth and rough microsomes. The authors concluded that renal microsomal enzymes are highly inducible by TCDD, with induction localized to the smooth endoplasmic reticulum of S₃ proximal tubule cells.

331. Fowler, B. A., Lucier, G. W., Brown, H. W., and McDaniel, O. S. (1973) Ultrastructural changes in rat liver cells following a single oral dose of TCDD. Environ. Health Perspec. 5:141-148.

The histological changes in rat liver tissue following an acute dose of TCDD are described. Charles River rats (30 per group) were administered 5 or 25 ug/kg TCDD in acetone-corn oil by gavage. Five rats per group were killed at each of six time points between 1 and 28 days after treatment. Liver tissue was removed and prepared for light microscopy. The only changes observed in hepatic tissue samples from treated rats compared to controls was a proliferation of the smooth and rough endoplasmic reticulums of parenchymal cells. The authors concluded that these changes were consistent with a cellular attempt at detoxification and were probably related to induced changes in RNA and protein synthesis.

332. Fowler, D. L., and Mahan, J. N. (1978) The Pesticide Review 1977. U. S. Department of Agriculture, Agricultural Stabilization and Conservation Service. 44 pp.

[Review article.]

333. Foy, C. L. Picloram and related compounds. In Herbicides: Chemistry, degradation, and mode of action. Vol. 2. eds., P. C. Kearney and D. D. Kaufman. (New York: Marcel Dekker Inc., 1976) pp. 777-813.

[Review article.]

334. Fox, J. L. (1979) Research solving body's detoxifying system. P-450 enzymes rival the immune system of the body in complexity and may explain how toxins such as dioxin exert their effects. Chem. Eng. News June 4, 1979, pp. 24-26.

[Review article.]

335. Fox, R. P. (1979) Air Base Defense In The Republic of Vietnam 1961-1973. Washington, D.C., U.S. Air Force, 278 pp.

[Review article.]

336. Frank, R., Sirons, G. J., and Ripley, B. D. (1979) Herbicide contamination and decontamination of well waters in Ontario, Canada, 1969-1978. Pest. Monit. J. 13(3):120-127.

The authors report on suspected herbicide contamination of 239 wells in Ontario, Canada. Well contamination was divided into 5 categories: 1) a spill of concentrated herbicide in or near the well, 2) a spill of diluted herbicide in or near the well, 3) herbicide drift during spraying, 4) contamination as a result of storm runoff, and 5) subterranean movement of herbicide into a well. One liter composite samples from several pumpings from each well were collected and analyzed by gas-liquid chromatography. A total of 61 wells were contaminated by 2,4-D primarily as drift from spraying and storm runoff. Concentrations of 2,4-D found in wells ranged from 0.1 ug/l to 14.6 mg/l. Twenty-five wells were contaminated with 2,4,5-T primarily from herbicide spraying. Six wells received picloram contamination from spraying, storm runoff and subterranean movement which ranged from 0.1 - 1.5 ug/l. Only one well was found to be contaminated by dalapon as a result of a spill of diluted herbicide. The authors noted that contamination was usually due to improper handling of herbicides. In cases where wells were decontaminated, 2,4-D was removed rapidly while picloram was difficult to remove. Ease of removal of dalapon and 2,4,5-T were not reported.

337. Fries, G. F., and Marrow, G. S. (1975) Retention and excretion of 2,3,7,8 tetrachlorodibenzo-p-dioxin by rats. J. Agr. Fd. Chem. 23:265-269.

The pharmacokinetics of TCDD elimination and tissue distribution were studied in the rat. Sprague-Dawley rats were fed a diet which contained 7 or 20 ppb of [¹⁴C]-TCDD for 42 days and then were fed a control diet for the subsequent 28 days. Rats were killed at 2 week intervals during the experiment and the liver and carcass were each homogenized and analyzed for radioactivity. Neither dose of TCDD was lethal to any rats. Food intake and weight gain were decreased during TCDD exposure and recovery occurred when the control diet was reinstated. Liver weights were increased to a greater extent in the low dosage group than the high dosage group. TCDD concentrations were

highest in the liver and the liver contents accounted for 85% of the dose administered to male rats and 70% of the dose administered to females. Half-lives for TCDD were calculated for liver clearance and whole body clearance as 11-15 days and steady state retention by the whole body was estimated at 10.5 times the daily intake. The authors concluded that fluctuations in their data resulted from low specific activity of the [¹⁴C]-TCDD preparation and inherent assumptions applied to the kinetic models probably introduced deviations in their calculated parameters from actual values.

338. Frohberg, H., Gleich, J., and Hofmann, A. (1975) Investigation on the embryotoxic effect of 2,4,5-T in NMRI mice. Teratology 10(3):309.

[Abstract, only.]

339. Fujita, K., Fujita, H. M., and Funazaki, Z. (1975) Chromosomal abnormality caused by 2,4,5-T. J. Jpn. Assoc. Rural Med. 24(2):77-79.

The authors studied the clastogenic effects of 2,4,5-T in human cell culture. Adult human leukocytes cultured with 10⁻⁴ to 10⁻⁵ 2,4,5-T having 0.09 ppm TCDD as a contaminant. Approximately 100-200 cells were examined at each dose level tested and chromatid breaks, isochromatid breaks, deletions + free fragment dicentrics, + rings were measured. Chromatid breaks appeared to increase with increasing doses of 2,4,5-T. The authors concluded that 2,4,5-T induced chromosome aberrations in human cells in culture. However, the authors could not determine whether this effect was due to 2,4,5-T or to the TCDD contaminant.

340. Fullerton, P. M. (1969) Toxic chemicals and peripheral neuropathy: Clinical and epidemiological features. Proc. Roy. Soc. Med. 62:201-204.

[Review article.]

341. Fullerton, R. W., Carlson, M. B., and Nolting, A. R. (1974) Final report on the 2,4,5-T scientific workshop. Washington, DC, Mar. 8-9. 45 pp.

[Background material.]

342. Funderburk, H. H., Jr., and Bozarth, G. A. (1967) Review of the metabolism and decomposition of diquat and paraquat. J. Agr. Food Chem. 15(4):563-567.

[Review article.]

343. Furst, J. R. (1980) Statement Before the White House Interagency Work Group on Long Term Health Effects of Phenoxy Herbicides and Contaminants. Sept. 22, 1980. 8 pp.

[Testimony.]

344. Gage, J. C. (1968a) The action of paraquat and diquat on the respiration of liver cell fractions. Biochem. J. 109(5):757-761.

The effect of diquat on rat liver cell oxidation-reduction systems was studied in vitro. Subcellular fractions were prepared from male Alderley Park rat liver cells and incubated in the presence of diquat and various metabolic substrates and cofactors. The products formed were quantitated spectrophotometrically. In the presence of ADP, 1.0 mM diquat produced a slight (25%) increase in oxygen consumption which was not blocked by adding amytal or antimycin A. Diquat (1.0 mM) also stimulated oxygen uptake by mitochondrial fragments in the presence of antimycin A or amytal, with certain substrates, including NADH and beta-hydroxybutyrate, but not succinate. Diquat stimulated NADH oxidase and NADPH oxidase activities in microsomal preparations and in the soluble cell fraction. The microsomal effect was not inhibited by carbon monoxide or para-chloromercuribenzoate, and the hydrogen peroxide generated during diquat oxidation by molecular oxygen was degraded by microsomal catalase. Paraquat was also studied in these experiments. The authors concluded that diquat caused stimulation of oxygen utilization by interacting with microsomal NADPH dehydrogenase and transient dipyridilium-free radicals generated during the reaction were likely to be responsible for diquat toxicity in animal cells. The authors also concluded that diquat probably did not penetrate intact mitochondrial membranes.

345. Gage, J. C. (1968b) Toxicity of paraquat and diquat aerosols generated by a size-selective cyclone: effect of particle size distribution. Br. J. Ind. Med. 25(4):304-314.

The toxicity of diquat from inhalation exposure was evaluated in 5 laboratory species. Diquat dichloride aerosol, with mean particles sizes of 2.5 micron or greater, was delivered to an exposure chamber. Two male Alderley Park rats were exposed to 25 ug/l of diquat in air (1 rat for 15 minutes and 1 for 30 minutes). Other groups of 8 rats (4 per sex) were exposed to 0.5 ug/l, 1.06 ug/l, or 2 ug/l diquat concentrations. Exposure to each atmosphere was for 15 daily 6 hr. periods. Groups of 10 mice (5 per sex), 8 guinea pigs (4 per sex), 2 female rabbits and 1 male beagle were exposed to 1.06 ug/l diquat for 15 daily 6 hour periods. The general condition of the animals and after sub-acute exposure of rats, hematological analyses, and necropsies were performed and organs were weighed. No signs of toxic effects were observed after acute exposure of rats or subacute exposure of any species except rats. Female rats that were exposed to the highest concentration initially showed signs of noisy breathing and histopathological signs of pulmonary irritation, edema, hyperplasia and macrophage infiltration. No other organs were affected. The toxic effects of paraquat aerosol exposure were also described. The authors concluded that a concentration of 0.5 mg/m³ of respirable diquat aerosol would not constitute an excessive exposure.

346. Gaines, T. B., Holson, J. F., Nelson, C. J., and Schumacher, H. J. (1974) Analysis of strain differences in sensitivity and reproducibility of results in assessing 2,4,5-T teratogenicity in mice. Tox. Appl. Pharmacol. 33:174-175.
- [Abstract, only.]
347. Gale, T. F., and Ferm, V. H. (1973) Effects of the herbicide 2,4,5-T and Pyrazon embryogenesis in the hamster. Anat. Rec. 175(2):503.
- [Abstract, only.]
348. Galston, A. W. (1979) Herbicides: A mixed blessing. Biosci. 29(2):85-90.
- [Review article.]
349. Galston, A. W. (1974) The ungreening of South Vietnam. Natur. Hist. 83(6):10,12,14.
- [Editorial.]
350. Galston, A. W. (1971) Some implications of the widespread use of herbicides. Bioscience 21:891-892.
- [Editorial.]
351. Gandenberger, G. F. (1980) Letter on the Proposed Statement at Hearing, 22 Sept., to Office of the General Counsel, U. S. Dept. of Health and Human Services, Washington, DC. 2 pp.
- [Background material.]
352. Garattini, S. (1977) TCDD poisoning at Seveso. Biomedicine 26:28-29.
- [Editorial.]
353. Garcia, J. D., and Rhodes, M. J. (1979) Residues of 2,4,5-T in the American coot (Fulcia americana). Bull. Environm. Contam. Toxicol. 23:231-235.

The authors report on levels of 2,4,5-T and TCDD found in tissues of the American coot, a waterfowl. Twenty-nine coots were collected from August through December 1976 at White River Lake, Texas, a reservoir which receives runoff from a watershed that was treated with 2,4,5-T in June 1976. The application rate of 2,4,5-T was 0.56 kg 2,4,5-T acid

equivalent/ha. Coot breast muscle, fat, gizzard, liver, brain, and heart were analyzed for 2,4,5-T and TCDD residues by gas chromatography (detection limit unspecified). No TCDD was detected in any of the samples. In addition, no 2,4,5-T was detected in brain and heart samples. Residues of 2,4,5-T were detected in the breast muscle of 14 birds, in the fat of 12 birds, and in the liver and gizzards of 5 birds. The authors did not present a breakdown of contamination by individual bird. The range of 2,4,5-T in breast tissue varied from 8-1,338 ppb. Levels higher than 1,000 ppb were found in only 2 of the 14 birds that had 2,4,5-T residues. All other birds had levels below 500 ppb. The authors speculated that the 2 birds with the highest residue levels probably received 2,4,5-T exposure elsewhere. Fat residue levels in birds ranged from 5-30 ppb; liver residues ranged from 2-30 ppb in 3 birds and were 68 and 118 ppb in 2 birds. Gizzard residue levels ranged from 6-21 ppb in 3 birds and were 36 and 41 ppb in 2 birds. When residue levels among tissues were compared, residues in liver in August were significantly higher ($p < 0.05$) than in other tissues. Residue levels in breast tissue were significantly higher ($p < 0.05$) than other tissues in October and November. No differences among tissues were observed in December. The authors concluded that use of 2,4,5-T at recommended rates would not present any serious hazard to coots.

354. Gardiner, J. A. Substituted uracil herbicides. In Herbicides: Chemistry, degradation, and mode of action. Vol. 1., P. C. Kearney and D. D. Kaufman, eds., (New York: Marcel Dekker, Inc., 1975) pp 293-321.

[Review article.]

355. Gasiewicz, T. A., and Neal, R. A. (1978) Tissue distribution and excretion of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and effects upon clinical chemical parameters in the guinea pig. Fed. Proc. Fed. Am. Soc. Exp. Biol. 37(3):501.

[Abstract, only.]

356. Gasiewicz, T. A., and Neal, R. A. (1979) 2,3,7,8-Tetrachlorodibenzo-p-dioxin tissue distribution, excretion, and effects on clinical chemical parameters in guinea pigs. Toxicol. Appl. Pharmacol. 51:329-339.

The tissue distribution and kinetics of excretion of TCDD in the guinea pig are described. Adult male guinea pigs were administered 2.0 ug/kg of [³H]-TCDD, intraperitoneally, as a single dose. Animals were killed within 1-15 days after dosing and tissues were analyzed for radioactivity. Other groups of guinea pigs were administered 0.5 ug/kg of TCDD, intraperitoneally. Excretion of radioactivity was monitored in urine and feces, collected for 23 days after the injection and these data were analyzed pharmacokinetically. Groups of guinea pigs were administered 1.0 ug/kg of TCDD and blood chemistry analyses were

performed on blood samples collected during the 14-day period after dosing. The results of these analyses were compared to those of pair-fed controls. Tissue levels of TCDD were greater than 0.3% of the dose per gram tissue for adipose, adrenals, liver, spleen, intestine and skin, 1 day after administration. Liver contained 3 fold higher levels on day 15, and levels for the adrenals, kidneys, and lungs also increased. TCDD excretion was linear over 23 days, with a calculated half life of 30.2 days. TCDD treatment produced elevated albumin, total protein, iron, urea nitrogen, cholesterol and triglyceride levels, compared to levels for pair-fed controls. The authors concluded that the increasing tissue levels they observed resulted from mobilization of TCDD from fat deposits to other tissues.

357. Gehring, P. J., and Betso, J. E. Phenoxy acids: effects and fate in mammals. In Chlorinated Phenoxy Acids and Their Dioxins, ed. C. Ramel (Ecol Bull. No. 27, Stockholm: Swedish Natural Research Council, 1978) pp. 122-133.

[Review article.]

358. Gehring, P. J., Kramer, C. G., Schwetz, B. A., Rose, J. Q., and Rowe, V. K. (1973) The fate of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) following oral administration to man. Toxicol. Appl. Pharmacol. 26:352-361.

The pharmacokinetics of 2,4,5-T clearance was described for five male volunteers. Five mg/kg of 2,4,5-T (with less than 0.05 ppm TCDD) was ingested by each subject and blood, urine, and fecal samples collected during the subsequent 96 hours were analyzed for 2,4,5-T content. The data for the log of plasma levels plotted as a function of time suggested first order kinetics for absorption and for clearance. The half-lives were calculated as 0.75 hours for absorption of 2,4,5-T and 23.1 hours for plasma clearance, and the volume of distribution was calculated to be 0.079 liter/kg. To determine plasma binding of 2,4,5-T, plasma samples were dialyzed against solutions of 2,4,5-T and the concentration of 2,4,5-T in the plasma fraction after 8 hours at 37°C was measured. Over 98% of 2,4,5-T was recovered bound to plasma protein, yielding an apparent volume of distribution (adjusting for fact that only a small proportion of 2,4,5-T is in plasma water) of 6.1 l/kg. Neither recovery of 2,4,5-T and ether extract of acid hydrolyzed urine nor chromatograms of the extracts revealed any metabolites. The urinary excretion rate was the same as the rate of plasma clearance. Over 88% of the dose was recovered in the urine while less than 1% was excreted in the feces. The pharmacokinetic data were used to extrapolate theoretical plasma levels of 2,4,5-T after various exposure regimens after repeated administrations of 2,4,5-T, plasma levels were estimated to plateau in 3 days.

359. Geissbuhler, H., Martin, H., and Vos, G. The substituted ureas. In Herbicides: Chemistry, degradation and mode of action. Vol. 1 P.C. Kearney and D.D. Kaufman, eds, (New York: Marcel Dekker Inc., 1975) pp. 210-291.

[Review article.]

360. Geldmacher-V., Mallinckrodt, M., and Lautenbach, L. (1966) Two cases of fatal poisoning (suicide) with chlorinated phenoxyacetic acids (2,4-D and MCPA). Archiv. Toxikol. 21:261-278.

[Foreign language.]

361. Geldmacher-V., Mallinckrodt, M., and Schussler, F. (1971) Toxicity of diuron 1-(3,4-dichlorophenyl)-3,3-dimethylurea (diuron) and its metabolism in man. Arch. Toxikol. 27(3-4):187-192.

The acute toxicity and metabolism of diuron is described following a case of human ingestion. A 39-year-old woman ingested 38 mg/kg diuron and 20 mg/kg aminotriazole as the herbicide preparation, Ustinex PA, in a suicide attempt. No signs of poisoning were observed in the patient upon admission to a hospital and no sugar or albumin were detected in the urine collected a few hours (exact number not given) after ingestion of herbicide. Urinary metabolites detected in the urine sample by thin layer chromatography were 1-(3,4-dichlorophenylurea) and 1-(3,4-dichlorophenyl)-3-methylurea, and a small amount of 3,4-dichloroaniline. Non-altered diuron and non-conjugated phenolic metabolites were not detected and no analysis of conjugated metabolites was performed. Unmetabolized aminotriazole was excreted. The authors concluded that diuron metabolism in man involved sequential demethylation of the N-methyl groups and then hydrolysis.

362. Gianotti, F. (1977) Chloracne in children from 2,3,7,8-tetrachlorodibenzo-p-dioxin. Ann. Dermatol. Venereol. (Paris) 104:825-829.

Clinical and histologic descriptions are given of dermatologic lesions observed in children exposed to TCDD from the Seveso accident. In general, the children had erythematous hyperkeratotic lesions on the face and limbs several days after the accident, which developed into chloracne in 2 months. Histologically, the nodular lesions showed epidermal hyperplasia. Hypercanthosis, metaplasia of the sweat glands, and horny cysts on the extremities were observed. The skin also contained foreign body granulomas. The total number of children affected, or even the ages of all the children whose clinical descriptions were presented, were not provided. The authors concluded that these symptoms resembled epidemic follicular keratosis, which was observed in about 2,000 children in Switzerland in 1946, and suggested that the 30-year follow-up of the Swiss children would provide useful predictive information for the Seveso children.

363. Gile, J. D., Collins, J. C., and Gillett, J. W. (1980) Fate of selected herbicides in a terrestrial laboratory microcosm. Environ. Sci. Technol. 14(9):1124-1128.

The authors describe the fate of 2,4,5-T and bromacil in a model terrestrial laboratory ecosystem. Model ecosystems were prepared using sand, clay, and Jiffy Mix, and water. After allowing 1 week for equilibration, Douglas fir seedlings, red alder seedlings, and rye grass were planted. When growth of rye grass was established, earthworms, pillbugs, and mealworm larvae were added, followed by snails and crickets. A vole was added after treatment. Twenty-six days after planting, 2-¹⁴C-labeled bromacil or ring-labeled ¹⁴C-2,4,5-T isooctyl ester were applied as a foliar spray (0.28 kg/ha) to the ecosystem model. Both animals and plants were analyzed for uptake of herbicides or their metabolites. The experiment was terminated after 22 days for bromacil and 20 days for 2,4,5-T. In the experiment, 91% of the applied 2,4,5-T was recovered, while only 69% of the applied bromacil was recovered. The measurement of recovered bromacil was complicated by the location of the ¹⁴C label: the C-2 carbon position is readily converted to CO₂. Concentrations of ¹⁴C materials were greatest in the upper 5 cm of soil: 0.12 ppm bromacil and 0.19 ppm 2,4,5-T. In ryegrass plants, at experiment termination, 2.5 ppm 2,4,5-T and 15 ppm bromacil were detected. 2,4,5-T was mainly present as extractable metabolites, while bromacil recovered was mostly extractable parent. In Douglas fir seedlings, most of the bromacil and 2,4,5-T detected was present in the roots with only a small amount in the stems. However, bromacil was present as extractable parent, while 2,4,5-T was present as extractable metabolites. Residues of both herbicides were greatest in snail feces and snails. In voles, the heart, lung, liver, GI tract, kidney, brain, and carcass were examined, and the majority of ¹⁴C-herbicide was found in the gastrointestinal tract (0.18 ppm bromacil, 0.20 ppm, 2,4,5-T). Bromacil was present mainly as bound residue, while the majority of 2,4,5-T recovered was metabolites. The authors did not make any conclusions on the environmental effects of these pesticides. However, it appears that neither herbicide accumulates in any of the plants or animals studied under the conditions of the experiments.

364. Glass, B. L., and Edwards, W. N. (1974) Picloram in lysimeter runoff and percolation water. Bull. Environ. Contam. Toxicol. 11(2):109-112.

The authors investigated the transport of picloram in surface runoff and percolation water from a lysimeter treated with 2.24 kg/ha in March 1970. Surface runoff and percolation water were analyzed for picloram by gas liquid chromatography. Cumulative rainfall in the area was 62.5 cm for the experimental period. Picloram was detectable in runoff water up to 7 months after treatment. No picloram was detected in runoff 1 year after treatment. The total picloram removed by this means was 0.13 mg, 0.007% of the total applied treatment. The largest amount (14.5 ppb) of picloram occurred in runoff water collected during the first month after application and decreased during each successive month. In percolation water, picloram was initially detected 2.4m

beneath the soil at 1.0 ppb one year after application. The highest amount of picloram detected in percolation water was 1.2 ppb observed during months 13 and 17. The authors concluded that movement through the soil column does not appear to be a major route of transport for picloram.

365. Goldmann, P. J. (1975) Initial study of persons exposed to dioxin after an accident on November 13, 1953 in Ludwigshaven, Germany. Working Paper, joint NIEHS/IARC Working Group.

[Not available.]

366. Goldmann, P. J. (1973) Severe, acute chloracne, a mass intoxication due to 2,3,7,8-tetrachlorodibenzo-dioxin. Der Hausarzt 24(4):149-152.

A summary is provided of the health problems observed in workers that contacted TCDD following an accident in the Baden Aniline and Soda Factory (BASF) in Germany in 1953. A total of 55 patients developed chloracne, including a worker who was only in contact with exposed animals and the son of another worker. Only 42 of the patients were included in this study (the reasons for eliminating the remainder were not provided). Dermal lesions were the only symptom in half of the 42 persons. The symptoms observed along with the dermal lesions in the other 21 persons were: central nervous system disorders (7 cases), tracheobronchitis (5), and hepatitis (4). Symptoms were described for the 14 most severe cases of the 42 cases studied; these 14 workers were unable to work for 5-24 months. Common symptoms for this group were bronchitis (6 cases), pain in arms and legs (6), liver damage (6), and headaches (5). These 14 workers were unable to work for 5-24 months. One person who visited the facility briefly in 1958 developed fatal pancreatic necrosis. This person also developed chloracne, but the authors did not suggest an etiology for the fatal lesion. The authors concluded that TCDD was the acnegenic agent released during the factory accident.

367. Goldmann, P. J. (1972) Extremely severe acute chloracne due to trichlorophenol decomposition products. A contribution to the perna problem. Arbeitsmedizin Socialmedizin Arbeitshygiene 7(1):12-18.

A summary is presented of the health of workers exposed to vapors released during an accident in a 2,4,5-trichlorophenol factory (Badischen Aniline and Soda Factory) in Germany in 1953; selected aspects of specific cases were also presented. A group of 21 patients had chloracne only. This group included the son of one worker (the son never entered the plant); one case in which comedones and sebaceous cysts remained 18 years later; two cases of chronic conjunctivitis and blepharitis; one case with involvement of the Meibomian glands, which lubricate the margin of the eyelid and are ontogenetically related to the sebaceous glands and their involvement was considered a subsymptom of chloracne. Some patients with only chloracne were unable to work

for up to 5 years. Skin and internal organ disorders were observed in an additional 14 persons. Disorders of internal organs involved the liver in four cases and inflammation of respiratory passages in five cases. One fatal case of pancreatitis occurred in the group. In general, an increased susceptibility to infection was noted in this group. The third group of seven patients had dermatologic and central nervous system disorders. Overall tiredness and neuromuscular weakness were characteristic of all members of this group. Included in this group were three cases of polyneuritis. Cases of neurasthenia occurred in all groups. Residues of TCDD were detected in the factory, and in 1969 the facility was demolished, because all previous decontamination procedures had failed. The demolition team showed no adverse symptoms. The authors concluded that TCDD exposure was responsible for chloracne and some of the internal and neurological disorders.

368. Goldstein, H. E., and Long, J. F. (1958) Observations on domestic animals exposed to herbicide spray applications of 2,4,5-T and dalapon. Proc. North Cent. Weed Control Conf. 15:28-29.

[Not available.]

369. Goldstein, J. A., Hickman, P., Bergman, H., and Vos, J. G. (1973) Hepatic porphyria induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Res. Com. Chem. Path. Pharmacol. 6(3):919-928.

Porphyrogenic properties of TCDD in the mouse are described. Male C57BL/6 mice (12-18 per group) were administered weekly doses of 1, 5 or 25 ug/kg TCDD in acetone-corn oil (1:6) orally for 4 weeks. Seven days after the last dose, mice were killed and the livers were analyzed for delta-aminolevulinic acid (ALA) synthetase by column chromatography, for porphyrins by thin-layer chromatography or fluorometry, and for iron by a spectrophotometric method. Liver tissue was also examined for histopathologic changes. A 2,000 fold increase in uroporphyrin levels was observed in livers of the highest dosage group, as 8- and 7- carboxyporphyrins. Half of the mice in this group died 2-3 days before they were scheduled to be killed, and no difference in uroporphyrin levels were observed in the 2 subgroups. Twelve mice were given a single oral dose of 150 ug/kg TCDD and 21-25 days later their liver uroporphyrin levels were elevated 4,000 fold over control levels. ALA synthetase was elevated 225% and iron by 160% in the 25 ug/kg dosage group and 260% in the single dosage group. The only changes observed in the 2 low dosage groups were increased liver weights. Hepatic necrosis and lipid accumulation were observed in the groups in which porphyria occurred, but no increase in liver weight occurred (liver weights were not given relative to body weight) nor any evidence of hemosiderosis. The authors concluded that TCDD was the most potent porphyrogen known.

370. Goldstein, N. P., Jones, P. H., and Brown, J. R. (1959) Peripheral neuropathy after exposure to an ester of dichlorophenoxyacetic acid. JAMA 171(10):1306-1309.

Three case reports are presented of peripheral neuropathy which developed in 2 men and 1 woman after exposure to 2,4-D. The patients ranged in age from 50 to 65 years. Exposure occurred while spraying weeds with 2,4-D herbicide; their legs or arms had become wet with the liquid herbicide. Health problems in all three patients developed within 2 days of exposure. Symptoms included nausea, numbness, and weakness of fingers and toes, muscle aches and fatigue, and fasciculation of limb muscles. The fatigue and muscle pain continued to worsen until all 3 patients were no longer able to walk normally. Neurologic examination revealed total or partial lack of tendon reflexes, absence of joint sensation, and, in one case, fasciculation of arm and leg muscles. Electromyographic tests on all 3 patients showed denervation in several extremity muscles. Conduction velocities in the ulnar nerve were measured and found to be slower than normal. Other physiologic function tests were performed, and all results were normal. These clinical findings were compared to results of animal experiments that found similar peripheral nerve dysfunction following exposure to 2,4-D. The authors concluded that the peripheral neuropathy observed in these patients was a direct result of cutaneous exposure to 2,4-D.

371. Grant, W. F. (1979) The genotoxic effects of 2,4,5-T. Muta. Res. 65:83-119.

[Review article.]

372. Grant, W. F. (1973) Cytological effects of environmental mutagens - pesticides. Mutat. Res. 21:221-222.

[Abstract, only.]

373. Grant, W. F. (no date) Cytological effects of environmental mutagens - pesticides. Abstract. Proc. Am. Environ. Mut. Soc., 1st International Conference: pp. 221-222.

[Abstract, only.]

374. Green, S., and Moreland, F. S. (1975) Cytogenetic evaluation of several dioxins in the rat. Toxicol. Appl. Pharmacol. 33:161.

[Abstract, only.]

375. Green, S., Moreland, F., and Sheu, C. (1977) Cytogenetic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on rat bone marrow cells. FDA By-Lines 6:242-294.

The authors studied the in vivo cytogenetic effects of TCDD on rat bone marrow cells. Male and female Osborne-Mendel rats (8 per group) received doses of 0.25, 0.5, 1, 2, or 4 ug TCDD/kg body weight by gavage two times per week for 13 weeks. No control group was included in the experiment. Bone marrow cells were obtained from the test animals and 50 metaphases per animal were examined for chromosome aberrations. Mitotic inhibition was scored in 1000 cells per animal. Numbers of chromosome aberrations at 1, 2, or 4 ug/kg were compared with numbers of aberrations at 0.25 ug/kg in a t-test. Significant increases in chromosome aberrations were observed at 2 and 4 ug/kg in males and at 4 ug/kg in females. However, the values of 2.36% chromosome aberrations (2 ug/kg, males) and 2.75% (4 ug/kg, females) are within the usual control range of 2-3%. Only the value of 4.65% for the group of males treated with 4 ug/kg can be regarded as biologically significant. The authors concluded that TCDD does produce chromosome aberrations in rats, that this is a weak effect.

376. Greenlee, W. F., and Poland, A. (1979) Nuclear uptake of 2,3,7,8 tetrachlorodibenzo-p-dioxin in C57BL/6J and DBA/2J mice. J. Bio. Chem. 254(19):9814-9821.

Uptake of TCDD by hepatic nuclei of methylcholanthrene (MC)-responsive and non-responsive mice in vivo and in vitro is described. C57BL/6J and DBA/25 mice were administered 10 mmol/kg of [³H]-TCDD and were killed 0.5h to 2 weeks later. Radioactivity associated with the nuclear and cytosol fractions of hepatic cells and microsomal 7-ethoxycoumarin O-deethylase activity were determined. The specific binding capacity of the cytosol fraction for [³H]-TCDD was determined in vitro as the amount of radioactivity that could be displaced from the receptor by adding excess cold TCDD (or active congener). In vitro nuclear binding was studied by incubating hepatic nuclei with cytosol that had been pretreated with [³H]-TCDD. Specific nuclear binding capacity was estimated as the difference between total nuclear uptake of radioactivity and nonspecifically bound nuclear radioactivity. Non-specific radioactivity was measured as the amount of radioactivity that was taken up by nuclei exposed to cytosol whose [³H]-TCDD content was displaced from the receptor by an excess of cold TCDD congener. From 1-8 hours after [³H]-TCDD was administered to C57BL mice, the specific cytosol binding capacity was half of its initial value; by 16 hours recovery to control values occurred. Nuclear uptake peaked at 8 hours and at 2 weeks remained elevated by 25%. Microsomal enzyme activity was elevated between 1 and 14 days after TCDD was administered. In vivo uptake of [³H]-TCDD in C57BL hepatic nuclei was 5 times the uptake by DBA nuclei and was inhibited almost completely by administering an excess nonradioactive active TCDD congeners, but not by inactive analogs in vitro. Nuclear uptake from [³H]-TCDD bound cytosol did not occur at 0°C and at 25°C was proportional to the amount of [³H]-TCDD bound to cytosol. In vitro uptake by C57BL nuclei was 14

times greater when [³H]-TCDD-bound C57BL cytosol rather than DBA cytosol was the [³H]-TCDD source and was reduced by 80-90% by inactivating charged C57BL cytosol with heat or with hyppsin. The ability of structural analogs of TCDD to compete for nuclear uptake in vitro correlated highly with the ability of the same compounds to compete with specific cytoplasmic binding. The authors concluded that nuclear binding of TCDD is an essential event in the process of mono-oxygenase enzyme induction and nuclear TCDD uptake and binding are mediated by the cytosol receptor.

377. Greer, G. S. (1977) The effects of 2,4,5- trichlorophenoxyacetic acid on Swiss-Webster mice. Proc. Ark. Acad. Sci. 31:46.

[Not available.]

378. Greig, J. B. (1979) The toxicology of 2,3,7,8-tetrachlorodibenzo-p-dioxin and its structural analogues. Ann. Occup. Hyg. 22:411-420.

[Review article.]

379. Greig, J. B. (1972) Effect of 2,3,7,8-tetrachlorodibenzo-1,4-dioxin on drug metabolism in the rat. Biochem. Pharmacol. 21:3196-3198.

The effect of TCDD administration to the rat on zoxazolamine paralysis time and hexobarbital sleeping time are described. A dose of 200 ug/kg TCDD in arachis oil was administered orally to male Porton rats. The duration of paralysis induced by 100 mg/kg zoxazolamine hydrochloride intraperitoneally 1-3 days after TCDD was determined. Male and female rats were administered 200 ug/kg TCDD in dimethyl sulfoxide and after 1-3 days sleeping times induced by 150 mg/kg hexobarbital in males and 75 mg/kg in females were measured. Zoxazolamine paralysis time was reduced about 54% and 75%, 1 and 3 days after TCDD treatment, respectively. Hexobarbital sleeping times were significantly extended 1 day after TCDD treatment and by 3 days were doubled in treated rats of both sexes. The increase in hexobarbital sleeping times occurred in starved rats treated with TCDD and was not evident in rats 89 days after TCDD treatment. The authors concluded that TCDD caused an increase in zoxazolamine metabolism and decrease in hexobarbital metabolism and were unable to determine whether these effects were related to dioxin toxicity.

380. Greig, J. B., and De Matteis, F. (1973) Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on drug metabolism and hepatic microsomes of rats and mice. Environ. Health Persp. 5:211-219.

The effects of TCDD on hexobarbital and zoxazolamine metabolism and on cytochrome P-450 levels and difference spectra are described for the rat and mouse. Portland rats and C57BL/6 and DBA/Z mice were

administered 200 ug/kg TCDD in arachis oil, orally and hexobarbital-induced sleeping times were measured at intervals between 12 hours and 2 weeks after TCDD was administered. Plasma barbital levels at waking were measured in female rats. Microsomes from livers of rats taken 3 days after TCDD treatment were assayed for zoxazolamine and hexobarbital metabolism and the cytochrome P-450 protein levels and difference spectra were established. Within 1 day of TCDD treatment, barbital sleep time in rats was increased 46% over times for vehicle controls and by 2 weeks this increase was 870% over controls. In C57BL mice 20 days after TCDD treatment, barbital sleep times were 300% of controls and zoxazolamine paralysis time was 15% of controls. Concomitant changes in barbital and zoxazolamine metabolism were demonstrated in vitro. The effect on sleeping time, but not the effect on paralysis time was blocked by administering ethionine to rats prior to barbital or zoxazolamine. Waking barbital levels were the same in rats 3 days after TCDD treatment and in controls. Liver weights and microsomal cytochrome P-450 levels increased in TCDD treated rats, while microsomal protein levels (per gram of liver) were unchanged. The pH dependence of the pyridine difference spectra was the same for microsomes from methylcholanthrene- and TCDD-treated rats but not for controls. TCDD-treated microsomes showed a type II difference spectra in the presence of aniline and of hexobarbital. The authors concluded that TCDD produced changes in cytochrome P-450 that resembled the effects of methylcholanthrene and the TCDD effects on hexobarbital and zoxazolamine metabolism resulted from separate modes of action.

381. Greig, J. B., Jones, G., Butler, W. H., and Barnes, J. M. (1973) Toxic effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Fd. Cosmet. Toxicol. 11(4):585-595.

The acute toxicity of TCDD was studied in rats, guinea pigs, and chickens. TCDD was synthesized and administered by oral intubation in DMSO or arachis oil to White Leghorn chickens, Porton rats, and Porton guinea pigs, while control animals received only the vehicle. Mortality, growth rate, food consumption, hematology, liver weight and histology of major organs were evaluated. A single dose of 25-50 ug/kg of dioxin was lethal to chickens causing death 12-27 days later. (The number of chickens tested was not reported.) The treated animals showed diminished weight gain, labored breathing, and serous fluid in the pericardial sac, characteristic of chicken edema syndrome. Three groups of six guinea pigs received 2, 4, or 10 ug/kg TCDD orally. One animal in each of the two highest dosage groups died within 24 days and others (number not stated) in poor condition were killed. The treated guinea pigs showed a 15-30% decrease in body weight and although the gastrointestinal tract was distended with gas and contained little or no food, no other visceral abnormalities were observed. Rats given 200 ug dioxin/kg in DMSO also showed a loss in body weight and decreased food intake and weanling female rats given 25-200 ug/kg TCDD had lower or zero growth rate compared to vehicle control. Single doses from 30 to 500 ug/kg of dioxin were administered to groups of six rats. The LD₅₀ was not estimated. The mean time to death was 40.4 days in this experiment. No consistent changes were noted in treated rats that

died. Common findings included gross hemorrhage, pulmonary changes involving hemorrhages, edema or infection, jaundice, and bile duct enlargement. Toxic effects, weight loss, and death did not occur earlier in rats administered a 10 fold higher dose of TCDD orally (in arachis oil). Elevations in red cell count, hematocrit, hemoglobin content and leucocyte counts were observed in rats given 200 ug/kg dioxin. No plasma assays related to liver function were altered 1-3 days after dioxin treatment, while ratios of liver weight to body weights and water content of the liver were increased and bilirubin levels rose after 3 weeks. Hepatic lesions were observed 3 weeks after treatment and included the formation of multinucleate parenchymal cells. The degree of severity of hepatic lesions was the same after all doses of TCDD. Acute inflammatory lesions in the lung and congestion of the gastrointestinal mucosa were observed. The authors concluded that TCDD elicited its toxicity by interfering with the capacity of the liver cells to maintain their correct organization.

382. Greig, J. B., Taylor, D. M., and Jones, J. D. (1974) Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the stimulated DNA synthesis in the liver and kidney of the rat. Chem. Biol. Interactions 8:31-39.

The effect of TCDD on DNA synthesis in the kidney after treatment with folic acid or lead in vivo or in the liver after partial hepatectomy were evaluated in the rat. Porton strain rats were administered 10 or 200 ug/kg TCDD in dimethylsulphoxide (DMSO) in arachis oil by oral intubation and August-strain rats were administered 10 ug/kg TCDD in DMSO intraperitoneally. Lead acetate trihydrate (40 mg/kg) was administered in water, intraperitoneally. Folic acid (250 mg/kg) was administered intraperitoneally and for some rats was preceded by 24 and 48 hr. with doses of 20 mg/kg of 3-methylcholanthrene i.p. or by 24 hr. with 1 ml/kg carbon disulphide orally. [³H]-thymidine was administered 1 hour before the animals were killed. DNA was extracted from the kidney and liver and counted for incorporated radioactivity. Pretreatment with 10 or 200 ug/kg TCDD 1 or 3 days prior to performing a 70% hepatectomy did not alter DNA synthesis in the liver 24 hours after the operation, compared to controls not treated with TCDD. DNA synthesis was decreased in folate-stimulated kidneys by pretreatment 0-9 days earlier with either dose of TCDD. The effect disappeared in an experiment in which folic acid-treated rats were pair-fed with TCDD-treated rats. Pretreatment of 200 ug/kg TCDD 1 day prior to lead treatment caused a depression in DNA synthesis to the same extent (50%) as occurred in folate-treated kidneys. Peak ³H incorporation into DNA occurred 34 hr. after lead was administered for controls and TCDD-treated rats. Unlike TCDD, methylcholanthrene or carbon disulfide pretreatment did not produce a depression in DNA synthesis. The authors concluded that dioxin did not interact directly with DNA or block protein synthesis to exert its inhibitory effect but suggested that mediation by a hepatic humoral factor was possible.

383. Greim, H., and Loprieno, N. (1978) No permanent injuries at Seveso? Umschau 2:53.

The health effects observed in people exposed to TCDD released from an industrial accident in Seveso, Italy in July, 1976 and distributed as a cloud to the surrounding countryside is summarized. Half of the 734 people who lived in the zone (A) of highest contamination (surface soil concentration, 10 mg/kg TCDD) had ingested contaminated animal and vegetable food and 50% worked in agricultural occupations in zone A during the 15-20 day period after the accident and prior to evacuation. The 5,000 residents of zone B (0.01 mg/kg of TCDD in soil) were not evacuated. In the summer, chloracne was diagnosed in 607 of 30,000 children. In the fall, chloracne was unchanged in 278 of 467 children re-examined, had regressed in 66 cases, and was not classified for the remainder. The authors expected chloracne to heal within several months and that conditions other than chloracne or repeated exposure to toxic substances occurred. One hundred eighteen people were treated for ocular irritation and ocular disorders (of unspecified nature) occurred in four cases. No adverse effects to the liver, kidney, gastrointestinal tract, peripheral nervous system or to carbohydrate or porphyrin metabolism were detected in clinical blood analyses performed on all inhabitants of zones A and B and all workers at the factory where the explosion occurred. No chromosomal aberrations were detected in peripheral blood tests on 90 of the factory workers and 180 zone A residents. No malformations were observed in fetuses from 30 therapeutic abortions and 4 spontaneous abortions. Animal teratology was reported, in which births of 1 normal calf and 1 malformed calf, 8 stillbirths and 5 premature births occurred among 12 pregnant cows that fed on contaminated grass through the end of August. The authors concluded that severe embryotoxic effects should be taken into consideration in light of the animal effects, despite lack of observed effects in exposed people.

384. Grolleau, G., deLavour, E., and Siou, G. (1974) Effects of 2,4-D on the reproduction of quail and partridge after application of the product by spraying on eggs. Ann. Zool.-Ecol. Anim. 6(2):313-331.

[Not available.]

385. Gross, M. L. (1980) Direct testimony before the U.S. Environmental Protection Agency, FIFRA Docket Nos. 415 et al.

[Testimony.]

386. Grover, R. (1976) Relative volatilities of ester and amine forms of 2,4-D. Weed Sci. 24:26-28.

[Background material.]

387. Grover, R., Maybank, J., and Yoshida, K. (1972) Droplet and vapor drift from butyl ester and dimethylamine salt of 2,4-D. *20*(4):320-324.

[Background material.]

388. Grunow, W., and Boehme, C. (1974) Metabolism of 2,4,5-T and 2,4-D in rats and mice. Arch. Toxicol. *32*(3):217-225.

[Foreign language.]

389. Grunow, W., Boehme, C., and Budczies, B. (1971a) Renal excretion of 2,4,5-T in the rat. Health Aspects Pestic. *5*:130.

[Abstract, only.]

390. Grunow, W., Bohme, C., and Budczies, B. (1971b) Renal Ausscheidung von 2,4,5-T bei Ratten. Fd. Cosmet. Toxicol. *9*:667-670.

The rate of urinary excretion and metabolites excreted after a single dose of 2,4,5-T was administered to rats is described. Male Wistar rats were administered 50 mg/kg 2,4,5-T in peanut oil by gastric intubation. Urine was collected over the subsequent 7 days and was analyzed for unchanged 2,4,5-T by gas chromatography, for bound 2,4,5-T by acid-catalyzed hydrolyzing urine samples prior to gas chromatographic analysis and for N-(2,4,5-trichlorophenoxyacetyl)-glycine by thin-layer chromatography, following partial purification by ion exchange chromatography. Maximum rates of 2,4,5-T excretion occurred on day 2 in 5 of 7 rats. From 30-58% of the dose was excreted as free 2,4,5-T, 12-31% as bound 2,4,5-T and a total of 45-70% of the dose was recovered in the urine in 7 days. The glycin bound derivative was positively identified in the urine samples. The authors concluded that urinary excretion of 2,4,5-T was slower than the values published elsewhere for rats of 2,4-D excretion.

391. Guarino, A. M., James, M. O., and Bend, J. R. (1977) Fate and distribution of the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in the dogfish shark. Xenobiotica *7*(10):623-631.

The tissue distribution, biotransformation, and kinetics of excretion of 2,4-D and 2,4,5-T were studied in the dogfish shark, Squalus acanthias. Female dogfish sharks were administered [¹⁴C]-2,4-D or [¹⁴C]-2,4,5-T intravenously. Urine, bile, and blood were collected and analyzed for radioactivity, along with tissues. Urinary metabolites were separated and identified by thin-layer chromatography and confirmed by paper chromatography and amino acid analysis. Urine contained 53% of the administered dose of 2,4-D in 4 hr., 68% in 1 day, and 90% in 6 days. Two percent of the dose was excreted in bile in 6 days. From 94 to 98% of the radioactivity in urine and bile was

conjugated to taurine, 2-4% was present as 2,4-D acid and traces, 4% as two unidentified metabolites. The half-life of plasma clearance of 2,4-D was 44 min. Tissue levels for the kidney and liver were 2-40 times the plasma, and were lower than plasma levels for muscle, brain, and cerebrospinal fluid. Urine contained 24% of the administered dose in 4 hr., 57% in 1 day, and 70% by 4 days. Biliary excretion accounted for 7% of the dose in 4 days. From 91-98% of the biliary and urinary radioactivity was in taurine conjugates, 2-6% as 2,4,5-T, and traces to 5% in two unidentified metabolites. The half-life of plasma clearance was 41 min. and tissue distribution showed the same pattern of distribution as described for 2,4-D. Plasma binding of both compounds was about 57% for concentrations up to 50 ug/ml herbicide. The authors concluded that the pharmacokinetics of 2,4-D and 2,4,5-T in the dogfish shark resembled those reported for some mammals, although only the dogfish shark excreted the compounds as conjugates.

392. Guenther, T. M., Fysh, J. M., and Nebert, D. W. (1979) 2,3,7,8-tetrachlorodibenzo-p-dioxin covalent binding of reactive metabolic intermediates, principally to protein in vitro. Pharmacol. 19(1):12-22.

The binding of TCDD to protein and to DNA in vitro is described. 2.0 uM [³H]-TCDD (58 Ci/mmol) in p-dioxane was added to 20 mg of deproteinized salmon sperm DNA and 5 mg of microsomal protein from 3-methylcholanthrene-pretreated C57BL/6N or DBA/2N mice. After incubation at 37°C for 45 minutes, DNA was isolated from the mixture, treated with degradative enzymes and separated on a Sephadex LH20 column as TCDD-metabolite-nucleoside complexes. Some mixtures were treated with 500 uM alpha-naphthoflavone or the isolated DNA was additionally digested with proteinase K. Protein was isolated from some mixtures by methanol precipitation and analyzed for radioactivity. More radioactivity bound to protein or DNA in the presence of microsomes from C57BL than DBA mice. Binding to the DNA fraction was decreased by 80% in proteinase K digests and was abolished when NADPH was not added to the incubation medium. NADPH was also required for protein binding and binding to both DNA and protein were decreased by more than 60% in the presence of naphthoflavone. The ratio of TCDD metabolite protein binding: DNA binding ratio was 120-440 and the rate of TCDD metabolism was calculated from its covalent binding to proteins at 100-250 f mol/min/mg microsomal protein. Two metabolites were identified by chromatography. The authors concluded that TCDD was metabolized to radioactive intermediates that bound covalently to cellular macromolecules. The authors proposed that this metabolite was an arene oxide produced by cytochrome P-450 and bound to the cytochrome protein so rapidly that a significant amount of further metabolism was precluded. The relative reactivity of TCDD in vitro to protein from the two mouse species corresponded well to both its relative teratogenicity and the levels of P-450 in the two species. The authors further suggested that TCDD toxicity in different species is related to the P-450 level for each species.

393. Guenther, T. M., and Nebert, D. W. (1978) Evidence in rat and mouse liver for temporal control of two forms of cytochrome P-450 inducible 2,3,7,8-tetrachlorodibenzo-p-dioxin. Europ. J. Biochem. 91:449-456.

TCDD induction of 2 hepatic enzymes in maternal and perinatal liver were correlated with the simultaneous appearance of 2 specific proteins. Sprague-Dawley rats and C57BL/6 mice were administered 20 ug/kg TCDD in para-dioxane, intraperitoneally 40 hours before they were killed. (In some cases mothers were treated 1 day before birth and livers of their 1-day-old offspring were examined). Livers were removed and microsomes were isolated and assayed for cytochrome P-450 spectrophotometrically by 2 methods, and for aryl hydrocarbon hydroxylase (AHH) and acetanilide 4-hydroxylase (A4-H) activities. Microsomal proteins were isolated by polyacrylamide gel electrophoresis. Between days 18 and 22 of gestation, AHH activity (per mg microsomal protein) doubled and A4-H activity increased 4 fold in TCDD-treated rats. Based on mg cytochrome P-450 protein, AHH activity decreased between day 20 and 22 and A4-H activity increased at this time. Concomitant with an increase in AHH between days 18 and 20 were increases in cytochrome P₁-450 and in a protein-staining band of 54,000 molecular weight, identified by electrophoresis. The increase in A4-H activity between day 20 and 22 was accompanied by increases in a cytochrome P-450 and a 56,000 molecular weight protein. Compared to activities of non-TCDD-treated rats of the same age, AHH was induced 200 fold and 35 fold in 18 day fetuses and mothers, respectively and A4-H was induced 15 fold and 4 fold in 22 day fetuses and mothers, respectively. Analogous changes occurred in mice studied with the same protocol. The authors concluded that TCDD induced 2 structurally distinct forms of cytochrome P-450 which correspond to 2 separate enzyme activities which are expressed at different points in fetal development (separated by 2 days).

394. Guenzi, W. D., and Beard, W. E. (1976) Picloram degradation in soils as influenced by soil water content and temperature. J. Environ. Qual. 5(2):189-192.

The authors studied the degradation of picloram in five soils: Glendale (sandy clay loam), Panoche (clay loam), Palouse (silty clay loam), Molakai (clay), and Ephrata (sandy loam). Soil samples (20g each) were air-dried and passed through a 2-mm sieve. Two replicate flasks received 0.2 mg, carboxyl-labeled ¹⁴C-picloram for each soil sample. Evolution of ¹⁴CO₂ was measured as an indication of microbial activity. Picloram degradation in Glendale, Panoche, and Palouse soils was very low at 5°C, increased gradually at 10°C, increased rapidly at 30°C, and decreased at 50°C. In Molokai and Ephrata soils, degradation of picloram increased to 50°C. Little degradation of picloram occurred in sterile soils indicating that microbial activity was probably responsible for picloram degradation. When soil samples were exposed to alternating wet and dry cycles designed to simulate soil conditions, degradation decreased with successive "dry" cycles. No relationship was found between soil factors (pH, surface area, organic matter, (texture) and picloram degradation. Picloram degradation also

decreased as soil water content decreased. The authors concluded that picloram degradation seemed to be primarily microbial.

395. Guiney, P. D., Yang, K. H., Seymour, J. L., and Peterson, R. E. (1978) Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the distribution and biliary excretion of polychlorinated biphenyls in rats. Toxicol. Appl. Pharmacol. 45:403-414.

The effects of TCDD on polychlorinated biphenyl distribution and biliary excretion are described. Male Holtzman rats were administered a single oral dose of 10 or 25 ug/kg TCDD in acetone-corn oil (1:19). Ten days later, 6 mg/kg of 2,5,2',5'-[³H]-tetrachlorobiphenyl (4-CB) or 2,4,5,2',4',5'-[³H]-hexachlorobiphenyl (6-CB) was administered intravenously to TCDD-treated rats and blood, and bile samples were collected and liver biopsies were removed periodically during the next 4 hours. Fat, muscle and skin tissue were removed at the end of 4 hours and all samples were analyzed for radioactivity. Some experiments were terminated 1 hour after the PCB injection and the liver was analyzed for radioactive metabolites by hexane extraction procedure. In other experiments bile collected 2 hours after the PCB injection was extracted for polar radioactive PCB metabolites; these metabolites were administered intravenously and biliary excretion over 4 hours was followed by the methods used for 4-CB and 6-CB. TCDD treatment resulted in dose-related increases in liver weight and increased liver retention of PCB radioactivities, decreases in PCB biliary excretion and bile flow, and no effect on PCB plasma clearance and body weight. The ratio of hexane-extracted PCB and metabolites to the unextracted polar metabolites, for 4-CB was unaltered and for 6-CB was decreased by TCDD. Radioactivity in the skin was lower and in the liver was higher in TCDD-treated rats than in controls. The rate of biliary excretion after 4-CB metabolites were administered was the same as the rate when 4-CB was administered. Hepatic retention of hexane-extractable PCBs and of polar metabolites were increased by TCDD treatment. The authors concluded that TCDD inhibited biliary PCB excretion, the major route of PCB elimination; this effect could increase the half-life of PCB in the body and could increase apparent toxicity by delaying elimination of toxic metabolites.

396. Gunby, P. (1979a) Dispute over some herbicides rages in wake of Agent Orange. JAMA 241(14):1443-1444.

[Editorial.]

397. Gunby, P. (1979b) Plenty of fuel for Agent Orange dispute. JAMA 242(7):593-597.

[Editorial.]

398. Gupta, B. N., Vos, J. G., Moore, J. A., Zinkl, J. G., and Bullock, B. C. (1973) Pathological effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in laboratory animals. Environ. Health Persp. 5:125-140.

Pathological changes resulting from acute, daily and weekly exposures of rats, guinea pigs, and mice were compared. CD rats were administered a single dose of 5-100 ug/kg TCDD or 3-31 daily doses of 0.1-10.0 ug/kg or 6 weekly doses of 0.2-5.0 ug/kg TCDD. Hartley guinea pigs were administered 1 dose of 3 ug/kg or 8 weekly doses of 0.008-1 ug/kg TCDD and CD-1 mice were administered 1 dose of 1-50 ug/kg TCDD. All doses were administered in acetone-corn oil by gastric intubation. Necropsies, performed on all animals, included histological examination of most tissues and liver, kidney, bone, and whole body examinations under ultraviolet light to detect porphyrin accumulation. A single dose of 100 ug/kg TCDD was lethal to 43% of rats, with death occurring within 18-21 days--50 ug/kg caused 7% mortality. Over 90% of rats given 16-31 daily doses of 10 ug/kg and guinea pigs given 1 dose or 1.0 ug/kg weekly for 4-5 weeks died, all within 15-32 days. No mortality resulted in groups given lower doses under these regimens or in mice. The thymus of all 3 species were atrophied. Liver lesions were severe only in the rat. These lesions included degenerative changes and the appearance of multinucleated giant hepatocytes. Hemorrhages were seen in various organs of rats and guinea pigs that received lethal doses of TCDD. Other lesions in the rat included renal and thyroid degeneration, spleen and ovarian follicle megakaryocytes, and lymphoid depletion. In guinea pigs, hyperplasia of the urinary bladder mucosa and adrenal atrophy occurred. Porphyria did not occur in rats or guinea pigs. The authors concluded that the hepatotoxic and porphyrogenic actions of TCDD were varied in different species.

399. Guseva, Y. N. (1956) On the pharmacology of 2,4-dichlorophenoxyacetic acid. Farmakologiya i toksikologiya. 19(4):41-44.

The acute and subacute toxicities of 2,4-D were examined in cats, rabbits, rats, mice, and frogs. 2,4-D sodium salt in aqueous solution was administered subcutaneously as a single dose to groups of 10 mice or as 22 daily doses of 5 mg/kg to groups of 6 mice. The LD₅₀ was 220 mg/kg and no clinical signs of toxicity were observed for 2 months after subacute treatment. Rabbits were administered doses of up to 5 ml of a 5% solution of 2,4-D per kg body weight intravenously. The only changes observed were temporary muscle stimulation, slower respiration, and dilation of the pupils. Urethane-anesthetized cats and rabbits were administered 20-30 mg/kg or 250-300 mg/kg 2,4-D and blood pressure and the rate of respiration were recorded. Both parameters were decreased transiently, and atropinization occurred. The functioning of the heat in situ of immobilized frogs was inhibited by a minimum of 2.5-5.0 mg of 2,4-D per frog and the effect was not blocked by 0.5 mg of atropine. Vasoconstriction of peripheral vessels of rabbit ears at concentrations of at least 0.4% 2,4-D applied dermally. At concentrations above 0.5 mg/ml, 2,4-D applied directly to isolated rabbit intestine produced a myotropic effect. No other details of methods were provided for these frog and rabbit experiments. The remaining

studies were mentioned without details of methods provided. 2,4-D at doses of 0.1-10 mg/kg did not alter efferent function in mice, determined by actography and at doses of 1-30 mg/kg did not alter frog sciatic nerve fatigue curve. At doses of 10-50 mg/kg, swimming time in mice was not changed. The latent time between administration of 10 mg/kg of strychnine to mice (10 per group) and the onset of convulsions was prolonged from 9 minutes in control group to 12 and 14 minutes in groups that received 50 and 100 mg/kg 2,4-D respectively. Strychnine was lethal to all mice. Sensitivity of rats to pain from electric current was unaltered by subcutaneous doses of 10-100 mg/kg 2,4-D. Blood catalase activity was increased and serum cholinesterase was decreased by 25-250 mg% 2,4-D in vitro. The authors concluded that the toxicity of 2,4-D was comparatively low.

400. Gutenmann, W. H., and Lisk, D. J. (1970) Metabolism and excretion of Bromacil in milk of dairy cows. J. Agric. Food Chem. 18(1):128-129.

Biotransformation and excretion of bromacil by the cow was studied. Diets that contained 5 and 30 ppm bromacil were administered to Holstein cows (one cow per dosage) for 4 days. Milk, urine, and feces was collected from the cow that was fed the 5 ppm diet and milk was collected from the second cow for 10 days after the experimental diets were introduced. Fresh rumen fluid and the 10,000 G supernatant fraction of homogenized beef liver were each incubated with bromacil. Bromacil and metabolites were analyzed in the biological fluids and incubation mixtures by gas chromatography. A total of 113.5 mg and 681 mg of bromacil were recovered in the milk of the cows given the 5 ppm and 30 ppm diets, respectively. No bromacil was detected in milk collected more than 1 day after the experimental diet was discontinued or in any urine or fecal samples. No bromacil metabolites were detected in incubates with rumen fluid for 7 hours or liver homogenate for 1 hour. The authors concluded that bromacil was excreted in the milk within 1 day of intake and was not metabolized. The proportion of the dose that was recovered in milk was not calculated.

401. Guthrie, F. E., Shah, P. V., and Moreland, D. E. (1974) Effects of pesticides on active transport of glucose through the isolated intestine of the mouse. J. Agr. Ed. Chem. 22(4):713-715.

The effects of 2,4,5-T, diuron, and about 30 other pesticides on intestinal glucose transport were studied in vitro. Midgut sections from 6 week old male mice were filled with the pesticide in a buffer containing [¹⁴C]-glucose as well as 0.3% acetone and 0.03% tween 80. After incubation at 30°C for 80 min., the radioactivity in the serosal fluid, luminal fluid, and gut tissue was analyzed. In the presence of 0.1 mM of 2,4,5-T or diuron, which were both reported elsewhere to uncouple oxidative phosphorylation, the percentage of radioactivity associated with the gut was significantly higher than for the glucose control, while the amounts in the luminal fluid were not significantly altered from controls and only diuron caused a significant decrease in radioactivity in the serosal fluid. The authors concluded that in general,

pesticides that inhibited oxidative phosphorylation also usually inhibited glucose transport. The significance of these preliminary findings in vitro with a single dose of each compound in vivo toxicity has yet to be established.

402. Gyrd-Hansen, N., and Dalgaard-Mikkelsen, S. (1974), The effect of phenoxy herbicides on the hatchability of eggs and the viability of the chicks. Acta Pharmacol. Toxicol. 35(4):300-308.

The effects of 2,4-D (dimethylamine salt), 2,4,5-T (dimethylamine salt), mechlorprop, and dichlorprop on chicken egg hatchability, teratogenicity, and chick survival were studied. The effects of pure compounds of over 99% purity were compared with those of commercial products of 93% purity (except for 2,4,5-T, with 58% purity). Two methods of administration were used. Aqueous solutions of compounds were injected into the egg yolks of unincubated eggs (35 per dose). Other eggs were immersed in 1% or 5% aqueous solutions of the test compound for 10 seconds at 10 or 10°C. For each treatment group, 6 unhatched, fully developed chicks were stained with alizarin red and examined for skeletal malformations. The hatched chicks were weighed weekly and on the fourth week were killed and observed macroscopically. For the injection study in general, all herbicides showed the same pattern of effects; at least 1 mg per egg of pure compound produced a minimal effect of hatchability and at least 2 mg/egg was required to decrease survival of hatched chicks through 4 weeks of age. The weights of surviving chicks in treated and control groups were similar at 4 weeks. The lowest concentration used in the immersion studies failed to produce any adverse effects in hatchability or chick survival and the higher dose of each compound had minimal effects. The most common anomalies of the embryos treated by injection of the 4 compounds were gastroschisis and growth retardation. The embryos of immersed eggs showed few anomalies. No skeletal malformations were observed. The only malformation in hatched chicks were bended toes, which occurred more frequently in treated than control groups. No major differences in survival or hatchability were seen in groups treated with commercial products compared with those treated with pure compounds of 2,4-D or 2,4,5-T. The commercial product of mechlorprop and the pure chemical dichlorprop each were more toxic than the other preparation of the same product. Two other compounds were tested, 4-chloro-2-methylphenoxy-acetic acid and 2,7-dichloro-dibenzo-p-dioxin. The former showed similar toxicity to the other phenoxy compounds, whereas the dioxin was about 100 times more potent. The authors estimated that a 1% solution of herbicide spray would expose a 60 gm egg to 2 mg of herbicide if the entire surface were exposed, but from the current results, no deleterious effects would be expected from this exposure. Furthermore the embryotoxic response of chicken embryos by phenoxy herbicides corresponds to the effects observed by others in rodents.

403. Haag, D., Goerttler, K., and Preiss, D. (1975) The influence of non-cytotoxic concentrations of the herbicide 2,4-dichlorophenoxyacetic acid on the DNA synthesis in cultured vertebrate cells. Arch. Toxicol. 33:91-102.

The effects of 2,4-D on DNA synthesis by embryonic muscle cells are described. Primary cultures of embryonic chicken skeletal muscle cells were maintained for 8 days, and then cultured in medium with 2,4-D sodium salt for 22 or 44 hr. Controls were cultured with sodium chloride for up to 44 hr. The cells were then fixed, dried, and stained for fluorescence microscopy. DNA fluorescence was measured with a photometer and then used to derive curves of DNA synthesis. After 44 hr., but not 22 hr., cultures treated with 2.5-50 mM 2,4-D showed a lack of polar orientation and increased nuclear to cytoplasmic area ratio, compared to controls. After treatment medium was replaced with control medium, these cells resumed a normal morphology. Treated cells contained nucleoli that did not fluoresce and heteropycnotic nuclear chromatin. At 0.5 mM, and above, 2,4-D produced significantly more nuclear necrosis by 44 hr. than observed in controls. The number of DNA synthesizing cells increased after 22 and 44 hrs. of treatment with 2.5mM, 2,4-D, but not in controls. In treated cultures, the number of cells in G₂ phase decreased. The authors concluded that 2,4-D exerted its effects on the chromosomal level.

404. Hall, S. M. (1972) Effects on pregnant rats and their progeny of adequate low protein diets containing 2,4,5-trichlorophenoxyacetic acid (2,4,5-T or p,p'-DDT). Fed. Proc., Fed. Am. Soc. Exp. Biol. 31:726.

[Abstract, only.]

405. Halprin, K. M. (1980) Chloracne recognition and its significance. Presented at the 2d Continuing Education Conference on Herbicide Orange, Washington, DC, May 28-30.

A description of the pathogenesis and clinical symptoms of acneiform lesions is provided. The author also commented on chloracne related to the use of "Agent Orange" in Vietnam. The only feature that distinguishes chloracne from acne is the development of the characteristic acneiform eruption within 1-3 months after exposure to certain halogenated compounds. Chloracne that developed after industrial exposure to these compounds cleared within 1-5 years. The authors indicated that the type of acne condition that would have been likely to develop from herbicide exposure in Vietnam should have been obvious to a physician, but was not observed by a team of physicians who examined troops in the field in the last 3 years of the war to assess skin problems. This condition was likewise not observed in Air Force RANCHHAND personnel who were heavily exposed to "Agent Orange" and were examined annually. The authors concluded that acneiform eruptions from "Agent Orange" use in Vietnam would have appeared within 1-3 months of exposure, and in 1980 (the time of the report), would remain in 10 percent of the patients. In 90 percent of the patients only scars,

which are indistinguishable from those of other types of acne, would remain.

406. Hamada, N, and Peterson, R. E. (1978) Effect of microsomal enzyme inducers on 2,3,7,8-tetrachlorodibenzo-p-dioxin induced depression in the biliary excretion of ouabain in rats. Drug Metal. Dispos. 6(4):456-464.

The effects of various drugs on TCDD-inhibition of biliary excretion of ouabain are described. Male Holtzman rats were administered 10 or 25 ug/kg TCDD or 25 ug/kg [³H]-TCDD in acetone-corn oil (1-19) as a single oral dose. Other drugs were administered in propylene glycol, intraperitoneally on days 6 through 9 or only day 10 following TCDD administration, or 4 days prior to TCDD. These drugs included phenobarbital, PB(50 mg/kg/day); 3-methylcholanthrene, 3-MC (20 mg/kg/day); pregnenolone-16-alpha-carbonitrile, PCN (75 mg/kg/day); and spironolactone, S(75 mg/kg/day). On day 10 (or day 20, following drug administration on day 10), following TCDD administration, [³H]-ouabain was injected intravenously and blood and biliary samples and liver samples were collected and counted for radioactivity. Mortality was determined for rats administered 100 ug/kg TCDD and then 75 mg/kg/day of PCN or S daily for 19 days, starting 1 day after TCDD was given. TCDD treatment caused a decrease in biliary excretion and of blood clearance of ouabain. Neither PB nor 3-MC altered these parameters. PCN treatment on days 6-9 caused both parameters to be elevated, in control and in TCDD-treated rats, while S treatment on days 6-9 reversed the TCDD effect but did not change the control rates. When PCN or S were administered on day 10, no effects on TCDD-inhibited biliary ouabain excretion were observed 6 hours later. PCN, but not S, reversed the TCDD effect on day 20; likewise, PCN, but not S, when administered 4 days prior to TCDD, reversed the TCDD effect. Survival times for rats were 14.5 days for 50% of rats treated with 100 ug/kg TCDD, 14.3 for the TCDD and S-treated group and 10.0 for the TCDD and PCN-treated group. Body weight reductions occurred in all 3 groups. PCN and S treatments before or after TCDD did not influence the concentration of [³H]-TCDD in the liver. The authors concluded that TCDD inhibition of biliary excretion, by PCN and by S, was the only known instance of drug reversal of an effect of TCDD.

407. Handler, P. (1980) Testimony before the Subcommittee on Medical Facilities and Benefits of the Committee on Veterans' Affairs, U. S. House of Representatives, Sept. 16., 1980. 11 pp.

[Testimony.]

408. Hanify, J. A., Metcalf, P., Nobbs, C. L., And Worsley, K. J. (1981) Aerial spraying of 2,4,5-T and human birth malformations: an epidemiological investigation. Science: 212.

Hanify et al (1981) investigated the incidence of birth malformations in relation to regions of New Zealand where spraying of 2,4,5-T had occurred during the same time period. Hospital records were used to identify births in specific areas of New Zealand where spraying had taken place during 1972 to 1976. Areas of low, intermediate, and high averaged annual spray density were determined. Time periods with spraying (1972-1976) versus those without spraying (1959-1965) were identified. Two tests of the data were performed. In the first, a control group of all babies born between 1 January 1960 and 31 August 1966 who were not exposed to 2,4,5-T and a study group of all babies born between 1 September 1972 and 31 August 1977 were identified. Incidence ratios and 90 percent confidence interval were constructed for commoner malformations. Defects of the heart, hypospadias and epispadias, talipe, and "all birth malformations" exhibited incidence ratios significantly different from 1. In the second test, incidence rates of "all birth malformations" were plotted against average annual exposure to putative teratogen with a decay factor of $f = 1.0$. Regions of high, low, and intermediate spray density were distinguished as were the years of spraying and non-spraying. The authors noted that incidence rate appears to be positively associated with exposure both across areas and across years. The authors also conducted an analysis utilizing binary regression to further investigate the correlation between individual malformations and exposure, where a malformation was considered to be a dichotomous variable (1 if malformation present, 0 if malformation absent). Exposure was considered in terms of three variables: (1) year, (2) month of the year, and (3) hospital catchment area. The difference between the average exposure value for malformed babies and the exposure value for all babies, suitably normalized, was used as the test statistic. Correlation between malformations exposure were derived in terms of three variables: year, month of the year, and hospital catchment area. The authors decided that a necessary condition for evidence of a correlation between spray and exposure would be tests with year and hospital catchment area significant at the p is less than .05 level. Given this criteria, the results of the tests conducted by the authors showed (1) no identifiable association for talipes when the decay factor, f , equals 0.25, but not when the decay factor, f , equals 1.00 and (3) no identifiable association for cleft lip with or without cleft palate, for isolated cleft palate, for malformations of the heart as a group, or for malformations of the male genitalia.

409. Hansen, W. H., Quaife, M. L., Habermann, R. T., and Fitzhugh, O. G. (1971) Chronic toxicity of 2,4-dichlorophenoxyacetic acid in rats and dogs. Toxic. Appl. Pharmacol. 20:122-129.

The authors attempted to determine the effects of long term feeding of 2,4-D to dogs and rats. Technical grade 2,4-D was obtained from Dow Chemical Company and contained no detectable TCDD (1 ppm detection

limits) and had a purity of 99.12 %. In the rat feeding study, groups of 25 male and 25 female 3-week old Osbourne-Mendel rats received 0, 5, 125, 625, or 1,250 ppm 2,4-D in their diet for 2 years. No analytical check on the 2,4-D content of the diet was reported by the authors. Storage conditions for the diet formulation also were not reported. Statistical analyses applied to the data were not presented. During the 2-year study, no significant differences in survival rates were observed between control and treated animals. Mean body weights and organ-to-body weight ratios of major organs were not significantly different at the time of termination of the experiment. Hemoglobin, hematocrit, and white blood cell count of both control and treated animals were within the normal range. However, at the end of the study, rats treated with 5, 625, or 1,250 ppm 2,4-D showed a tendency toward macrocytosis and slight polychromasia which was not present in the control animals. Tumor incidence in the rats did not reveal any "target organ" types according to pathological interpretation. Statistical analysis of tumor incidence showed a statistically higher incidence of tumors in male rats fed 1,250 pp 2,4-D and a trend toward increased tumor formation with log dose in females. Pathological interpretation of the data, however, did not show a carcinogenic effect. While trends towards increased tumor incidence in this study show a possible carcinogenic effect, no clear conclusions can be drawn. Only a small number of animals were used in the study and the dose range was not broad enough to show any kind of toxicological effect. In the dog feeding study, 3 male and 3 female 6-8 month old Beagles were fed 0, 10, 50, 100 or 500 ppm 2,4-D in their diet for 2 years. None of the lesions occurring in the dogs was believed to be due to 2,4-D ingestion. However, only small numbers of animals were used and the length of time of the study was not sufficient to prove a negative effect. In the rat reproductive study, male and female rats were fed 0, 100, 500, or 1500 ppm 2,4-D in their diet for 3 generations and a total of 6 litters. Twenty female and 10 male Osbourne-Mendel rats composed each test group. Fertility, numbers of pups surviving to weaning and weanling weights were measured as well as liver aliesterase and acylamidase in animals (10 males and 10 female) from the F₂₆ litter. Both enzymes were assigned in the 0, 100, and 500 ppm groups only. No effects on fertility or average litter size were observed in 2,4-D treated rats. However, the percentage of pup surviving to weaning decreased in the 1500 ppm group. Lower dose levels did not appear to effect fertility, litter size, or survival to weaning. The measured liver enzymes were not altered by 2,4-D treatment.

410. Haque, R., Deagen, J., and Schmedding, D. (1975) Binding of 2,4-dichloro- and 2,4,5-trichlorophenoxyacetic acids to bovine serum albumin. A proton magnetic resonance study. J. Agric. Food Chem. 23(4):763-766.

The nature of the binding of 2,4-D and 2,4,5-T to protein was studied using nuclear magnetic resonance (NMR). Bovine serum albumin (BSA) was dissolved in deuterated water (D₂O) and 2,4-D and 2,4,5-T (as the potassium salts were also dissolved in D₂O and the ¹H NMR spectra of

all three compounds were obtained. BSA at 4 mg/ml, was added to solutions of 3-12 mg/ml 2,4-D and to solutions of 3-12 mg/ml 2,4,5-T in deuterated water and the changes in the line widths in the resonance peaks were determined. All line widths for the phenoxy-acids broadened in the presence of BSA, with the smallest increase in the peak corresponding to HOD and the largest increase in the methyl peak. No changes in the chemical shifts of any of the resonance peaks occurred when BSA was added to either herbicide. The authors concluded that a weak binding occurred between the non-ring methyl group of each herbicide and BSA.

411. Hardell, L. (1979) Malignant lymphoma of histiocytic type and exposure to phenoxyacetic acids or chlorophenols. Lancet 1(8106):55-56.

The author describes a pilot study of male patients with exposure to phenoxyacetic acids or chlorophenols who were admitted to the University Hospital in Umea, Sweden, from January to September of 1978. Fourteen out of 17 men who were admitted for treatment of malignant lymphoma of the histiocytic type were employed in occupations that were likely sources of exposure to the chemicals being studied. Eleven of them reported that they had been repeatedly or chronically exposed to phenoxyacetic acids or chlorophenols. All but one patient were exposed to chemicals that often are contaminated by dibenzodioxins and dibenzofurans. A 64-year-old man who had been exposed to 2,4-dichlorophenoxyacetic acid (2,4-D) developed a retroperitoneal tumor. Six men aged 83, 68, 66, 56, and 56, who had been exposed to both 2,4-D and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) developed tumors of the sinus frontalis; left parotic region; ileum; left femur; and right groin, pelvis, and paravertebrae; respectively. A 38-year-old man who had been exposed to 2,4-D, 2,4,5-T, and chlorophenol developed a tumor in the left groin. Two other men, aged 75 and 63, who had been exposed to chlorophenol only, developed tumors of the left side of the neck and the left submandibular region, respectively. A 72-year-old man who had been exposed to pentachlorophenol had developed a tumor of the bladder, and a 55-year-old man who received exposure to 4-chloro-2-methylphenoxyacetic acid (MCPA) had a retroperitoneal tumor. The author postulated that the immunosuppressive effect of dioxins, particularly TCDD, may be related to the occurrence of malignant lymphomas of histiocytic type.

412. Hardell, L. (1977) Malignant mesenchymal tumours and exposure to phenoxy acids - a clinical observation. Lakartidningen 74:2753.

[Foreign language.]

413. Hardell, L., Eriksson, M., and Lenner, P. (1980) Malignant lymphoma and exposure to chemical substances, especially organic solvents, chlorophenols and phenoxy acids. Lakartidningen 77(4):208-210.

A case-control study examined exposure to phenoxy acids or chlorophenols contaminated by polychlorinated dibenzodioxins or dibenzofurans, and to a lesser extent organic solvents, in men aged 25-85 with malignant lymphoma who were admitted to the Department of Oncology in Ömeå, Sweden, from 1974-1978. Two controls for each case were chosen based on age, sex, occupation, place of residence, and in the case of deceased cases, year of death. Controls for deceased cases watched within 5 years of age; for cases who died in 1978, controls had 1977 deaths lymphoma were histopathologically verified; classification as Hodgkin's disease was done according to Lukes and Butler (1966); as non-Hodgkin's lymphoma, by a modification of Lukes and Collins (1975). Total number of cases was 169; (62 deceased) 60 of these had Hodgkin's disease and 109 non-Hodgkin's lymphoma (4 of them unclassifiable). Total number of controls was 335. Questionnaires sent to patients, next of kin and employers inquired as to leisure time activities, exposure to chemicals, medicine intake, and smoking habits. Employers verified both the ingredients of compounds and actual exposure. Phenoxy acid exposure (primarily 2,4-D, 2,4,5-T, and picloram) occurred in forestry and agriculture. Chlorophenol exposure occurred in work with cutting oils, leather products, and wood protection agents; the last group was classified as either low-grade (less than 1 week or repeated brief exposure for less than 1 month) or high-grade exposure. For organic solvents, special consideration was given to so-called "high-grade" solvents (e.g., benzene, trichloroethylene, styrene) with demonstrated mutagenic effect. Exposures to any combination of the three groups were considered separately. Possible latency periods between exposure and development of tumors called for two separate analyses; for one, cases with less than 5 years' exposure were excluded. The following numbers of exposures were determined. For exposure to phenoxy acids only, 108 cases were unexposed, 31 were exposed for less than 90 days, and 10 were exposed for more than 90 days. Corresponding controls were: unexposed, 303; less than 90 days, 20; and more than 90 days, 4. For exposure to chlorophenols only, 83 cases were unexposed, 14 had low-grade exposure, and 25 high-grade. Controls had 277 unexposed, 19 low-grade, and 9 high-grade. For exposure to organic solvents only, 60 cases were unexposed, 10 had low-grade exposure, and 40 had high-grade. Controls had 222 unexposed, 33 low-grade, and 48 high-grade. In addition, 23 controls had exposure to both organic solvents and either phenoxy acids or chlorophenols; 7 controls had the same exposure. Exposure to either phenoxy acids or chlorophenols comprised 36.1 percent of the cases and 9.6 percent of the controls. The authors indicate that there is no decisive proof that malignant lymphoma can be chemically induced. They could show no significant dose-response relationship for phenoxy acids; there seemed to be a relative risk of 9.3 for high-grade exposure to chlorophenols, and of 2.5 for low-grade exposure; exposure to high-grade organic solvents alone or in combination with either phenoxy acids or chlorophenols, did show increased risk compared to exposure to other solvents. No noticeable difference was seen between the incidence of

Hodgkin's and non-Hodgkin's lymphoma. After accounting for congenital factors, induced immunity deficiencies, and known links between benzene and malignant lymphoma, the authors suggest that there may be a relationship between the incidence of malignant lymphoma and exposure to chlorophenols, or phenoxy acids that are contaminated with PCDDs or PCDFs.

414. Hardell, L., and Sandstrom, A. (1979) Case-control study: Soft-tissue sarcomas and exposure to phenoxyacetic acids or chlorophenols. Br. J. Cancer 39:711-717.

The authors performed a retrospective study to determine a causal relationship between phenoxyacetic acid and the development of soft tissue carcinoma. The case population consisted of 21 living and 31 deceased male patients. Four matched controls were selected for each case. The living patients were matched to persons from the National Population Registry of Sweden for sex, age, and place of residence. The deceased patients were matched to controls obtained from the National Registry for Causes of Death by sex, age, and year of death. Information on exposure, work environment, and health habits i.e. smoking were obtained through mail and telephone questionnaires, 36.5% of the patients were exposed to chlorophenols and/or phenoxyacetic acids. Calculation of relative risk in the matched material was based upon principles by Miethinen 1970. The effect of matching for control of confounding factors was estimated as the quotient of the relative risk in the matched to unmatched material. The relative risk for exposure to chlorophenol and/or phenoxyacetic acids was found to be 6.2 in the matched group and 5.7 in the unmatched. The relative risk for phenoxyacetic acid only was found to be 5.3. In addition the author states no apparent differences were observed in patient or control populations between smokers and non-smokers. The author concluded an increased risk for soft tissue sarcoma related to the use of phenoxyacetic acids or chlorophenols and that "it was unlikely that" confounding factors influenced the results. It is not possible however to correlate the development of soft tissue sarcoma to either phenoxyacetic acid or chlorophenol exposure due to the mixed exposure of these and other herbicides.

415. Hardell, L., and Sandstrom, A. (1978) Malignant mesenchymal soft-tissue tumors and exposure to phenoxy acids or chlorophenols. Lakartidningen. 75:3535-3536.

This is the first report of a formal investigation prompted by a pilot study (Hardell, 1977) suggesting a relationship between exposure to phenoxyacids or chlorophenols and development of mesenchymal soft tissue tumors. The population under study consisted of all males hospitalized between 1970 and 1977 at the Oncological clinic at Umeaa, Sweden, and diagnosed as having mesenchymal soft tissue tumors. Eight controls were chosen for each living case based on age, sex, and residence. For deceased cases, ten controls were matched according to age, sex, and year of death based on the cause of death register.

Investigation of occupation, chemical exposure, smoking habits, etc., was made by using responses to questionnaires by patients, next of kin, and employer. Of 260 people studied (52 cases, 208 controls; 31 deceased cases) 36.5 percent of cases had documented exposure to phenoxy acids or chlorophenols as compared to 9.2 percent of controls. The authors reported that phenoxy acids in wide use during the study period included 2,4-D, 2,4,5-T, and 4-chloro-2-methylphenoxyacetic acid. Exposure to phenoxy acids was difficult to evaluate; replies from employers were obtained for less than half of the patients. After subtracting the number of patients and controls with documented exposure to chlorophenols, it was found that of 45 patients, 13 had been exposed to phenoxy acids, compared to 14 of 201 controls. Exposure times varied from 3 to 27 years. The relative risk to exposure was calculated to be 5.3. Confounding factors, such as smoking or exposure to DDT or chain saw fumes did not, according to the authors, contribute to the increased relative risk, but no data was provided that supported this conclusion. The influence of exposure to diesel oil or pesticides could not be evaluated. The authors concluded that the use of chlorophenols or phenoxy acids contributed to an increased risk from soft tissue sarcoma, although no evaluation of the effect of specific substances could be made.

416. Harman, L. E. (1971) "Chapter 21: Skin Diseases in United States Military Personnel Serving in Vietnam," In The Skin. (Amsterdam: International Academy of Pathology) pp. 423-434.

Dermatology problems seen in a field hospital in Vietnam in 1967 are described. Of the total new patients with skin problems, 3.8 percent were diagnosed as having acne. Soldiers often indicated that pre-existing acne worsened about 6 weeks after arriving in Vietnam. The author did not mention chloracne, nor did he associate acne with any particular military assignment or location. [Other common skin lesions in Vietnam were caused by fungus and bacterial infections.]

417. Harrigan, E. T. (1970) Calibration Test of the UC-123K/A/A45Y-1 Spray System. Technical Report ADIG-TR-70-36. Armament Development and Test Center, Eglin AFB, Florida. 160 p.

[Background material.]

418. Harris, M. W., Moore, J. A., Vos, J. G., and Gupta, B. N. (1973) General biological effects of TCDD in laboratory animals. Environ. Health Perspect. 5:101-109.

The effects of acute and subchronic administration of TCDD were studied in the guinea pig, rat, and mouse. TCDD (more than 99% pure) in acetone-corn oil was administered by gastric intubation to Hartley guinea pigs, CD rats and CD-1 mice. Mortality, body weight gains, food consumption, and organ weights were recorded. Single doses of 1 or 5 ug/kg TCDD to rats had no effect on body weight during the next 8

weeks, while a 25 ug/kg dose caused a transient decrease in body weight gain compared to controls during the first 1-2 weeks after treatment. Doses of 50-100 ug/kg and in some experiments 25 ug/kg had a dose-related deleterious effect on body weight gain and food consumption and caused death in some rats. Daily doses of 1 or 10 ug/kg of TCDD for 31 days or weekly doses of 5 ug/kg for 6 weeks administered to rats caused significant decreases in weight gain, while 0.1 ug/kg daily doses or 0.02 or 1.0 ug/kg weekly doses were ineffective. Mortality and significant weight loss were observed in guinea pigs that received a single dose of 3 ug/kg or 1 ug/kg weekly doses for 8 weeks. At 25 ug/kg weekly doses, C57B1/6 mice showed significant decreases in weight gain and one of seven mice in the group died, whereas single doses of up to 50 ug/kg TCDD were ineffective in CD-1 mice. Decreased thymus weights were observed in all three species at doses below those which elicited decreases in body weight. Repeated doses of TCDD were cumulative and did not increase the threshold amount required to elicit an effect. Death was delayed in all species and female rats appeared more sensitive to TCDD than males. Although food consumption was usually reduced in animals exposed to TCDD, the authors indicated that this reduction was not adequate to account for the severe losses in body weight.

419. Harrison, D.D., Miller, C.I., and Crews, R.C. (1979) Residual levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) near herbicide storage and loading areas at Elgin AFB, Florida. Air Force Armament Laboratory. Technical report AFATL-TR-79-20.

[Background material.]

420. Hart, E. R., and Valerio, M. G. (1972) Teratogenic effects of 2,4,5-T in mice. Toxicol. Appl. Pharmacol. 22:317.

[Abstract, only.]

421. Hawkins, S. F. (1980) The in vivo effect of paraquat and diquat on intermediary metabolism in mouse lung. Dissertation Abstracts International: Section B 40(10):4763-4764.

[Abstract, only.]

422. Hawkins, S. F., Medina, M. A., and Stavinoha, W. B. (1979) The acute in vivo effect of paraquat and diquat on intermediary metabolism in mouse lung. Fed. Proc. 38(3) Pt. 1:582.

[Abstract, only.]

423. Hay, A. (1979) Accidents in trichlorophenol plants: A need for realistic surveys to ascertain risks to health. Ann. N.Y. Acad. Sci. 320:321-324.
[Review article.]
424. Hay, A. (1978a) Dioxin meeting recommends cancer study. Nature 271:202.
[Editorial.]
425. Hay, A. (1978b) Dioxin source is "safe." No reference.
[Editorial.]
426. Hay, A. (1978c) Vietnam's dioxin problem. Nature 271:597-598.
[Editorial.]
427. Hay, A. (1977a) Seveso solicitude. Nature 267:384-385.
[Editorial.]
428. Hay, A. (1977b) Tetrachlorodibenzo-p-dioxin release at Seveso. Disasters 1:289-308.
[Review article.]
429. Hay, A. (1976a) Seveso: the aftermath. Nature 263:836-838.
[Review article.]
430. Hay, A. (1976b) Toxic cloud over Seveso. Nature 262:636-638.
[Review article.]
431. Hayes, W. J. (1975) Toxicology of Pesticides (Baltimore: The Williams & Wilkins Co.) pp. 138-148.
[Review article.]
432. Hayes, W. J. (1964) Toxicological problems associated with use of pesticides. Industry and Tropical Health 5:118-132.
[Review article.]

433. Heene, R. (1968) Histochemical and morphological findings in experimental 2,4-Dichlorophenoxy Acetate (2,4-D) myopathy in warm blooded animals. Acta Neuropath. 10:166-169.
[Foreign language.]
434. Helling, C. S., Isensee, A. R., Woolson, E. A., Ensor, P. D. J., Jones, J. R., Plimmer, J. R., and Kearney, P. C., (1973) Chlorodioxins in pesticides, soils, and plants. J. Environ. Qual. 2(2):171-178.
[Not available.]
435. Henck, J. W., et al. 2,3,7,8-tetrachlorodibenzo-p-dioxin - acute oral toxicity in hamsters. Toxicol. Appl. Pharmacol. (pre pub. copy)
[Not available.]
436. Henig, R. M. (1979) Congress calls for 2,4,5-T ban after dramatic herbicide hearings. BioSci. 29:453-454.
[Editorial.]
437. Herbicide Assessment Commission Amer. Assoc. for the Advancement of Science. (1970) Summary of Presentations.
[Not available.]
438. Herbicides: More research on 2,4,5-T. Chem. Eng. News. 49(20):11. 1971.
[Editorial.]
439. Hewitt, W. R., Pegg, D. G., and Hook, J. B. (1976) Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on renal function in rats in vitro. Toxicol. Appl. Pharmacol. 37(1):177.
[Abstract, only.]
440. Hickey, G. C. (1974) The effects of herbicides in South Vietnam. Part B. Working papers: Perceived effects of herbicides used in the highlands of South Vietnam. National Academy of Sciences - National Research Council. AD-779 027. 23 pp.
[Background material.]

441. Higginbotham, G. R., Huang, A., Firestone, D., Verrett, J., Ress, J. and Campbell, A. (1968) Chemical and toxicological evaluations of isolated and synthetic chloro derivatives of dibenzo-p-dioxins. Nature 220:702-703.

The effects of TCDD in the chick embryo bioassay were briefly reported. The report also described results of the isolation and identification of compounds produced from the pyrolysis of chlorophenols and their biological activity in relation to chick edema factor, hexachloro-dibenzo-p-dioxin. TCDD was prepared by chlorinating dibenzo-p-dioxin and 0.05 ug/egg was injected into the air space of fertilized eggs. After 21 days of incubation, 100% of the embryos were dead compared to 10-15% of the non-treated and vehicle controls. Other compounds tested showed only a fraction of the potency of TCDD. The authors suggested that chick edema factor could arise in fat samples during heating operations used to produce commercial fats from chlorophenols present as contaminants from their use as pesticides.

442. Highman, B., Gaines, T. B., and Schumacher, H. J. (1977) Retarded development of fetal renal alkaline phosphatase in mice given 2,4,5-trichlorophenoxyacetic acid. J. Toxicol. Environ. Health 2(5):1007-1018.

The level of alkaline phosphatase detected histochemically in fetal mouse kidneys was determined to evaluate renal functional development in fetal mice which develop cystic kidney in response to maternal 2,4,5-T treatment. Pregnant CD-1 and hybrid mice were administered 60-120 mg/kg 2,4,5-T (less than 0.05 ppm dioxin contaminant) by gavage in acetone-corn oil (1:9) on days 6-14 of gestation. On day 17 or 18 a region of each fetus which included the kidneys was excised, prepared histologically and slices were stained by a cobalt sulfide method to visualize alkaline phosphatase. The numbers of resorptions were recorded and fetuses were examined for cleft palate. Maternal blood was analyzed for hematologic and blood chemistry parameters by automated methods. Maternal toxicity was observed at the highest dose. Alkaline phosphatase was localized principally in clusters of tubules in the inner cortex and along the brush border of the convoluted tubules in day 17 (untreated) fetal kidneys. Groups treated with 2,4,5-T had a statistically significant increase in the frequency of fetuses with diminished or no alkaline phosphatase present. The renal pelvis was described as dilated or cystic in some fetuses of 2,4,5-T-treated mice but not of control mice, but the incidence was not reported. No cleft palates occurred in fetuses from control groups. For each dose group, about 20% of the mice with normal alkaline phosphatase levels had cleft palate, while 40 to 70% of the fetuses with low alkaline phosphatase levels had cleft palate. Fetuses with cleft palate tended to weigh less than fetuses from the same treatment group with normal palates. Hematologic and clinical chemistry tests of maternal blood revealed no differences among treatment groups. The incidence of viable fetuses decreased from 93% in control groups to 74% and 59% for the 90 and 120 mg/kg dose groups. The frequently concomitant occurrence of cleft palate and decreased fetal weight indicated to the authors that 2,4,5-T produces toxic effects at several indepen-

dent loci. The incidence of lowered alkaline phosphatase levels in treated fetuses on day 18 was more lowered than on day 17 and the authors concluded that renal abnormalities caused by 2,4,5-T resulted from a general retardation in development. This conclusion would be greatly strengthened by a demonstration of a stage when renal development is complete in 2,4,5-T-treated fetuses or neonates.

443. Highman, B., Gaines, T. B., and Schumacher, H. J. (1976a) Sequential histopathologic, hematologic and blood chemistry changes induced in mice by a technical and purified preparation of 2,4,5-trichlorophenoxyacetic acid. J. Toxicol. Environ. Health 1(3):469-484.

The effects of 2 preparations of 2,4,5-T administered to pregnant mice on maternal health was evaluated. Dihybrid female mice derived from a cross of (C57BL X A/JAX) hybrids and (C3H X BALB) hybrids were administered 60 or 120 mg/kg 2,4,5-T in acetone-corn oil (1:10) by gavage on day 6-15 of gestation. Two preparations of 2,4,5-T were administered: a technical preparation (97.0% purity, with less than 0.05 ppm TCDD) was administered to 314 mice and a purified preparation (99% purity, with less than 0.005 ppm TCDD) to 64 mice. Of the control mice, 23 received no treatment and 73 received vehicle only. Nonpregnant mice were included in the study in treatment and control groups. Mice were killed from 6 hours to 11 days after the first dose was administered; moribund mice were also killed. Tissues were prepared for light microscopy and blood was analyzed for hematological parameters and blood chemistries. The frequencies of lesions were tabulated for mice in various groups by clinical condition (normal, sick, or moribund). In general, lesions were most frequent and severe in moribund mice and mild or absent in mice that appeared normal. Differences between treated and control mice were not analyzed for statistical significance. Histopathologic changes included rarefaction of myocardial fibers, and necrosis of the outer myocardium, thymus atrophy (involution was considered to be associated with pregnancy), splenic atrophy, bone marrow and lymph node lesions, thyroid hyperplasia (by day 17 of gestation), hepatic glycogen depletion during treatment, and increased leukocyte counts, urea nitrogen levels and lactate dehydrogenase and glutamic oxaloacetic transaminase activities. Plasma calcium levels fell in moribund mice with severe myocardial rarefaction. The incidences of lesions of the spleen, bone marrow, and lymphatic organs was higher in mice treated with purified 2,4,5-T than with the same dose of the technical preparation. The incidence of fetal deaths or malformations were not given. The authors concluded that the observed hemoconcentration in treated mice indicated that 2,4,5-T caused a hemolytic effect (in addition to its effect on bone marrow; 2,4,5-T and not contaminants of the technical preparation were responsible for the observed toxicity; and the maternal toxicity was not a factor in causing fetal abnormalities because they only occurred immediately prior to maternal death.

444. Highman, B., Gaines, T. B., and Schumacher, H. J. (1976b) Renal alkaline phosphatase activity in fetal offspring of maternal mice given 2,4,5-trichlorophenoxy acetic acid. Toxicol. Appl. Pharmacol. 37:145.

[Abstract only.]

445. Highman, B., Gaines, T. B., Schumacher, H. J., and Haley, T. J. (1976) Strain differences in histopathologic, hematologic and blood chemistry changes induced in mice by a technical and purified preparation of 2,4,5-trichlorophenoxyacetic acid. J. Toxicol. Environ. Health 1(6):1041-1054.

The toxicity of 2,4,5-T was described in pregnant and nonpregnant mice. Pregnant and nonpregnant CD-1 mice from several laboratories and dihybrid mice from a mating of (C57BL X A/JAX) hybrids with (C3H X BALB) hybrids were administered 60-140 mg/kg 2,4,5-T by gavage daily "on days corresponding to days 6 through 14 of pregnancy" (which was not explained further for nonpregnant mice). Two preparations of 2,4,5-T were used, including a technical preparation of 97.9% purity and less than 0.05 ppm dioxin, and a purified preparation of 99% purity with less than 0.005 ppm dioxin. Controls received only the acetone-corn oil (1:9) vehicle. Animals were killed 1-11 days after the first dose and were necropsied. Blood samples were removed from some CD-1 mice for hematology and blood chemistry analyses. Histopathological lesions included myocardial fiber rarefaction; myocardial necrosis; thymic and splenic atrophy; bone marrow hypocellularity, congestion and hemorrhages, and lymph node lesions and thyroid hyperplasia. In males, urothelial hyperplasia and testicular degenerative changes were also observed. CD-1 mice from 1 laboratory (NCTR), showed toxicity from 60 mg/kg doses while CD-1 mice from another laboratory (CRBL) were insensitive to doses of 90-120 mg/kg 2,4,5-T, which the authors attributed to a latent epizootic infection in NCTR mice. Another group of CD-1 mice (with comparable response to 2,4,5-T as CRBL mice) were more resistant to 2,4,5-T than nonpregnant female dihybrids or male dihybrids and severe lesions in CD-1 mice were restricted to moribund mice. Although no fetuses were examined in this study, the incidences of cleft palate in these strains after 2,4,5-T exposure obtained in an unpublished study were reported to be divergent from the relative maternal sensitivity to 2,4,5-T toxicity. In general, pregnant mice showed no increased sensitivity to 2,4,5-T compared to non-pregnant mice. In clinically normal, treated mice hemoconcentration and elevated lactate dehydrogenase levels were observed. The authors concluded that maternal toxicity by 2,4,5-T was unlikely to have caused fetal abnormalities reported in other studies; 2,4,5-T and not other components in the preparation probably cause the observed toxic effects; large differences in sensitivity to 2,4,5-T were demonstrated for different strains of mice; and development of bladder epithelial hyperplasia in dihybrid treated males indicated a potential for carcinogenicity of 2,4,5-T.

446. Hiles, R. A., and Bruce, R. D. (1976) 2,3,7,8-tetrachlorodibenzo-p-dioxin elimination in the rat: first order or zero order? Fd. Cosmet. Toxicol. 14(6):599-600.

The authors challenge the validity of the assumption made in previous reports that TCDD elimination followed first-order kinetics. Data presented previously (Allen et al., 1975; Piper, et al., 1973) was analyzed using 2 models: 1 representing zero-order kinetics, in which the rate of TCDD clearance was assumed to be independent of TCDD concentration and the concept of half-life is not applicable, and a model for first-order kinetics. The data fit the model based on zero-order kinetics better than the model for first-order kinetics. The point at which the 2 models predicted very divergent values was for TCDD concentrations measured after 24 days after administration. Neither report presented data for this time period. The authors concluded that the order of the elimination process for TCDD could not be determined by the previously published data and the presentation of biological half-lives was invalid until first-order kinetics are verified.

447. Hill, E. V., and Carlisle, H. (1947) Toxicity of 2,4-dichlorophenoxyacetic acid for experimental animals. J. Indust. Hyg. and Toxicol. 29(2):85-95.

The acute and subchronic toxicities of 2,4-D and several salts were studied in several species after oral, parenteral, and inhalation exposures. The oral LD₅₀ of the sodium salts of a purified 2,4-D preparation was 375 mg/kg in the mouse, 666 mg/kg in the rat, 1000 mg/kg in the guinea pig, and 800 mg/kg in the rabbit. Intraperitoneal LD₅₀ values were the same as the oral values for the mouse and rat and lower for the other species. The maximum tolerated doses in monkey were 214 mg/kg orally and 428 mg/kg intraperitoneally. In the rabbit, an intravenous dose of 400 mg/kg caused acute ventricular fibrillation in half the animals' myotonia. Smaller animals showed symptoms of muscular incoordination, stiffness and paralysis, followed by stupor, coma, and death. Monkeys developed vomiting after large oral doses were administered. The sodium and ammonium salts of a crude (commercial) preparation of 2,4-D and the purified 2,4-D (acid) showed the same toxicity as the sodium salt of purified 2,4-D. Toxicity was the same for 2,4-D when dissolved in either n-butyl alcohol or tributylphosphate. Various subchronic doses of 2,4-D were administered to dogs. The doses were cumulative and symptoms included stiff gait, large and sudden drops in leukocyte, lymphocyte and platelet counts, and bleeding gums. Dogs that died received a cumulative dose of 300 mg/kg or more. Large subchronic oral doses of 0.1 percent 2,4-D in feed to rats, 1.0 gm or 2,4-D to guinea pigs over 12 days or 6000-8000 mg-min per liter of vapors in air to guinea pig were survived. Gross and histologic examination of rats, guinea pigs that received fatal doses of 2,4-D, and rabbits exposed subchronically revealed renal lesions and pulmonary edema and hemorrhages. Exposed dogs were susceptible to liver damage and also had renal lesions and lymphoid necrosis. The authors concluded that 2,4-D has a low order of toxicity and estimated that a 75 kg man would tolerate a dose of 15 grams and calculated a human LD₅₀ of 28 gm.

448. Hinsdill, R. D., Thomas, P. T., and Couch, D. Immunotoxicity assessment of dioxins. In: Inadvertent modification of the immune response. The effects of foods, drugs and environmental contaminants. Office of Health Affairs, FDA, Washington, D.C. pp. 76-79.

[Background material.]

449. Hobson, L. B. (1980) Remarks presented at the 2d Continuing Education Conference on Herbicide Orange, Washington, DC, May 28-30.

[Background material.]

450. Hodge, H. C., Downs, W. L., Panner, B. S., Smith, D. W., Maynard, E. A., Clayton, J. W., and Rhodes, R. C. (1961) Oral toxicity and metabolism of diuron (N-(3,4-dichlorophenyl)-N',N'dimethylurea) in rats and dogs. Fd. Cosmet. Toxicol. 5:513-531.

Animal studies of the acute, subchronic, chronic and reproductive toxicities, oncogenicity, potential for skin irritation and sensitivity, and metabolism of diuron are presented. Male rats were administered single doses of diuron in suspension in peanut oil orally and the LD₅₀ was calculated. Wistar-derived rats were used for a series of feeding experiments, except for the 2-week and 3-month studies in which Charles River rats were used. For the 2-week study, male rats were administered 10 daily oral doses of 1 g/kg diuron. For the other oral rat studies, diets that contained 25-8000 ppm diuron were fed for 1, 3, 15, 23 or 24 months. Food consumption, hematological parameters and mortality were assessed during the study. Rats were necropsied and organs were weighed at the end of the feeding period. Dogs (6 per group) were fed 25-2400 ppm diuron for 24 months and for a preliminary study 1 dog was administered 8 mg/kg diuron orally in a gelatin capsule daily for 1 month and another dog was administered diuron for 2 months in doses that increased from 8 mg/kg/day to 120 mg/kg/day over a 2-month period. For a 3 generation reproductive study, rats were fed 125 ppm diuron in the diet and numbers of births, and weanlings and weight gains during lactation were recorded for all generations and histology was evaluated in the second litter of the third generation. Tissue levels of diuron were determined in rats and dogs fed diuron for 2 yrs. and urinary metabolites were identified in urine from dogs by thin layer chromatography and ultraviolet spectrophotometry. The LD₅₀ was 3.4 g/kg and 10 doses of 1g/kg to rats resulted in reduced weight gains. The spleens of rats, after 1-10 doses, were large and dark, with numerous foci of blood formation in the spleen and bone marrow. In the short-term rat feeding studies, mortality (60%) occurred only at the highest dose and reductions in hematocrits and growth retardation occurred in groups given at least 2000 ppm diuron. Histology resembled findings after acute administration. The only effect in dogs fed diuron for 1-2 months was weight loss and anemia from the 120 mg/kg feeding regimen. In the long-term studies, growth retardation occurred at the highest dose only. The incidences of mortality in treated groups were not compared to controls statistically. The authors did not attribute any observed increase in mortality to diuron treatment.

Sporadic decreases in hemoglobin levels were observed in rats over the 2-year study and the only changes observed in tissues of treated groups were increased splenic weights. No deleterious cellular effects were observed in spleen and bone marrow cells. No adverse effects on reproduction occurred. One litter (F_{2b}) showed growth depression which the authors did not attribute to diuron treatment. In chronically treated dogs, only the highest dosage group showed persistent weight losses, anemia, increased liver weights and erythroid hyperplasia of the spleen and bone marrow. Sulphemoglobin was detected in blood from dogs and rats fed high dietary doses of diuron. No diuron accumulation was observed in tissue, as only a minute amount of the dose was recovered in tissues. Tissue levels were related to the dosage administered and only about 10% of the estimated daily doses were recovered in urine and feces. (The fate of the ingested dose was not indicated.) The principal urinary metabolite was identified as N-(3,4-dichlorophenyl)-urea, and others present in smaller amounts were N-(3,4-dichlorophenyl)-N'-methylurea, 3,4-dichloroaniline, 3,4-dichlorophenol and unmetabolized diuron. The distribution of metabolites in urine were the same from rats after 1 year of feeding as after 2 yr. The authors mentioned that skin irritation and sensitization tests were negative but did not present the methods or results from these experiments. The authors concluded that diuron did not produce adverse effects at dietary doses below 500 ppm fed for 1-3 months or toxicity or oncogenicity or reproductive effects over 3 generations at doses of 125 ppm or less fed chronically. The metabolic pathways for diuron were concluded to be the same as those found in soil and plants, with sequential removal of methyl groups, followed by hydrolysis of the substituted urea to its aniline derivative.

451. Hodge, H. C., Maynard, E. A., Downs, W. L., and Coye, R. D. (1958) Chronic toxicity of 3-(p-chlorophenyl)-1,1-dimethylurea (Monuron). Am. Arch. Ind. Health 17:45-47.

The authors studied the effects of monuron on rats and dogs in a 2-year feeding study. Weanling albino rats (Rochester strain, 30 males and 30 females per group) were fed 0, 0.0025%, 0.025%, and 0.25% monuron in their diet for 2 years. Purity of the compound, storage of formulated diet, and an analytical check on the diet formulation were not reported. Male rats fed 0.25% showed a slight depression in growth after 1 month. Approximately a 20 g difference was observed between treated and control males; females also had a slight growth depression but this was less than seen in the males. No numerical data were presented to evaluate this conclusion. According to the authors, approximately 70-90% of both treated and untreated animals died by the end of the second year. The high mortality was due to viral epidemics within the animal colony. Therefore, results reported are data from a severely limited number of animals. Because of this, the study cannot be used as a good indicator of the effects of monuron. Hematological examinations showed slight anemia in both males and females exposed to 0.25 percent monuron. Urine sugar and protein levels were within the normal range. Incidence of tumors were also within the historical-normal colony occurrence. No numerical data of results were presented. In the young adult Beagle dog feeding study, groups of 1 male and 1

female dog were given 0, 2.5, 12.5, and 25 mg/kg monuron in their feed for one year. Neither the number of animals nor the length of the study was sufficient to make a reliable interpretation of this study. According to the authors, urine sugar and protein, blood profiles and weights of major organs were within the normal range during the study. No increases in tumor incidence were observed.

452. Hofmann, H. T. (1957) Neure Erfahrungen mit hochtoxischin chlorkoleminasextoffin. Naumiun. Schnidebergys Arch. Exp. Path. Pharmak. 232:228-230.

[Foreign language.]

453. Holden, C. (1979) Agent Orange furor continues to build. Science 205:770-772.

[Editorial.]

454. Holmberg, B. (1975) Biological aspects of chemical and biological weapons. Ambio 4(5-6):211-216.

[Review article.]

455. Homberger, E., Reggiani, G., Sambeth, J., and Wipf, H. K. (no date) The Seveso accident: its nature, extent and consequences. Ann. Occup. Hyg. 22:327-366.

The authors describe the environmental effects of TCDD contamination that resulted from the factory accident in Seveso, Italy, in 1976 and present human birth rate and morbidity data for the affected region. A constant decline in the birth rate from 17.1 per thousand in 1973 to 12.6 per thousand in 1977 was found in the Seveso region, which the authors attributed to psychological factors that led to an increase in the use of voluntary birth control methods. No increase in the death rate occurred in the Seveso region after the accident. Three days after the accident, animal deaths occurred near the factory, and animals in a progressively larger area died in the subsequent months. In general, herbivores were affected first, probably from ingesting contaminated vegetation. Birds appeared to die rapidly, while rabbits and small animals suffered from anorexia, apathy, gastrointestinal hemorrhages, pulmonary edema, and depletion of fat stores before death. Small animals succumbed before sheep and goats, and cattle and horses were affected last. As part of the decontamination program, animals were slaughtered, and traces of TCDD were detected in the livers and fat of some, although no systemic effects were observed. Wildlife in the area closest to the factory were exterminated to prevent contamination of other areas. In the autumn when the grass died, TCDD was transported to the soil. Damage to the flora from the TCDD cloud released during the accident was limited to phytotoxic damage to some broad-leaved plants. An estimated 80 percent of the released TCDD

adhered to foliage, grass, and crops. In a defined study area in the most contaminated sector (zone A), from 28 percent to 49 percent of the deposited TCDD was cleared from the soil in an 18-month period, while minimal transfer of TCDD from the soil into crops was observed. Decontamination procedures were described; natural decontamination is estimated to take from 6 to 8 years to complete. The authors concluded that the only significant health effect identified to date as attributable to TCDD exposure was mild to moderate chloracne in a sensitive segment (youth) of the population. The area remains contaminated with TCDD, although the levels are lower than immediately after the accident.

456. Honchar, P. A., and Halperin, W. E. (1981) 2,4,5-T, trichlorophenol, and soft tissue sarcoma. Lancet Jan. 31:268-269.

The authors reviewed studies conducted by Zack and Suskind (1980); Cook, Townsend, and Ott (1980); Ott Holder, and Olson (1980); and the US Department of Health, Education, and Welfare's National Center for Health Statistics (1975). The four studies dealt with mortality rates among workers at Dow Chemical USA or the Monsanto Company who were exposed to 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) or 2,4,5-trichlorophenol (TCP), both of which are contaminated with TCDD. Comparing the four cohorts, the authors found that three of 105 deaths, or 2.9 percent, were due to soft tissue sarcoma. This compares to a rate of 0.07 percent of all US males aged 20-84 in 1975. One man (Zack and Suskind) died in 1978 with malignant fibrous histiocytoma of soft tissue origin; he was 58. He had been exposed to TCP at Monsanto in 1949 and developed chloracne, a standard symptom of TCDD intoxication. Another man (Cook et al.), who died at 53 of fibrosarcoma, had worked on TCP production at Dow Chemical and had facial dermatitis; no diagnosis of chloracne was made, however. The third case (Zack, unpub.) involved a man who had worked on 2,4,5-T synthesis at Monsanto and had no history of chloracne. In 1972, at the age of 49, he died of generalized liposarcoma. According to Ott et al., one cohort of 2,4,5-T workers at Dow contained no case of soft tissue sarcoma. When considered separately, the rates of soft tissue sarcoma occurrence in the four cohorts do not show an excess risk. However, the authors feel that these three cases may represent a common pattern and suggest that their method of analysis may prove valuable in detecting trends among small cohorts of workers with common occupational exposure.

457. Hood, R. D., Patterson, B. L., Thacker, G. T., Sloan, G. L., and Szczech, G. M. (1979) Prenatal effects of 2,4,5-T, 2,4,5-trichlorophenol and phenoxyacetic acid in mice. J. Environ. Sci. Health (3C)13:189-204.

The teratogenic effects of 2,4,5-T were studied in the mouse. Pregnant CD-1 mice were administered 800-900 mg/kg of 2,4,5-T (with 0.01 ppm TCDD contaminant) in honey-water (1:1) by gastric intubation on a single day from day 8 to 15 of gestation. Other pregnant mice received 250-300 mg/kg on 3 consecutive days (days 7-9, 10-12, or 13-15) by the same route. On day 18 of gestation fetuses were removed, weighed, and

examined grossly. Some fetuses were also examined for visceral malformations or for skeletal malformations of alizarin red stained and cleared fetuses. Fetuses of mice treated on day 14 or 15 or days 13-15 with 2,4,5-T weighed 35% (significantly) less than vehicle-treated controls or non-treated controls. Treatment on day 14 also resulted in a 4 fold increase in incidences of deaths and of resorptions over both control groups. Treatment on day 11 or days 13-15 consecutively produced gross malformations in 22-24% of the fetuses, including decreased jaw length and exencephaly, while less than 1% of the controls were affected. Fused ribs and malformed vertebrae were observed in 13% of the fetuses treated on days 13-15 but this value was not (statistically) significantly different from both control groups which had no skeletal malformations or cleft palates. Treatment on days 9 or 11 or 14 or days 10-12 or 13-15 all caused incidence of cleft palate of over 35%. Phenoxyacid and 2,4,5-trichlorophenol were tested in these experiments as well, but caused none of the effects reported for 2,4,5-T. The authors concluded that both the carboxyl group and the chlorinated aromatic ring are necessary for teratogenic and fetotoxic activity. The statistical tests applied to the data include arcsin transformation of means and ANOVA and Gabriel's multiple range tests and rank sum methods. These tests are relatively insensitive as they did not distinguish between frequencies of zero percent incidence of cleft palate (in both controls) and 35.3% from day 14 treatment or 10% frequency of prenatal mortality (controls) and 48.2% from day 12 treatment. Assuming the standard deviations were not excessive, these differences have biological significance and the statistical tests do not aid in interpreting the data for risk assessment.

458. Hook, G. E. R., Haseman, J. K., and Lucier, G. W. (1975) Induction and suppression of hepatic and extrahepatic microsomal foreign-compound-metabolizing enzyme systems by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Chem. Biol. Interactions 10:199-214.

The effects of TCDD on several enzyme systems that metabolize foreign compounds were studied in the rat, rabbit, and guinea pig. TCDD was administered by stomach tube. Subsequently, microsomes were isolated from various tissues and assayed for different enzyme activities. At doses of TCDD that did not elicit any aberrant health effects (0.2-5 ug/kg), TCDD induced benzpyrene hydroxylase activity and cytochromes P-450 and b5 in the rat liver, which persisted for 73 days after a single dose was given. The response in enzyme induction was independent of the age of the treated rat. Actinomycin D partially blocked the inductive effect of TCDD. TCDD treatment inhibited N demethylases in adult male rats, but not in females or in young males. The effect was not observed 35 days after the single dose of TCDD was given. TCDD treatment did not alter cholesterol or phospholipid concentrations in hepatic microsomes, but increased the proportions associated with rough endoplasmic reticulum relative to smooth endoplasmic reticulum. Extrahepatic enzymes of the rat, rabbit and guinea pig were induced by TCDD, but the specific enzymes and tissue sources of the enzymes that responded to TCDD were different for the 3 species. The authors suggested that TCDD either operates through a receptor or interacts

directly with an operator gene to explain the ability of minute amounts of TCDD to induce enzymes.

459. Hook, G. E. R., Orton, T. C., Moore, J. A., and Lucier, G. W. (1975) 2,3,7,8 tetrachlorodibenzo-p-dioxin-induced changes in the hydroxylation of biphenyl by rat liver microsomes. Biochem. Pharmacol. 24(3):335-340.

TCDD-induced biphenyl 2- and 4-hydroxylase activities of rat liver microsomes are described. Charles River rats were administered a single dose of TCDD in acetone-corn oil by stomach tube. Microsomes were prepared from the liver and assayed for cytochrome P-450 content, biphenyl hydroxylation in the 2 and 4 positions, and for testosterone hydroxylation in the 2 and 6 beta positions and 7 and 16 alpha positions. TCDD at a dose of 0.2 ug/kg in females and 1.0 ug/kg in males produced increases in 2- and 4-hydroxylation and in cytochrome P-450 content. The inductive effect of 25 ug/kg on biphenyl hydroxylases persisted 73 days after TCDD treatment, the last time point examined. Biphenyl-2- and 4-hydroxylase levels were induced to the same absolute levels in rats between 10 and 335 days of age, even though 10 day old rats had lower initial biphenyl-2-hydroxylase levels than older rats. Actinomycin D administration partially blocked the TCDD effect 18 hours after the 2 agents were administered. TCDD treatment resulted in 4-17 fold elevations in the Vmax values of both biphenyl hydroxylases and an increase in the apparent Kn for the 2-hydroxylase to the same value as was shown for the 4-hydroxylase. TCDD treatment also resulted in decreased testosterone hydroxylation at the 2 beta- and 16 alpha positions. The authors concluded that TCDD is one of the most potent and longlasting inducers of biphenyl hydroxylases in the rat and suggested that separate enzymes may be responsible for biphenyl hydroxylation in the 2- and 4-positions.

460. Hook, J. B., Bailie, M. D., Johnson, J. T., and Gehring, P. J. (1974) In vitro analysis of transport of 2,4,5- trichlorophenoxyacetic acid by rat and dog kidney. Fd. Cosmet. Toxicol. 12(2):209-218.

2,4,5-T uptake by renal cortical slices of dogs and rats was characterized. Renal cortical slices from dogs and Sprague-Dawley rats were incubated in the presence of ¹⁴C-labelled compounds and uptake of radioactivity into tissue was determined at the end of the exposure period. The presence of 2,4,5-T did not alter N-methylnicotinamide (NMN) uptake, but caused a dose-dependent competitive decrease in para-aminohippurate (PAH) uptake. The slice to medium (S/M) ratios for 2,4,5-T were 14 in rat tissue and 9 in dog tissue. Both ratios were decreased by addition of probenecid and PAH, but only the ratio for rat tissue was dependent upon potassium concentration. Maximum uptake of 2,4,5-T by kidney slices from 10 day-old rats (S/M ratio = 10) was below the level for adult rat tissue. The authors concluded that prolonged half-lives for 2,4,5-T in young rats compared to adult rats and in dogs can be explained by their differences in rates of uptake by the kidney, the major route of 2,4,5-T excretion.

461. Hook, J. B., Cardona, R., Osborn, J. L., Bailie, M. D., and Gehring, P. J. (1976) The renal handling of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in the dog. Fd. Cosmet. Toxicol. 14:19-23.

Renal excretion of 2,4,5-T by the dog and the rat was studied in vivo and in vitro in the presence of metabolic substrates and inhibitors. Sodium pentobarbitone-anesthetized mongrel dogs were administered 2,4,5-T by intravenous infusion and urine was collected from urethral catheters. Blood samples were collected at the midpoint of each clearance period and inulin, para-aminohippurate (PAH) and 2,4,5-T clearances were determined. PAH secretion was significantly reduced by administration of 5-10 mg/kg 2,4,5-T with no change in glomerular filtration rate. Infusion of sodium acetate increased 2,4,5-T clearance in a dose-related manner, but 2,4,5-T clearance never reached the rate of inulin clearance. Administration of acetazolamide, mannitol and saline also caused increases in 2,4,5-T clearance (below the rate of inulin clearance). Pretreatment of dogs with ammonium chloride for 1-3 days before the experiment and infusion of sodium bicarbonate to acidify the urine resulted in a substantial enhancement in 2,4,5-T clearance at urine pH values above 6.0. Renal slices were prepared and incubated with 2,4,5-T and PAH. Plasma added to the incubation medium increased PAH uptake but reduced 2,4,5-T uptake by half. The authors concluded that factors that influenced 2,4,5-T clearance included, along with active renal transport, a urinary pH-dependent process as passive diffusion, and plasma-protein binding of 2,4,5-T with a higher affinity for 2,4,5-T than the affinity for the kidney transport system.

462. Hook, J. B., McCormack, K. M., and Kluwe, W. M. (1978) Renal effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin. In Pentachlorophenol: Chemistry, pharmacology and environmental toxicology. K. R. Rao, ed., (New York: Plenum Press). pp. 381-388.

The effect of TCDD on chloroform-induced renal toxicity was studied in the mouse. Male ICR mice were administered 1.6 or 16 ug/kg TCDD intraperitoneally. After 72 hours, from 0.5-25 ug/kg of chloroform was administered and 24 hours after chloroform treatment, blood was collected and analyzed for blood urea nitrogen (BUN) and serum glutamate oxaloacetate transaminase (SGOT) activities. Other mice were killed 3 days after TCDD treatment and liver and kidney microsomal preparations were assayed for epoxide hydratase, aryl hydrocarbon hydroxylase, and biphenyl-2- and -4-hydroxylases. TCDD treatment resulted in significant elevations in all enzyme activities in both tissues; all control and induced enzyme activities were higher in liver microsomes than in kidney microsomes. BUN and SGOT levels were unaltered by TCDD. TCDD-treated mice (number of mice not reported) had increased liver-to-body weights, but not kidney-to-body weights. Chloroform administered to control mice or TCDD-treated mice did not alter kidney-to-body weight ratios. Other effects of TCDD on renal function presented in previous publications were reviewed. The authors concluded that TCDD produced metabolic changes in the kidney but not physiological effects or potentiation of chloroform toxicity; any decrease in renal function was considered nonspecific and was attributed to a decline in the general health of the animal.

463. House, W. P., Goodson, L. H., Gadberry, H. M., and Dockter, K. W. (1967) Assessment of ecological effects of extensive or repeated use of herbicides. Advanced Research Projects Agency, Department of Defense, ARPA No. 1086, DDC No. AD 824314. 372 pp.

[Background material.]

464. Huddle, F. P. (1969) A technology assessment of the Vietnam defoliant matter: a case history. Report to the Subcommittee on Science Research and Development of the Committee on Science and Astronautics. US House of Representatives, 91st Congress, Prepared by the Science Policy Research Division, Legislative Reference Service, Library of Congress, Washington, DC. 73 pp.

[Background material.]

465. Hudson, A. J. (1977) Amyotrophic lateral sclerosis and toxic hydrocarbons. [letter to the editor] Arch. Neurol. 34:721.

[Editorial.]

466. Huff, J. E., Moore, J. A., Saracci, R., and Tomatis, L. (1980) Long-term hazards of polychlorinated dibenzodioxins and polychlorinated dibenzofurans. Environ. Health Perspec. 36:221-240.

[Review article.]

467. Huff, J. E., and Wassom, J. S. (1974) Health hazards from chemical impurities: Chlorinated dibenzodioxins and chlorinated dibenzofurans. Inter. J. Environmental Studies. 6:13-17.

[Review article.]

468. Huff, J. E., and Wassom, J. S. (1973) Chlorinated dibenzodioxins and dibenzofurans. Environ. Health Perspec. 5:283-313.

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469. Hughes, J. S., and Davis, J. T.. (1963) Variations in toxicity to bluegill sunfish of phenoxy herbicides. Weeds 11:50-53.

[Background material.]

470. Hughes, R. D., Millburn, P., and Williams, R. T. (1973) Biliary excretion of some diquatery, ammonium cations in the rat, guinea pig and rabbit. Biochem. J. 136(4):979-984.

Biotransformation and urinary and biliary excretion of diquat by the rabbit, guinea pig, and rat are described. Anesthetized female animals with cannulated bile ducts were administered an intraperitoneal dose of 14 μmol (cation)/kg of diquat dichloride to Dutch rabbits, 13 $\mu\text{mol}/\text{kg}$ to English guinea pigs and 40 $\mu\text{mol}/\text{kg}$ to Wistar rats. Rats were maintained under anesthesia for 24 hr. and the remaining animals for 3 hr. Bile and urine samples were collected and analyzed for radioactivity. Metabolites were separated from the parent compound by paper chromatography and by ion exchange chromatography, followed by colorimetric analysis. In 3 hrs. the percentages of administered diquat excreted were 3% and 5% in bile of rabbits and guinea pigs, respectively and 64% and 45% in urine, respectively. Excretion by the rat was 1.4% in bile at 3 hr., 2.2% in bile at 24 hr., and 82% in urine in 24 hr. Half of the radioactivity in rat bile and all in the urine was unmetabolized diquat. Two other biliary metabolites were separated but not identified. In the rabbit and guinea pig, 89 and 72% respectively of urinary radioactivity was recovered as unmetabolized diquat and the remainder chromatographed with the metabolite in rat bile. After renal ligation, 6.1% of diquat administered to rats was excreted in bile in 3 hr., compared to 1.4% in non-ligated controls. Excretion of 4 other compounds were studied. The authors concluded that biliary excretion of dications with molecular weights below 500-600, accounted for excretion of less than 10% of the administered dose.

471. Hull, A. C., Jr. (1971) Effect of spraying with 2,4-D upon abundance of pocket gophers in Franklin Basin, Idaho. J. Range Manage. 24:230-232.

[Background material.]

472. Hunter, J. H., and Young, A. L. (1972) Vegetative succession studies on a defoliant-equipment test area. Technical Report AFATL-TR-72-31. Air Force Amament Laboratory, Eglin Air Force Base, Fla.

[Background material.]

473. Hurtt, W., and Danoro, R. A. (1968) Biological effectiveness of still bifluid and orange. Air Force Armament Laboratory, Air Force Systems Command, Eglin AFB, Fla. Technical Report No. AFATL-TR-68-122.

[Background material.]

474. Hurtt, W., and Darroro, R. A. Comparison Test of defoliants. Vol II. Appendix III. Biological effectiveness of still bifluid and Orange. Air Force Armament Laboratory, Air Force Systems Command, Eglin AFB, Fla. Publication No. ADTC-TR-69-30.

[Background material.]

475. Hussain, S., Ehrenberg, L., Lofroth, G., and Gejvall, T. (1972) Mutagenic effects of TCDD on bacterial systems. Ambio 1:32-33.

The authors evaluated the mutagenicity of TCDD in three bacterial systems: 1) reversion to streptomycin independence in Escherichia coli Sd-4, 2) reversion to histidine independence in Salmonella typhimurium strains TA 1530 and TA 1532 which respond to mutation by base pair substitution and frame shift mutation, respectively and 3) prophage induction in E. coli K-39. TCDD (99% purity) in DMSO was added to suspension cultures of lag phase E. coli Sd-4 at 0.5, 1, 2, and 4 ug/ml for 1 hour. Mutation frequencies appeared to increase at 2 ug/ml. However, the two replicate cultures reported had a wide variation in frequencies of mutation (34×10^{-8} and 256×10^{-8}), but not in survival, 18% and 11% respectively. Since the variation was so wide it is difficult to make conclusions about the result of the study. No further testing was done. In addition, lag phase cells were exposed in this experiment. Other investigators have found that this is not the most sensitive stage for mutagenic effects to occur. In S. typhimurium TA1530, no increased numbers of revertants were observed at 1 and 10 ug/ml TCDD for 1 hour. Survival values were 90 and less than 1% respectively. In strain TA1532 increased mutation frequency was observed when TCDD caused bacterial survival to decrease to about 1%. No numerical data were presented to analyze the author's results. However, in this test results which have survival rates of less than 10% are not generally recognized as being a reliable indicator of a positive result. In the prophage induction experiment TCDD in DMSO was incubated at 0, 0.5, 1, 1.5, and 2.5 ug/ml for 30 minutes with E. coli K-39 () cells. E. coli K-49 cells were used as indicator cells and incubated with the treated, washed K-39 () cells for 2 hours. Numbers of replicate cultures were not reported. The solvent DMSO appeared to have an effect on prophage induction. In the controls without DMSO, 7.3×10^{-5} plaques/ml were observed while only 1.9×10^{-5} plaques/ml were observed with DMSO. The significance of this effect could not be calculated because of lack of data. At 0.5 ug/ml, TCDD induced 5.4×10^{-5} plaques/ml, about a two-fold increase compared to DMSO controls. No other concentration of TCDD increased the numbers of plaques observed. As a result of lack of data from replicate cultures, no statistical analysis of results, and poor presentation of experimental design, the conclusion of the authors i.e., that TCDD is mutagenic in S. typhimurium TA1532 and is a weak inducer of prophage, cannot be relied on.

476. Hwang, S. W. (1973) Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the biliary excretion of indocyanine green in rat. Environ. Health Perspec. 5:227-244.

Hepatobiliary function was evaluated in rats administered TCDD. Male CD rats were administered a single dose of 5 or 25 ug/kg TCDD perorally in acetone-corn oil. Controls received vehicle only. Biliary excretion and bile flow were studied in 4 rats per treatment group 1, 7 and 16 days after treatment by measuring indocyanine green (ICG) excretion. Rats were anesthetized and after indocyanine green was administered intravenously bile was collected from cannulated bile ducts for 20 minutes and plasma was collected periodically over the 20 minute collection period. The levels of ICG in the blood and bile samples and in the liver at the end of the experiment were determined spectrophotometrically. Significant increases in bile flow and in liver weights of treated rats over controls occurred 7 and 16 days after TCDD treatment. The rate of ICG excretion was decreased in treated rats 7 and 16 days after treatment. All of these effects were greater after the 25 ug/kg dose of TCDD than after the 5 ug/kg dose. ICG concentrations in the blood and liver were higher in treated rats than controls and bile concentrations were lower in treated rats. Liver-to-plasma concentration ratios of ICG were decreased by the high dose, indicating a dose-dependent inhibition of hepatic uptake of ICG. Bile-to-liver ratios, an indication of ICG excretion into bile, was inhibited to the same extent by both doses. Plasma ICG clearance decreased in a dose-dependent manner after TCDD treatment. The decrease in clearance was more marked after 16 days than after 7 days. The authors concluded that TCDD produced a long-lasting decrease in hepatobiliary excretion which could alter the elimination of actively secreted anions.

477. Hwang, S. W., and Schanker, L. S. (1973) Absorption of organic arsenical compounds from the rat small intestine. Xenobiotica. 3(6):351-355.

The absorption of cacodylic acid by the small intestine of the rat was determined. The small intestines of entobarbital-anesthetized male Charles River derived rats were exposed, ligated and injected lumenally with sodium cacodylate. The incision was then closed and absorption was allowed to take place for up to 5 hours. After the absorption period was terminated, the intestinal content was assayed for the proportion of the dose that was not absorbed and the tissue samples were analyzed for arsenous oxide. The half time for the apparent first-order absorption of cacodylic acid (administered at a concentration of 5mM) was 1 calculated at 201 minutes. Other arsenicals that were studied included carbarsone and tryparsamide. The rates of absorption for the 3 compounds correlated with the chloroform-water partition coefficients for these compounds, but not with the molecular weights. Absorption was not saturated in the concentration range of 1-100 mM. The authors concluded that cacodylic acid was absorbed at a moderate rate compared to rates for other drugs, and that absorption was probably by simple diffusion through a lipid phase.

478. Innes, J. R. M., Ulland, B. M., Valerio, M. G., Petrucelli, L., Fishbein, L., et al. (1969) Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: a preliminary note. J. Natl. Cancer Inst. 42(6):1101-1114.

The authors evaluated 120 pesticides for carcinogenicity in a bioassay in mice. Test compounds included the following: 2,4-D isopropyl ester, 2,4-D isooctyl ester, 2,4-D, diuron, 2,4,5-T, and monuron. All compounds tested were commercial formulations. No purity information on these preparations was presented by the authors. Males and females of two strains of mice, (C57BL/6 x C3H/Anf)F₁, and C57BL/6 x AKR)F₁, were used in the study and received the maximally tolerated dose for each compound: 46.4 mg/kg, 2,4-D isopropyl ester, butylester, isooctyl ester or acid, 21.5 mg/kg 2,4,5-T, 464 mg/kg diuron, 1000 mg/kg 2,4-D acid, or 215 mg/kg monuron. Starting at 7 days and until 4 weeks of age, each mouse received daily doses of the compound in 0.5% gelatin by stomach tube. After weaning, 18 mice of each sex per strain received approximately the maximally tolerated dose in their diet for 18 months. Chemical analysis of diet formulations for correct amounts of test compound was not reported by the authors nor was storage length of treated feed reported. Both positive and negative controls were included in the study. None of the herbicides mentioned above caused a significant increase in tumors in either sex or strain of mice.

479. Interagency Work Group to Study the Possible Long-Term Health Effects of Phenoxy Herbicides and Contaminants. (1980a) [February, 1980 report] 18 pp.

[Background material.]

480. Interagency Work Group to Study the Possible Long-Term Health Effects of Phenoxy Herbicides and Contaminants. (1980b) [March, 1980 report] 109 pp.

[Background material.]

481. Interagency Work Group to Study the Possible Long-Term Health Effects of Phenoxy Herbicides and Contaminants. (1980c) [August, September, and October, 1980 report] 139 pp.

[Background material.]

482. Interagency Work Group to Study the Possible Long-Term Health Effects of Phenoxy Herbicides and Contaminants. (1980d) Summary Report of the Public Meeting of the Interagency Work Group to Study the Possible Long-Term Health Effects of Phenoxy Herbicides and Contaminants. Sept. 22, 1980. 72 pp.

[Background material.]

483. International Agency for Research on Cancer. (1977) IARC monographs on the evaluation of the carcinogenic risk of chemicals to man: Some fumigants, the herbicides 2,4-D and 2,4,5-T, chlorinated dibenzodioxins and miscellaneous industrial chemicals. 15:41-299.

[Review article.]

484. International Agency for Research on Cancer. (1976) IARC monographs on the evaluation of carcinogenic risk of chemicals to man: Some carbamates, thiocarbamates and carbazides. 12:167-176.

[Review article.]

485. Irish, K. R., Darrow, R. A., and Minarck, C. E. (1969) Information manual for vegetation control in Southeast Asia. Mis. Pub. 33. Department of the Army, Fort Detrick, Frederick, Md. 71 pp.

[Not available.]

486. Isensee, A. R. Bioaccumulation of 2,3,7,8-tetrachlorodibenzo-para-dioxin. In Chlorinated Phenoxy Acids and Their dioxins. C. Ramel, ed., (Ecol. Bull. No 27, Stockholm: Swedish Natural Science Research Council, 1978) pp. 255-262.

[Review, article.]

487. Isensee, A. R., and Jones, G. E. (1971) Absorption and translocation of root and foliage applied 2,4-dichlorophenol, 2,7-dichlorodibenzo-p-dioxin, and 2,3,7,8-tetrachlorodibenzo-p-dioxin. J. Agric. Food Chem. 19(6):1210-1214.

[Background material.]

488. Isensee, A. R., and Jones, G. E. (1975) Distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in aquatic model ecosystems. Environ. Sci. Technol. 9(7):668-672.

The authors studied the bioaccumulation of TCDD in an aquatic ecosystem. Concentrations of 0.0001-7.45 ppm ring-labeled ¹⁴C-TCDD were added to soil samples in aquaria which were filled with 4 liter water. Duplicate tanks were used for each concentration. One day later about 100 daphnids, 8 snails, a few strands of algae, and 10 ml of water containing aquatic microorganisms were added to the tanks. Water samples were removed at 2-day intervals for scintillation counting. At 30 days, samples of daphnids were removed and 2 mosquito fish added to each tank. On day 33, all organisms were removed and analyzed for ¹⁴C-TCDD. Two fingerling catfish were then added to each tanks and

were harvested 6 days later. At 3.17 ppm, TCDD in water reached equilibrium in 4 days while 0.1 and 0.001 ppm TCDD did not reach equilibrium until day 15. No toxicity to aquatic organisms was observed. However, fish were only treated for 3-6 days which may not be a long enough; since TCDD toxicity is delayed in most organisms. TCDD concentrations in the organisms approached concentrations that were in the soil samples. Water concentrations averaged 0.015% that in the soil. Bioaccumulation averaged from $\frac{2}{3}$ - 2.6×10^4 for snails, mosquito fish, and daphnids, and $4-9 \times 10^3$ for duckweed, algae, and catfish, indicating that TCDD has the potential to accumulate in the environment. Highly significant correlation coefficients ($r = 0.94$) were obtained between TCDD in organisms and in water, suggesting that bioaccumulation of TCDD by aquatic organisms is dependent on TCDD available in the water.

489. Isensee, A. R., Kearney, P. C., Woolson, E. A., Jones, G. E., and Williams, V. P. (1973) Distribution of alkyl arsenicals in model ecosystem. Environ. Sci. Technol. 7:841-845.

The authors describe the behavior of cacodylic acid in a model aquatic ecosystem. ^{14}C -Cacodylic acid (10.6 ppb) was added to aquarium tanks containing 4 liters of "standard reference water", and 10 of sandy loam soil was added to each treated and control tank. Organisms added to each tank included 3 fish, 10 snails, a few strands of algae, 30 Daphnia, and a few ml of old aquarium water that contained diatoms, protozoa, and rotifers. Fish, Daphnia, snails, and algae were exposed to cacodylic acid for 3, 29, 32, and 32 days, respectively. Lower food chain organisms (algae and Daphnia) bioaccumulated more cacodylic acid than did higher food chain organisms, indicating that cacodylic acid does not biomagnify in the food chain. Bioaccumulation ratios were 1,635, 1,658, 419, and 21 for algae, Daphnia, snails, and fish, respectively. According to the authors, bioaccumulation of cacodylic acid was probably due to adsorption by the organisms. Algae and Daphnia have a higher surface area to mass ratio than fish or snails and therefore could adsorb more cacodylic acid. The authors concluded that these results show that cacodylic acid does not have a high potential to biomagnify in the environment.

490. Jackson, S. (1980) Agent Orange - Bibliography, (National Library of Medicine Literature Search, No. 80-30) 9 pp.

[Bibliography.]

491. Jensen, N. E. (1972) Chloracne: 3 cases. Proc. Roy. Soc. Med. 65:21-22.

Chloracne was described in two men exposed to 2,4,5-trichlorophenol and in the son of one of the men. Three years before this incident, an explosion had occurred in a factory that produced 2,4,5-trichlorophenol, and the factory had been dismantled and buried when 70 maintenance workers at the site developed chloracne. (In these workers, chloracne remained severe for 1-1.5 years and still persisted in some after 3 years). Several large tanks, however, were repeatedly cleaned and put into use again. Two men who set up one of these tanks for a new use developed chloracne on the face, trunk, and arms 1 to 2 months after working with the tank. No other clinical symptoms were reported, and both still had chloracne 11 months after it was initially diagnosed. Three months after his father's symptoms developed, the 4-year old son of one worker developed chloracne with the same clinical pattern. The authors suggested that the child developed chloracne after touching his father's work clothes. The serum lipid levels and liver function tests of the patients were normal. Rabbits housed in the decontaminated tanks before their re-use failed to develop skin lesions. In an addendum, the authors mentioned that the wife of the other worker developed facial chloracne 11 months after her husband was exposed to TCDD.

492. Jensen, N. E., Sneddon, I. B., and Walker, A. E. (1972) Tetrachlorodibenzodioxin and chloracne. Trans. St. Johns Hosp. Derm. Soc. 58(2):172-177.

A brief description of four cases of chloracne is given, and a brief review of circumstances that caused chloracne in the past is presented. The four cases were described in another publication (Jensen, N.E. Proc. Roy. Soc. Med. 65:21-22) and involved two maintenance workers and two members of their families. The workers were exposed to equipment from a trichlorophenol plant in England where an explosion had occurred. An analysis of sebum from the forehead of one patient 1 year after exposure did not reveal TCDD. The analysis was by gas liquid chromatography, which can detect 1 part TCDD per 10 million of sample. Based on the simultaneous 1-day exposure of both workers to the contaminated equipment and the simultaneous development of symptoms, the authors concluded that chloracne resulted from exposure to a pocket of residual TCDD.

493. Jenssen, D., and Renberg, L. (1976) Distribution and cytogenetic test of 2,4-D and 2,4,5-T phenoxyacetic acids in mouse blood tissues. Chem. Biol. Interact. 14:291-299.

The authors tested 2,4-D and 2,4,5-T in a micronucleus test in mice, a test indicating chromosome breakage. Male CBA mice (8-12 weeks old) received a single 100 mg/kg intraperitoneal injection of 2,4-D or 2,4,5-T (TCDD less than 1 ppm). Three animals were used per treatment in the micronucleus test and killed 24 hours or 7 days after injection. Positive and negative control animals were included in the study. Approximately 2,000 bone marrow cells from each animal were analyzed for numbers of polychromatic erythrocytes with micronuclei. Plasma and cells of bone marrow and peripheral blood at 4 and 24 hours post injection were analyzed for 2,4-D and 2,4,5-T by gas chromatography in another group of two mice per treatment. No increase in polychromatic erythrocytes with micronuclei was observed in animals treated with 2,4-D or 2,4,5-T 24 hours or 7 days. However, a weak toxic effect on mitotic activity was observed in treated animals. Both compounds appeared in peripheral blood and bone marrow within 4 hours after injection. 2,4-D and 2,4,5-T levels in blood plasma declined by 24 hours. No more than 50% of the plasma levels of the herbicides were found in cell fractions. The authors concluded that results of the test were not a reliable indicator of a lack of mutagenicity because the 2,4-D and 2,4,5-T do not enter the target cells to an appreciable extent. However, since the data were in agreement with the known rapid excretion of these compounds, the authors also concluded that no cytogenetic hazard is connected with 2,4-D and 2,4,5-T.

494. Jirasek, L., Kalensky, J., and Kubec, K. (1973) Acne chlorina and porphyria cutanea tarda during the manufacture of herbicides. Part I. Cesk. Dermatol. 48(5):306-315.

The pattern of dermal toxicity that developed in 76 workers involved in the manufacture of 2,4,5-T and pentachlorophenol was described. All workers but two were male, and 66 percent were under 30 years old. Only six cases of chloracne began with acute dermatitis and sensitivity to the sun; the remainder of the cases involved development of chloracne in an "occult" manner. Inflammation occurred only in the most severe cases of acne. Porphyria cutanea tarda was detected in 11 persons and in two additional patients who were free of acne. In most patients, acne began with the appearance of comedones on the face above the cheek bones, but in 17 cases the first signs were papulopustules on the legs. In general, the eyebrows, eyelids and scalp were not affected. In five persons, the skin of the entire body was affected. In 19 cases, hypertrichosis, hyperpigmentation of the face, or both occurred without any evidence of a disorder of porphyrin metabolism. The extent of exposure and latency period between exposure and the appearance of symptoms varied considerably among workers. Fatigue, muscle pains, loss of appetite, headaches, and other subjective disorders were more frequent among patients with extensive skin symptoms. These and other symptoms were described in another report and are only mentioned in this report. Details of the manufacturing process were

described. The authors concluded that TCDD was responsible for the observed toxicity; the plant remained closed at the time of the report and construction of a new factory was being considered.

495. Jirasek, L., Kalensky, J., Kubec, K., Pazderova, J., and Lukas, E. (1974) Acne chlorina, porphyria cutanea tarda and other manifestations of general intoxication during the manufacture of herbicides, Part II. Cesk. Dermatol. 49(3):145-157.

A description of symptoms observed in 50 patients involved in the manufacture of 2,4,5-T was presented. The patients (except two) were among the group of 78 patients whose dermatological conditions were described in a previous report by the authors (Jirasek, et al., 1973). About 80 percent of the cases became ill during their period of employment in the manufacture of 2,4,5-T, while the remainder became ill 1-18 months after exposure had ended. Illnesses were identified between 1965 and 1968 and physical examinations were performed between 1967 and 1973. The average age of the study group was 35.9 years and over half of the patients with porphyria cutanea tarda were over 40 years old. In 11 patients uroporphyrin levels were elevated consistently and in 12 others were elevated intermittently. The levels gradually decreased from a mean of 1,043 (u)g/24 hours in 1969 to 235 (u)g/24 hours in 1973. Other porphyrin levels were not elevated. In 10 patients with elevated uroporphyrin levels, other symptoms of porphyria cutanea tarda also occurred primarily hyperpigmentation and hypertrichosis. Fluorescence of several tissues removed at autopsy was observed under UV light. Elimination of delta-amino levulinic acid was twice as high for exposed patients as for a control group in 29 patients. Lipid metabolism was altered in half of the patients and liver function tests were abnormal in 11 cases. The mean total blood protein levels were higher in all years for the exposed group than for a control group that was not described further. Mild to moderately severe neurological disorders were observed in 17 cases. All cases involved central neurological symptoms and 13 involved peripheral neuronal damage, usually in the legs. In 27 percent of the patients electroencephalogram results were interpreted as abnormal and in 1 patient as markedly abnormal. Five people had died at the time of the report, including two (ages 47 and 59) from bronchogenic carcinoma, which were too few cases to establish a causative link with exposure to TCDD. Arteriosclerosis occurred in one patient and the authors suggested that the disturbance in lipid metabolism in exposed patients may have created a predisposition for this disease. The authors concluded that TCDD exposure produced toxicity in many organ systems.

496. Jirasek, L., et al. (1976) Chloracne, porphyria cutanea tarda and other intoxications by herbicide. Der Hautarzt 27:328-333.

The author presents a summary of the illnesses in workers from a herbicide factory in Czechoslovakia that was contaminated with TCDD. All of the findings were presented in more detail in a previous report (see Jirasek et al., 1974).

497. Johnson, E. F., and Muller-Eberhard, U. (1977) Multiple forms of cytochrome P-450 from liver microsomes of rabbits treated with 2,3,7,8-tetrachlorodibenzo-p-dioxins. Fed. Proc. Fed. Am. Soc. Exp. Biol. 36(3):833.
- [Abstract, only.]
498. Johnson, J. E. (1971) The public health implications of widespread use of the phenoxy herbicides and picloram. BioSci. 21(17):899-905.
- [Review article.]
499. Joint NIEHS/IARC Working Group Report. (1978) Long-Term Hazards of Polychlorinated Dibenzodioxins and Polychlorinated Dibenzofurans. World Health Organization - International Agency for Research on Cancer, Lyon.
No. 78/001. 57 pp.
- [Review article.]
500. Jones, E. L., and Kizek, H. (1962) A technique for testing acnegenic potency in rabbits, applied to the potent acnegen, 2,3,7,8 tetrachlorodibenzo-p-dioxin. J. Invest. Dermatol. 9:511-517.

The histology and keratin content of rabbit ears treated with TCDD were studied. Doses of 0.3-10 (u)g of TCDD (synthesized from 2,4,5-trichlorophenol in the laboratory) were applied on 3 successive days to the inner surface of one ear of rabbits, while acetone only was applied to the other ear. Three biopsy samples were removed with a punch from the anterior, posterior, and middle regions of the ear 14 days after the first application was made. The biopsies were cleared of cartilage and digested with pepsin, and the dried weight after digestion was reported as the keratin content. Other biopsy specimens were stained for histopathological examination. A dose-related increase in keratin occurred after TCDD treatment, with three times more keratin in biopsy plugs from rabbit ears treated with 10 (u)g TCDD compared to the keratin levels in the other ear. The lowest dose, 0.3 (u)g, was ineffective in altering keratin levels but produced follicular dilatations. Large comedones were produced by 3 (u)g of TCDD in the rabbits that showed the largest increases in keratin content from this dose. Histologically, TCDD-treated ears showed a characteristic hyperkeratosis and hyperplasia of the surface epidermis. Few subaceous cells were present in tissues treated with high doses of TCDD, and the follicles from these tissues were filled with a keratinous mass. Keratin masses took the form of tree structures at lower doses and were oval, of typical comedone shape, at high doses. The authors concluded that the experimental system presented was appropriate for evaluating the dermatologic effects of acnegens. The method used to quantitate keratin is non-specific and probably does not remove 100 percent of non-keratin components, while leaving 100 percent of the original

amount of keratin. However, the results clearly confirm an acneiform response of this animal model to TCDD and support the contention that keratin production is increased in afflicted tissue.

501. Jones, G. (1975) A histochemical study of the liver lesion induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin) in rats. J. Pathol. 116:101-105.

Hepatic plasma-membrane associated enzymes were evaluated histochemically and hepatic morphology was examined histopathologically in rats administered TCDD acutely. Male Portland rats were administered 200 ug/kg TCDD in arachis oil orally. Between 1 day and 9 months later, groups of three treated and one control rat were killed and liver tissue was prepared for histology. Acid phosphatase, adenosine triphosphatase (AT Pase), 5-nucleotidase, and alkaline phosphatase were identified in liver slices and adjacent slices were stained with hematoxylin and eosin for histopathological examination. Typical TCDD-induced hepatic lesions were observed at 34 and 42 days, but not earlier. These changes included alterations in trabecular and sinusoidal patterns and cell size, the appearance of multi-nucleate cells, and fibrosis around the central vein. At 9 months, partial correction of these lesions was observed. AT Pase was diminished around the central vein along the canalicular borders of the parenchymal cells 3 days after TCDD treatment and by 5-8 days AT Pase was reduced in the midzone with normal localization of this enzyme only around the portal tracts. The sinusoids in the centrilobular zone showed dense staining. These changes in the AT Pase reaction persisted at 34 days and 42 days only in animals that also showed clinical signs of intoxication at this time. At 9 months, when surviving rats showed improvement of their clinical state, the AT Pase reaction was restored to normal. No significant and consistent changes in the quantity or distribution of the other enzymes were observed. The authors suggested that an increase in the sinusoidal AT Pase levels was indicative of an inflammatory reaction and that the loss of parenchymal AT Pase activity indicated loss in membrane function that precedes morphological changes and correlates with the clinical state of the animal. The authors concluded that the site of TCDD toxicity is the parenchymal cell plasma-membrane.

502. Jones, G., and Butler, W. H. (1974) A morphological study of the liver lesion induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats. J. Pathol. 112(2):93-97.

The development of multinucleate cells in the centrilobular zone of the liver of rats after TCDD administration is described. A dose of 200 ug/kg TCDD in dimethylsulfoxide or arachis oil was administered orally to groups of four Porton rats and one group was killed each week during the subsequent 10 weeks. Two vehicle-control rats were killed with each treatment group. Prior to death, the liver was perfused in situ with glutaraldehyde and then sliced and prepared for light and electron microscopies and at 1 week degeneration of parenchymal cells was

evident by light microscopy. Throughout the experiment nuclear morphology was normal and damage was restricted to the cytoplasm of the hepatic parenchymal cells of the centrilobular zone. Proliferation of smooth endoplasmic reticulum was evident, as well as fusion of plasma membranes, which were interpreted in relation to enzyme induction and the formation of multinucleate cells, respectively. Mural fibrosis of the central veins was also observed. The endothelial cells that line the sinusoids appeared normal. The authors concluded that TCDD induces membrane abnormalities at the canalicular borders of hepatocytes.

503. Jones, G., and Greig, J. B. (1975) Pathological changes in the liver of mice given 2,3,7,8-tetrachlorodibenzo-p-dioxin. Experientia 31:1315-1317.

Histopathological changes of the liver were described for mice. Male C57BL/6 mice were administered 250 ug/kg of TCDD by oral intubation in arachis oil and were killed 1 to 35 days later. The liver was weighed, homogenized, and analyzed for protein DNA, fatty acid, and cholesterol. The body weights of treated mice fell while the liver weight increased relative to body weight, which was attributed to the concomitant increase in hepatic lipid content. The protein and water contents of the liver decreased and the DNA content remained unaltered and was attributed to inflammatory infiltration. The hepatic lesions regressed in surviving mice and no multinucleate hepatocytes were observed. The authors concluded that the hepatic lesions in TCDD-treated mice differed substantially from those observed in the rat after TCDD administration and suggested that the fatty liver in the mouse could be the result of decreased food uptake. Eight days after the compound was given, the liver was larger and paler than controls and a progressive necrotic centrilobular lesion developed.

504. Jones, K. G., and Sweeney, G. D. (1980) Dependence of the porphyrogenic effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin upon inheritance of aryl Toxicol. Appl. Pharmacol. 53:42-49.

The induction of several hepatic enzymes and the porphyrogenic effects of TCDD was observed in hybrid mice that were backcrossed from genetically AHH (aryl hydrocarbon hydroxylase) responsive and non-responsive mice. Doses of 25 ug/kg TCDD in 1,4-dioxane were administered weekly to groups of 6-9 C57BL/6J (AHH responsive Ah/Ah) mice, (non-AHH responsive, ah/ah) DBA/2J mice and D2 (B6 D2) F₁/J (backcrossed) mice. Urinary porphyrins and hepatic cytochrome P-450 levels, AHH, uroporphyrinogen decarboxylase (UD) and aminolevulinic acid (ALA) synthetase activities were assayed. After 6 weeks of treatment, C57BL mice excreted 6 times as much porphyrin (comprised primarily of uroporphyrin) as DBA mice, which excreted primarily coproporphyrin. UD activity was inhibited only in C57BL mice. The genotype of backcrossed mice (from a cross of DBA mice with C57BL X DBA offspring) were categorized as AHH responsive (Ah/ah) or AHH non-responsive (ah/ah) from zoxazolamine paralysis times after beta-naphthoflavone pretreatment. Half of each group was administered TCDD weekly for 11 weeks. Only

mice with the Ah gene that controls AHH responsiveness showed elevated porphyrin excretion. Only these groups had significantly decreased UD levels, by 80% in Ah/Ah (C57BL) mice and 33% in Ah/ah mice. AHH activity and microsomal cytochrome P-450 levels were induced by TCDD in all groups and ALA-synthetase activity was unchanged in all groups. The authors concluded that porphyria susceptibility genetically segregates with the gene controlling AHH responsiveness and these phenotypes are controlled by the same gene or are closely linked. The levels of porphyrin excreted by Ah/Ah mice and Ah/ah mice after TCDD treatment were indistinguishable, which contrasts with the theory of Poland and Glover (1975) that the heterozygous mice have a lower affinity TCDD receptor than homozygous mice.

505. Jones, K. G., and Sweeney, G. D. (1979) Iron deficiency prevents liver toxicity of 2,3,7,8- tetrachlorodibenzo-p-dioxin. Fed. Proc. Fed. Am. Soc. Exp. Biol. 38(3):536.

[Abstract, only.]

506. Jorgenson, T. A., Rushbrook, C. J., and Newell, G. W. (1976) In vivo mutagenesis investigations of ten commercial pesticides. Toxicol. Appl. Pharmacol. 37(1):109.

[Abstract, only.]

507. JRB Associates, Inc. (1980) EPA 2,4,5-T/Silvex cancellation hearing: Review of direct testimony and supporting documents on Seveso Accident. Final report of EPA contract no. 68-01-6280: Assignment 1. 60 p.

[Background material.]

508. Jung, H. D., and Wolf, F. (1977) Contact eczema due to Selest 100 herbicide in forestry. Dtsch. Gesundheitswesen 32(31):1464-1467.

[Not available.]

509. Juzwiak et al. (1973) A study of the extent of the pesticide threat to the health of agricultural workers in Olesnicki County. PTL-MS 28(11):419-422.

The authors measured concentrations of 2,4-D sodium salt in air at a pesticide manufacturing plant and during spraying of agricultural fields. Five 40-60 minute air samples were collected from various locations in a 2,4-D warehouse. Methods of sample analysis were not specified. Concentrations in air averaged 0.0012 - 0.0036 mg/l with a range of 0.0005 - 0.0065 mg/l. The average concentrations of 2,4-D in air exceeded the maximum allowable concentration of 2,4-D for both the Soviet Union and the USA. Air samples collected during agricultural

spraying also exceeded recommended standards. Concentrations of 2,4-D and MCPA measured on tractors ranged from 0.003 - 0.026 mg/l while measurements taken 20-120 m from the field ranged from 0.0016 - 0.010 mg/l. The authors concluded that the exposure of warehouse and agricultural employees to harmful effects of this herbicide is high.

510. Kalra, S. K., and Chahal, K. S. (1979) Degradation of diuron (3-(3,4,-dichlorophenyl)-1,1,-dimethylurea) in cows. Ecotox. Environ. Safety 3(4):362-368.

Excretion and metabolism of diuron was studied in the cow. Four Holstein dairy cows were administered diets containing 5, 10, 25, or 50 ppm diuron for 33 days and milk, urine, and feces were collected daily for the first 5 days and weekly thereafter. Blood samples were collected 15 minutes, 1 hour, and 12 hours after the first feeding, daily for 3 more days and weekly thereafter. Diuron levels were determined in all biological fluids by a colorimetric method. Recovery of diuron was 79-89% and data was corrected accordingly. Metabolites were separated by thin layer chromatography. Toxic effects were seen after the highest 2 dosages and included pyrexia, reduced milk yield, and hemuresis. For all cows, from 33-43% of the doses of diuron were recovered in urine, 6-7% in feces and none in milk. The authors reported that "The major portion of the herbicide was excreted in urine, followed by feces, and the least in blood" and presented in a table entitled "Mean Percentage Excretion of Diuron Residues and Metabolites" values of 2-5% for blood for the 4 cows, but did not explain which blood samples were analyzed for these values. Two metabolites, 3-(3,4-dichlorophenyl)-1-methylurea and 3(3,4-dichlorophenyl) urea were detected in urine and accounted for 21-23% and 6-8% of the dose. Diuron was stated to be present in smaller amounts than the metabolites, although data presented in the table did not agree with this statement. About 35% of the dose of diuron was not accounted for in the samples analyzed. The authors concluded that diuron was metabolized by 2 N-demethylation steps, analogous to reactions in higher plants, and could be degraded by gut flora. The inconsistencies in the presentation of data obscure the findings of these experiments.

511. Kanazaki, H., Sera, T., Inoue, Y., and Takahashi, T. (1973) On the health disturbance of the inhabitants around a pesticide factory in Araki area in Kerume City. (Abstract). J. Jap. Ass. Rural Med. 22(3):198-199.

[Not available.]

512. Karickhoff, S.W., Brown, D.S., and Scott, T.A. (1979) Sorption of hydrophobic pollutants on natural sediments. Water Research 13:241-248.

[Background material.]

513. Kaufman, D. D., and Kearney, P. C. Microbial transformations in the soil. In Herbicides: Physiology, Biochemistry, Ecology. Vol. 2. ed., L. J. Audus, (New York: Academic Press, 1976) pp. 29-64.

[Review article.]

514. Kay, J. H., Palazzolo, R. J., and Calandra, J. C. (1965) Subacute dermal toxicity of 2,4-D. Arch. Environ. Health 11:648-651.

The subchronic dermal toxicity of 3 commercial formulations of 2,4-D were studied in rabbits. The dimethylamine salt, isooctyl ester or butyl ester of 2,4-D (other components of the formulations were not given) was applied to shaved and abraded or intact skin and covered for 7 hours per day with plastic. At the end of each daily application the compound was washed from the skin with soap and water. The compounds were applied as 0.6 to 3.1% 2,4-D acid equivalents in water or furnace oil and were administered 3 days per week for 3 weeks. Vehicle controls received oil or water only. Body weights, incidence of mortality, blood chemistries and hematology, behavioral changes, and local skin reactions were monitored. At the end of the treatment period, the animals were killed and organs were weighed and examined grossly and microscopically. Of 112 rabbits used in the study, 12 died during the experiment, but none of the deaths were attributed to 2,4-D treatment. All rabbits that received oil, including vehicle controls, experienced pain from the applications and local severe inflammatory reactions. The reaction produced by water or aqueous 2,4-D solutions was milder. No alterations were observed in treated rabbits compared to their controls in body weights, hematologic and clinical blood chemistry results, or weights or microscopic or gross pathology of any tissue (including peripheral and central nervous system tissues) except the skin. Inflammation of the skin was similar in incidence and degree for all water-treated groups, regardless of whether 2,4-D was administered. Inflammation in the oil-treated groups was more severe in the presence of 2,4-D. The authors concluded that exposure to 2 to 5 times the anticipated amount of 2,4-D expected from home or field use produced no significant adverse effects attributed to 2,4-D except local inflammation that was produced by the vehicles, alone.

515. Kay K. (1975) Conference on toxicology- epidemiology-health effects of pesticides. Clin. Toxicol. 8(3):289-300.

[Review article.]

516. Kaye, Scholer, Fireman, Hayes, and Handler (Hearing Attorneys for the Dow Chemical Co.). In re: 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T). Dow Prehearing Memorandum No. 2. FIFRA Docket No. 295 et al. U.S. Environmental Protection Agency; Before the Administration. January 18, 1974. 25 pp.

[Not available.]

517. Kaye, Scholar, Fireman, Hayes, and Handler (Hearing Attorneys for the Dow Chemical Co.). In re: 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Dow Prehearing Memorandum No. 3. FIFRA Docket No. 295 et al. U.S. Environmental Protection Agency; Before the Administration. February 22, 1974. 25 pp.

[Not available.]

518. Kearney, P. C., Woolson, E. A., and Ellington, C. P., Jr. (1972) Persistence and metabolism of chlorodioxins in soils. Environ. Sci. Technol. 6(12):1017-1019.

The authors studied persistence and metabolism of TCDD in two soil types, Hagerstown silty clay loam and Lakeland loamy sand. The major differences between the two soil types are that Hagerstown soil is high in organic matter and supports a high microbial population, and the Lakeland soil is low in organic matter soil and microbial activity. TCDD was added to 100 g portions of each soil at 1, 10, and 100 ppm. Both soils were analyzed at days 20, 40, 80, 160, and 350 for TCDD by gas chromatography. In addition, ¹⁴C-TCDD was applied to soil samples at 1.78, 3.56 and 17.8 ppm for a metabolism study. Between 50-70% of the TCDD applied to soil samples persisted for 350 days. Persistence of TCDD did not seem to vary with soil type. Concentration of TCDD had no effect on persistence in Lakeland soil, but increasing TCDD concentration seemed to result in larger residues in Hagerstown soil. No TCDD metabolites were found in treated soil after 1 year. The authors concluded that TCDD is relatively persistent in soils. However, the concentrations used in this study were 10⁴-10⁶ higher than what would occur in an actual field situation. Extrapolation of these data to such a situation may not be possible.

519. Kearney, P. C., Woolson, E. A., Isensee, A. R., and Helling, C. S. (1973) Tetrachlorodibenzodioxin in the environment: Sources, fate, and decontamination. Environ. Health Perspec. 5:273-277.

[Review article.]

520. Kehrer, J. P., Haschek, W. M., and Witschi, H. (1979) The influence of hyperoxia on the acute toxicity of paraquat and diquat. Drug Chem. Toxicol. 2(4):397-408.

The acute toxicity of diquat was determined in rats in the presence of oxygen concentrations of 20-100%. Male Fisher rats were administered a dose of 5-80 mg/kg diquat in saline intravenously and immediately transferred to chambers ventilated with 100% oxygen. Other groups of rats were administered 20 mg/kg diquat intravenously and then were exposed to chambers with 40, 60 or 80% oxygen. Mortality and LT₅₀ values (time until half of the group died) were recorded. Distribution of [¹⁴C]-diquat was determined in rats administered 20 mg/kg diquat intravenously by removing tissues from 1-10 hours after the injection

and counting radioactivity. Lungs removed from rats administered 10 mg/kg diquat were inflated, embedded, stained and examined by light microscopy. The LT_{50} values for rats in 100% oxygen decreased from 3041 min. with a 5 mg/kg dose to 87 min. for an 80 mg/kg dose. The LT_{50} values for a control group administered the same doses of diquat and then placed in room air was not reported. Death resulted from respiratory failure. The LT_{50} values for a dose of 20 mg/kg diquat decreased from 1454 min. in 40% oxygen to 809 min. in 100% oxygen. Diquat levels in plasma, lung, liver, and kidney were the same for rats exposed to room air as for rats exposed to 100% oxygen. At each time point, the lung and plasma had the lowest diquat concentrations of the 4 tissues and the kidney had the highest concentration. Perivascular edema was present in lungs from diquat-treated rats in high oxygen, but not in vehicle controls exposed to high oxygen or in diquat-treated rats exposed to room air. The effects of paraquat were also described. The authors concluded that a toxic interaction between diquat and oxygen occurred, the mechanism of which was unclear.

521. Keil, J. E., Caldwell, S. T., and Loadholt, C. B. (1977) Pesticide usage survey of agricultural, governmental, and industrial sectors in the United States, 1974. U. S. Environmental Protection Agency Report No. 540/9-78-007. 67 pp.

[Review article.]

522. Kenaga, E. E. (1975) The evaluation of the safety of 2,4,5-T to birds in areas treated for vegetation control. Residue Review 59:1-19.

[Not available.]

523. Kenaga, E. E. (1974a) 2,4,5-T and derivatives: Toxicity and stability in the aquatic environment. Down to Earth 30(3):19-25.

[Review article.]

524. Kenaga, E. E. (1974b) Toxicological and residue data useful in the environmental safety evaluation of dalapon. Residue Rev. 53:109-151.

[Review article.]

525. Khan, S. U., (1973) Interaction of humic acid with chlorinated phenoxyacetic and benzoic acids. Environ. Letters 4(2):141-148.

The authors studied the interaction of humic acid and 2,4-D or picloram. Humic acid is present in nearly all material soil and water systems. A finely ground sample of humic acid (1g) was mixed with 200 ml of a water solution containing 500 (u) mole 2,4-D or 300 (u) moles picloram. After 3 days of mixing, the humic acid was centrifuged and

the supernatant removed. This was followed by washing with 25 ml portions and one 400 ml portion of water until all the unbound herbicide was removed. The humic acid was then dried in an oven at 30°C. The amount of herbicide adsorbed was determined by subtracting the concentration of herbicide recovered in the solution from the initial concentration. Humic acid adsorbed 30 (u) moles/g 2,4-D and 18 (u) moles picloram/g. The authors concluded that these data suggest that herbicides applied to soils or water form complexes with humic substances, which would affect the activity, bioavailability, and persistence of the herbicides in soil and water.

526. Khanna, S. C., and Fang, S. C. (1966) Metabolism of ^{14}C -labeled 2,4-dichlorophenoxyacetic acid in rats. J. Agric. Food Chem. 14(5):500-503.

The tissue distribution and excretion of 2,4-D were studied in the rat. A single dose of 1-100 mg of [^{14}C]-2,4-D per rat was administered by stomach tube to Wistar rats (weighing 225-400 g). Radioactivity was determined in tissues and in feces, expired air and urine collected up to 144 hours after treatment. Tissue samples were also fractionated into subcellular components and the radioactivity associated with each fraction was determined. No radioactivity was detected as $^{14}\text{CO}_2$ or other metabolites in expired air and 93-96% of low doses (1-10 mg) of 2,4-D were excreted in the urine in 24 hours. At higher doses, less of the dose was recovered in the urine and maximum urinary excretion occurred between 24 and 48 hours. Of 12 tissues examined for radioactivity, the half-lives were below 1 hour for a 1 mg dose for the blood, liver, kidney, heart, and spleen and 3 hours for an 80 mg dose. One metabolite of 2,4-D was separated in tissue and urine extracts by a counter-current separation technique, but was not identified. The liver contained the highest level of metabolite. From 57% (in liver) and 86% (in lung) of the tissue radioactivity was in the soluble fraction, with less in the nuclear fraction and below 10% in mitochondrial and microsomal fractions. The radioactivity in the soluble fraction was ether extractable. The authors concluded that larger doses of 2,4-D were absorbed and excreted more slowly than smaller doses and little biotransformation or macromolecular binding of 2,4-D occurred.

527. Khara, K. S. (1976a) Distribution, metabolism and perinatal toxicity of pesticides with reference to food safety evaluation: A review of selected literature. Advan. Mod. Toxicol. Pt. 1:369-420.

[Review article.]

528. Khara, K. S. (1976b) Significance of metabolic patterns in teratogenic testing for food safety. Clin. Toxicol. 9(5):773-790.

[Review article.]

529. Khera, K. S. and McKinley, W. P. (1972) Pre- and postnatal studies on 2,4,5-trichlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid and their derivatives in rats. Toxicol. Appl. Pharmacol. 22:14-28.

The effects of 2,4,5-T, 2,4-D, and several of their derivatives on the teratology and postnatal development of the rat were studied. Pregnant Wistar rats were administered test compounds perorally in 0.5% aqueous gelatin or corn oil on days 6 to 15 of gestation. On day 22 of gestation, fetuses were weighed and examined for skeletal and visceral anomalies. Other litters were delivered and the offspring were monitored for body weight and gross defects until they reached 12 weeks of age. 2,4,5-T and its butyl ester contained less than 0.5 mg/kg TCDD. Maternal toxicity was observed only with the highest dose of 2,4,5-T, 150 mg/kg. No visceral defects were observed in fetuses of herbicide-treated groups. The four parameters measured, including number of viable fetuses, number of dead fetuses, average fetal weight, and percent skeletal malformations, were not altered by doses of 25 or 50 mg/kg of either herbicide. A statistically significant dose-related response was observed in these parameters at doses of 100 and 150 mg/kg. The moderate effect of each parameter at 100 mg/kg was statistically significant compared to controls except for the effect of 2,4,5-T on the number of viable fetuses. The highest dose, 150 mg/kg, produced maternal toxicity. The derivatives of 2,4-D tested included the isooctyl ester, the butyl ester, the butoxyethynol and dimethylamine salts. In general these derivatives produced significant decreases in fetal weight and percentage of malformed fetuses. 2,4,5-T butyl ester caused no deleterious effects of any parameter of either 50 or 150 mg/kg doses. The most common skeletal defects observed in affected animals were wavy ribs, additional ribs, and sternal malformations. Dilated renal pelvis was observed in 7-45% of fetuses from all treated groups and 20-35% of controls. The incidence of this defect did not correlate with any dose or compound treatment. No decrease in post-natal survival was observed in offspring of 2,4-D or 2,4,5-T treated rats. When offspring of 2,4-D and 2,4,5-T groups given up to 100 mg/kg dose were mated with other rats of the same generation and treatment group, no decrease in fertility was observed from data on numbers of conceptions and viable and dead fetuses. No data or description of methods was provided for this part of the study. The authors concluded that the teratologic effects observed in fetuses was not incompatible with life, since no compromise in survival, growth, or reproductive capacity were observed in treated offspring.

530. Khera, K. S., Huston, B. L., and McKinley, W. P. (1971) Pre- and postnatal studies on 2,4,5-T, 2,4-D, and derivatives in Wistar rats. Toxicol. Appl. Pharmacol. 19:369-370.

[Abstract, only.]

531. Khera, K. S., Whalen, C., Trivett, G., and Angers, G. (1979) Teratogenicity studies on pesticidal formulations of dimethoate, diuron and lindane in rats. Bull. Environ. Contam. Toxicol. 22(4-5):522-529.

The teratologic effects of diuron were studied in the rat. From 125 to 500 mg/kg of a commercial formulation containing 80% diuron (the identities of other ingredients were not known) were administered to pregnant Wistar rats from days 6-15 of gestation. On day 22, corpora lutea were counted and fetuses were weighed, examined for external malformations, and then stained with alizarin red and examined for skeletal malformations or fixed in Bouin's fluid and examined for visceral anomalies. The numbers of corpora lutea and live fetuses per pregnancy and the percentage of dead or resorbed fetuses were the same for the three diuron-treated groups as the values for the control group. A decrease in fetal weight of about 12% observed in the highest dosage group compared to the control group was statistically significant. A significant increase in the incidence of malformations over control levels (from 5% in controls to 10% with 250 mg diuron) was observed in the group given the middle dose of diuron but not in the group given the highest dose. The incidence of wavy ribs was also increased in the middle dose group. Maternal toxicity, measured as a decrease in maternal weight gain, was observed in the 500 mg/kg treatment group. The authors concluded that 250 mg/kg produced teratologic effects and 500 mg/kg caused maternal toxicity, while a single anomaly of delayed ossification of the calvarium produced by the lowest dose, only, may indicate that the low dose of 125 mg/kg may also be a toxic dose. This assessment of the lowest dose seems inappropriate, since the effect was not seen at higher effects and therefore was probably a random occurrence unrelated to treatment.

532. Khera, K. S., and Whitta, L. L. (1968) Embryopathic effects of diquat and paraquat in the rat. Ind. Med. Surg. 37:553.

[Abstract, only.]

533. Khubutiya, R. A., Ugulava, N. A. (1973) Cytogenetic effect of herbicides. Tr. Nauchno-Issled. Inst. Zashch. Rast. (Tislis) 25:97-99.

[Not available.]

534. Kimbrough, R. D. (1980) 2,3,7,8-tetrachlorodibenzodioxin (TCDD) - Toxicity in animals, relevance to human health with notes on 2,4,5-T, picloram, cacodylic acid and 2,4-D. Presented at the 2d Continuing Education Conference on Herbicide Orange, Washington DC, May 28-30.

[Review article.]

535. Kimbrough, R. D. (1979) The carcinogenic and other chronic effects of persistent halogenated organic compounds. Ann. N.Y. Acad. Sci. 320:415-418.

[Review article.]

536. Kimbrough, R. D. (1974) The toxicity of polychlorinated polycyclic compounds and related chemicals. CRC Critical Reviews in Toxicology 2:445-498.

[Review article.]

537. Kimbrough, R. D. (1972) Toxicity of chlorinated hydrocarbons and related compounds. Arch. Environ. Health 25:125-131.

[Review article.]

538. Kimbrough, R. D., Carter, C. D., Liddle, J. A., Cline, R. E., and Phillips, P. E. (1977) Epidemiology and pathology of a tetrachloro-dibenzodioxin poisoning episode. Arch. Environ. Health 32(2):77-86.

The health effects in animals exposed to soil sprayed with TCDD-contaminated salvage oil are described. Tissues from seven horses and four cats were examined histopathologically, and findings included vascular lesions, fibrosis of the liver, bile duct proliferation, hyperkeratosis, gastric ulcers, degenerative changes of the kidney, and edema and hemorrhage of the lungs. In addition to the presence of TCDD and trichlorophenol in the soil, reported in an earlier publication (Carter et al. 1975), polychlorinated biphenyls were found in soil sample analyses. The authors also briefly described the health effects in exposed humans, which were published elsewhere. The authors theorized that the lack of serious illness in humans, compared to the incidence in animals, reflected less absorption or lower dermal sensitivity in humans.

539. Kimmig, J., and Schulz, K. H. (1957a) Occupational acne due to chlorinated aromatic cyclic esters. Dermatologica 115:540.

The authors described the clinical symptoms of workers involved in the manufacture of 2,4,5-trichlorophenol and 2,4,5-T and experiments in rabbits designed to identify the acnegenic agent in the manufacturing process. The workers were classified on the basis of the severity of skin changes as severe (9 cases), moderate (14) or mild (8). The clinical course of chloracne, which appeared in the summer and fall of 1954 in the subjects, started as facial dermatitis and developed into acneiform eruptions on the face and neck and then, in severe cases, on the trunk, arms and legs, and genitals. Other symptoms observed in the affected workers were chronic blepharo-conjunctivitis, fatigue, and weakness of the legs. Evidence of liver damage was revealed in 3 cases

from clinical examination. No incidences of systemic symptoms or types of clinical tests used were presented. Skin symptoms remained 20 months after exposure to the toxic compounds was terminated. An additional instance of chloracne in a laboratory worker involved in the animal experiments (described below) was also mentioned. One hundred compounds involved in the synthesis of 2,4,5-T were tested for acne-genic potency. Each compound was dissolved in polyglycol and painted on the inside of one rabbit ear daily while the other ear served as the vehicle control. Active compounds produced inflammation in 2-4 weeks and several days later caused follicular swelling, hyperkeratoses, and cysts. Large cysts formed at later stages. Histologically, inflammatory infiltrations and edema of the cutis were observed and dilated follicles filled with keratinous material. Chlorinated dibenzofurans were potent hepatotoxins and acnegens but could not be isolated as byproducts of the manufacturing process. TCDD applied 3-4 times at 3-4 day intervals at doses of 0.005-0.01% was acne-genic and a single oral dose of 0.05-0.1 mg/kg was hepatotoxic and lethal to most rabbits in 20 days. TCDD was isolated as a byproduct of 2,4,5-T synthesis. Technical 2,4,5-trichlorophenol, but not pure trichlorophenol, was acne-genic. The authors concluded that TCDD was the acne-genic contaminant of trichlorophenol responsible for chloracne in exposed workers.

540. Kimmig, J., and Schulz, K. H. (1957b) Chlorinated aromatic cyclic ethers as the cause of so-called chloracne. Naturwissenschaften 44:337-338.

Experiments that identified the acne-genic properties of TCDD are summarized. The identity of chloracne and hepatic toxicity in workers involved in the synthesis of trichlorophenol from tetrachlorobenzene led the authors to investigate the acne-genic potencies of various chemical precursors and byproducts formed in the process in the rabbit. These compounds were synthesized for the study and one laboratory technician involved in the synthesis developed severe chloracne. Compounds were applied to the rabbit ear and inflammatory changes in 2 to 4 weeks and symptoms resembling acne were recorded. (The number of rabbits tested, the method of application, or a list of test substances were not provided.) TCDD was also administered in single oral doses of 0.05 to 0.5 mg/kg to rabbits and mortality and autopsy findings were reported. TCDD was the most potent acne-gen, producing acne at concentrations of 0.002-0.01%; larger dermal doses caused hepatic necrosis and premature death. Other active acne-genic compounds were trichloro-dibenzofuran and tetrachlorodibenzofuran. Inactive compounds included 2,4,5-trichlorophenol, pentachlorophenol, 2,3,4,-tetrachlorobenzene and various chlorinated diphenylethers and unsubstituted and monochlorinated dibenzofurans. Oral doses of TCDD produced death in all animals within 2 weeks. At autopsy, necrosis and fatty degeneration of the liver were observed. TCDD was successfully isolated from the by-products of the trichlorophenol process. The authors concluded that byproducts of the manufacturing process were responsible for causing chloracne in the factory workers.

541. King, C. T. G., Horigan, E. A., and Wilk, A. L. (1971) Screening of the herbicides 2,4,5-T and 2,4-D for cleft palate production. No reference: 233.

[Abstract, only.]

542. King, M. E., and Roesler, A. R. (1974) Subacute intubation study on rats with the compound 2,3,7,8-tetrachlorodioxin. IIT Research Inst. Report to EPA No. IITRI-L6073-12. 60 pp.

The subacute oral toxicity of TCDD in the rat is described. Sprague-Dawley rats (70 per group) were administered doses of 1 and 0.1 ug/kg/week of TCDD in acetone-corn oil (1:9) by oral intubation. TCDD was administered 2 times weekly on Tuesday and Thursday for 28 weeks, followed by a 12-week recovery period. Animals were weighed weekly and rats (6 per group) were killed after 2-28 weeks and necropsies were performed. The body weights of treated animals remained below the control rats during the treatment and recovery periods; for the higher dosage group, this difference reached statistical significance. The only organ weights, expressed relative to body weights, that were statistically different from controls were increased liver weights in all groups. Hepatic changes were observed histopathologically starting at 28 weeks of treatment (no animals were killed between 16 and 28 weeks). More males than females were affected by the lower dose and by the end of the recovery period, fewer animals were affected than at the end of the treatment period or after the first 4 weeks of the recovery period. Changes included increased numbers of lipid vacuoles in centrilobular hepatocytes and electron microscopic suggestions of smooth endoplasmic reticular proliferation (which was difficult to discern because the tissue fixation techniques used were not appropriate for electron microscopy). Mild hepatocellular necrosis, multinucleated hepatocytes, enlarged hepatocytes at the periphery of the lobule increased numbers of hemosiderin-containing macrophages no other organs were affected. The authors concluded that TCDD produced delayed, reversible hepatic toxicity in rats and the histopathologic lesion was suggestive of impairment in hepatic lipid transport or metabolism.

543. Kinoshita, F. K., and DuBois, K. P. (1970) Induction of hepatic microsomal enzymes by Herban^R, diuron and other substituted area herbicides. Toxicol. Appl. Pharmacol. 17(2):406-417.

The effects of subacute administration of monuron and diuron on induction of several rat hepatic microsomal enzymes is described. Female 53-day-old Holtzman rats (3 per group) were administered 10-200 mg/kg/day of diuron in carboxymethyl cellulose orally for 5 days. On the sixth day, rats were killed and whole liver homogenates were prepared and assayed for activity of the phosphorothioate detoxification (PTD) system and O-demethylase activities. Significant increases of 144% and 192% occurred in the PTD activity and of 254% and 335% in O-demethylase

activity at the 2 highest doses of diuron, 100 and 300 mg/kg, respectively. Male and female 30-day-old Holtzman rats (3 per group) were administered diets that contained 100-2000 ppm diuron for 1-13 weeks and then were killed and liver homogenates were assayed for various enzyme activities. Significant, sustained increases occurred in PTD activity of females fed 1000 and 2000 ppm diets and in O-methylase activity for all groups compared to the control group (whose treatment was not described). N-methylase activity was increased in all male groups and in females fed 500-2000 ppm at 1-3 weeks, but returned to control levels in all groups by week 6. Female 30- and 53-day-old rats (5-6 per group) were fed diets that contained 1000 ppm diuron for 1 week and then hepatic enzyme activities were determined. O-methylase activity was increased 135% in the younger group of rats only, and N-demethylase and PTD activities were unaltered in either age group. The induction potencies of other substituted urea herbicides were also studied. The authors concluded that diuron produced weak enzyme induction (relative to DDT and other pesticides not investigated in this study) and this induction was only observed within the first few weeks of the feeding regimen, and sex-related differences in the inductive response were evident.

544. Kirkland, G. L., Jr. (1978) Population and community responses of small mammals to 2,4,5-T. Research Note, USDA Forestry Service, Pacific Northwest Forest Range Experimental Station Report No. PNW 314. 6 pp.

[Not available.]

545. Klein, R. E., and Harrigan, E. T. (1969) Comparison of Defoliants Vol 1. Directorate of Test and Evaluation, Armament Development and Test Center, Air Force Systems Command, Eglin AFB, Fla. Report No. ADTC-TR-69-30.

[Background material.]

546. Klingman, D. L., Gordon, C. H., Yip, G., and Burchfield, H. R. (1966) Residues in the forage and in milk from cows grazing forage treated with esters of 2,4-D. Weeds 14(2):164-167.

The levels of 2,4-D in milk from cows that grazed 2,4-D sprayed pastures and 2,4-D levels in forage are reported. One pasture was sprayed with 2 lb per acre of 2,4-D isopropyl ester and another with 2 lb per acre of 2,4-D-2-ethylhexyl ester (twice the recommended agricultural rate). For each pasture, two cows were grazed for 6 days prior to spraying and for several weeks after spraying. The experimental protocol was repeated 3 years later and 1 additional cow per pasture was added 2 days after the spraying and another 4 days after the spraying. Milk collected from the exposed cows and forage samples from the fields were analyzed for 2,4-D by gas chromatography. The highest

levels of 2,4-D were obtained in milk samples collected in the afternoon of the first exposure day and on the subsequent day with peak levels of 0.05-0.06 ppm 2,4-D. No 2,4-D was detected on the third day after exposure to the isopropyl ester or 14 days after exposure to the 2-ethylhexylester (no samples were collected between the 3rd and 14th day). For the second experiment conducted 3 years after the first, peak levels were 0.03 ppm 1 day after spraying and for animals from both fields 0.01 ppm 2,4-D was detected in all samples for the 7 days after spraying and in milk of cows introduced to the fields 2 days after spraying. No 2,4-D was detected in milk from cows introduced 4 days after spraying (detection unit, 0.01 ppm). Forage levels decreased rapidly, from 58 ppm of 2,4-D (acid) immediately after the butyl ester was sprayed to 5 ppm after 1 week and from 37 to 14 ppm for the 2-ethylhexyl ester in 1 week. The levels of the esters in forage were substantially below the acid levels and only the acid form was detected in milk. The authors concluded that only low levels of 2,4-D would be expected in milk of cows grazing on 2,4-D-sprayed fields.

547. Knutson, J. C., and Poland, A. (1980) 2,3,7,8-tetrachlorodibenzo-p-dioxin: failure to demonstrate toxicity in twenty-three cultured cell types. Toxicol. Appl. Pharmacol. 54:377-383.

The toxicity of TCDD was assessed in 23 different cell types, in vitro. Cell types that were tested included tissues and species that exhibit sensitivity to TCDD toxicity in vivo. Cells were exposed to TCDD for usually at least 1 week. Concentrations of TCDD of 10^{-9} M were used, and in some cultures, 10^{-8} and 10^{-11} M concentrations were also tested. The effect of TCDD on cell morphology, cell viability, growth rate, and aryl hydrocarbon hydroxylase activity. Fourteen of 22 cell lines tested, showed no detectable enzyme induction 3 showed 2-3 fold increases in activity, 2 showed 7-9 fold increases and 3 showed 40-650 fold increases. TCDD produced no changes in cell morphology, viability, or growth rate in any of the 23 cell lines. The authors concluded that their results were not surprising, as cytosol receptor binding was necessary, but no necessarily sufficient for TCDD, in order to elicit a toxic response. Alterations introduced by the culture conditions or missing cell-cell interactions were suggested to explain the differences of in vivo and in vitro experiments.

548. Kociba, R. J., Keeler, P. A., Park, C. N., and Gehring, P. J. (1976) 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD): Results of a 13-week oral toxicity study in rats. Toxicol. Appl. Pharmacol. 35:553-574.

The subacute, oral toxicity of TCDD in the rat is described Sprague-Dawley rats (12 male and 12 female per group) were administered 0.001, 0.01, 0.1 or 1.0 ug/kg of TCDD in acetone-corn by oral gavage, 5 days per week for 13 weeks. Five rats of each sex was killed in each group and the remainder were observed for a 13-week post-treatment period before they were killed. Body weights, food consumption, general appearance, hematological parameters, urinalysis and blood chemistry analyses were described for the exposure and post-exposure periods and

complete pathological examinations were performed at necropsy. Treatment-related deaths occurred in only the highest dosage group; 4 females died during treatment, and 2 males and 2 females died after treatment ended. Aortic thrombosis and adrenal hemorrhage each occurred in 1 of these rats, but not in the rats in this group that were killed. Reduced body weights and food consumption occurred in the 2 highest dosage groups, and icterus and reduced activity were observed in the highest dosage group. Sex-dependent hematologic changes were observed in the highest dosage group and included decreased red blood cells, packed cell volume, and red blood cells in males. Increased urinary excretion of porphyrins and delta amino-levulinic acid and decreased creatinine excretion occurred from the highest dose. Blood chemistry changes from this dose included increased bilirubin, blood urea nitrogen, and alkaline phosphatase levels. Liver weights relative to body weights were elevated in all but the lowest dosage group and relative thymic weights were decreased from the 2 highest doses. Histopathologic changes occurred in the 2 highest dosage groups and included liver degeneration, lymphoid depletion of the thymus and morphological changes of reproductive organs suggestive of functional suppression. Hepatic levels of TCDD, measured by gas chromatography-mass spectrometry, were 0.3, 0.035, and 0.003 ug per gm liver at the end of 13 week exposures to 1.0, 0.1, and 0.01 ug/kg doses, respectively. The authors concluded that no discernable ill effects occurred from doses of 0.01 ug/kg/day of TCDD administered for 13 weeks to rats and that no additional effects were likely from higher doses, beyond those already described, because steady state body burdens were probably reached by 13 weeks.

549. Kociba, R. J., Keyes, D. G., Beyer, J. E., Carreon, R. M., Gehring, P. J. (1979a) Long-term toxicologic studies of 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD) in laboratory animals. Ann. N.Y. Acad. Sci. 320:397-404.

This paper summarizes the data presented in Kociba et al. (1978). No new information was reported.

550. Kociba, R. J., Keyes, D.G., Lisowe, R. W., Kalnins, R. P., Dittenber, D. D., Wade, C. E., Gorzinski, S. J., Mahle, N. H., Schwetz, B. A. (1979b) Results of a two-year chronic toxicity and oncogenic study of rats ingesting diets containing 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Fd. Cosmet. Toxicol. 17(3):205-221, 1979.

In a two-phase study, the author investigated the long-term toxicity and oncogenicity of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in rats. Groups of 7- to 8-week-old Sprague-Dawley rats were fed in their diet either 99 percent pure 2,4,5-T in acetone at doses of 3, 10, or 30 mg/kg (comparable to approximately 85, 230, and 677 ppm), or just acetone. Test groups consisted of 60 males and 60 females each, while the control group consisted of 96 males and 96 females. No TCDD was found in the 2,4,5-T as determined by gas chromatography-mass spectrophotometry (limit of detection was 0.33 ug/kg). The primary phase of

the study lasted 2 years, but 10 rats of each sex from each group were killed for a interim of the phase study at 118-119 days. This sub-chronic group was weighed once a week for the first 3 months from the start of the experiment; observations of general health and possible toxicological responses were made at these times. After the first 3 months body weights was recorded monthly, but other observations continued on the weekly schedule. Food consumption was recorded twice weekly. In the chronic group, 20 rats of each sex from test and control groups were observed and weighed on the same initial schedule; weekly observations continued to the 6th month. Food consumption was measured twice weekly for the first 4 months and thereafter for 1 week each month. In both subchronic and chronic phases, routine urinalysis (for specific gravity, pH, glucose, protein, ketones, bilirubin, and occult blood) and blood samples from the tail vein (for total erythrocyte count, total and differential leucocyte count, thrombocyte and reticulocyte count, packed cell volumes, and hemoglobin) were done for the subchronic phase on days 82 and 83, and for the chronic phase on days 89-90, 364-365, and 726-726. More in-depth urinalysis were performed on five rats of each sex from each of the subchronic groups three times before day 106 and just before the interim kill; and for the chronic phase, on four or five rats of each sex from each group after days 95-97, 188-190, 361-363, 564-566, and 698-700. This analysis measured creatinine, coproporphyrin, and uroporphyrin with or without -aminolevulinic acid. Serum samples from 10 rats of each sex per group were analyzed for urea nitrogen; glutamic-pyruvic transaminase (SGPT) and alkaline phosphatase activities (SAP); total, direct, and indirect bilirubin; and total protein, albumin, and globulin. Eyes were examined both by glass slides placed against the cornea under fluorescent light, and by preservation of eyes from five rats of each sex and group in Zenker's fixative. In addition, a complete gross pathological examination was made of representative sections of all major organs and tissues from all animals. Any grossly observed lesions or suggestion of tumor formation were preserved, and weights were recorded for liver, kidneys; brain, heart, thymus, spleen, and testes or ovaries/uterus. Portions of fat, liver, and kidney were saved from five rats of each sex and group for possible 2,4,5-T analysis. For the chronic phase, representative parts of the skull (including nasal turbinates and ear canal) were also examined. Peripheral blood and femoral bone marrow smears were also made for this group. Sections of tissues embedded in paraffin were stained with hematoxylin and eosin and examined histologically. Tissues from low-dose rats in the subchronic phase were not examined, as there was no evidence of treatment-related effects in the high-dose group. For this subchronic phase, there were no toxicologically significant deviations from controls for body weights, food consumption, mortality, palpable masses and general toxicological analyses, hematological, and urinary parameters, BUN, SGPT, SAP, bilirubin, protein, albumin, or globulin. There was a statistically significant increase in relative kidney weights for high-dose males. For the chronic phase, no toxicologically significant changes were found in body weights, food consumption, mortality in the lower dose groups, palpable masses and toxicological observations, hematological parameters, BUN, SGPT, SAP, bilirubin, protein, albumin, or globulin. In the high-dose group, males showed a

decrease in mortality in the latter part of the study, and median-dose females showed increases in the incidence of minimal degenerative inflammatory changes including focal renal tubular atrophy and renal aggregation of lymphoid cells; these changes were considered related to less severe chronic renal disease in these rats. These rats also showed an increase in total urine volume and in the excretion of coproporphyrin and uroporphyrin. Males receiving 10 mg/kg/day showed an increase in coproporphyrin excretion in the early part of the study. In the high-dose females coproporphyrin was shown to be increased in most samples; uroporphyrin decreased after 119 days but increased again after 566 days. In the lowest dose males and in the 3- and 10mg/gk females no significant differences were noted. There was a decrease in absolute heart weights of high-dose males, but this was of questionable significance due to an absence of any gross or histopathological changes. High and median-dose females showed both gross and microscopic evidence of increased mineralized deposits in the renal papillae or pelvis, on occasion accompanied by a localized reaction of the adjacent renal-pelvis epithelium. Females also showed evidence of increased pigment content in the cytoplasm of the proximal convoluted tubular epithelial cells. Males particularly showed a decrease in the severity of spontaneous chronic nephropathy and a secondary decrease in mineralization of pulmonary alveoli, myocardium, myocardial blood vessels, and gastric mucosa and muscularis. The increased renal pigment noted gave a higher positive reaction with Malloy's stain for iron. Lung changes evidenced in high-dose males may or may not have been treatment-related. There was some increase in focal pulmonary interstitial inflammation, focal accumulations of alveolar macrophages and cholesterol clefts; females in this group showed focal accumulation of secreted material in the alveoli. Cardiovascular changes occurring in high-dose females included dilated, flaccid ventricle; the opposite change occurred in males of this group. A variety of tumor types occurred in both control and test rats at an incidence level not considered toxicologically significant. Total numbers of tumors per group, average number per rats, and latency, were all comparable to control rats. With one exception, the incidence of individual tumor types was comparable between treated and control groups; however, the incidence of interfollicular C-cell adenoma was higher in females given the 3 mg/kg/day dose (10 tumors vs. none in controls). This increase was not believed to be treatment related since the incidence of C-cell adenoma in control rats was swollen than what would have been historically expected in this strain of rat (up to 17%).

551. Kociba, R. J., Keyes, D. G., Beyer, J. E., Carreon, R. M., Wade, C. E., et. al. (1978) Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats. Toxicol. Appl. Pharmacol. 46:279-303.

The authors studied the effects of TCDD in a 2-year feeding study in rats. Groups of 6-7 week old male and female Sprague-Dawley Spartan substrain rats (50 males and 50 females per group) were fed 0, 0.1, 0.01, or 0.001 ug TCDD/kg/day. TCDD (99 percent purity) in acetone was added to standard lab feed. A negative control group (86 males, 86

females) received standard diet. New stocks of feed with TCDD were prepared 6 times during the study. Periodically, feed samples were taken to analytically verify the TCDD content of the food. All rats were palpated and weighed on a monthly basis. Blood and urine samples were collected from 7-8 rats/sex/group at 3, 12, and 23 months. Urinary excretion of creatine, coproporphyrin, uroporphyrin and S-amino-levulinic acid were measured in 4-5 rats/sex/group at 3-4, 12, and 23 months. Serum samples were analyzed from 7 rats/sex/group for urea nitrogen, glutamic pyruvic transaminase, bilirubin, cholesterol, and triglycerides. Gross pathological examinations were conducted on all rats. Weight of major organs were measured and organ-to-body weight ratios calculated. Histological examinations were conducted on tissues from all rats in the control and high dose groups and select tissues from the other groups. Liver tissue was examined by electron-microscopy. Statistical analyses of the data included analysis of variance, Fischer exact test, and the Mantel-Haenszel test. Multiple toxicological effects were observed in animals ingesting diets containing 2200 ppt TCDD (0.1 ug TCDD/kg/day). These included increased mortality, decreased body weight gain, slight depression of some hematologic parameters, increased urinary excretion of porphyrins, and S-amino levulinic acid, increased serum alkaline phosphase, glutamic pyruvic transaminase, and gamma-glutamyl transferase, and morphological changes in hepatic lymphoid, respiratory, and vascular tissues. A statistically significant (Fisher's exact test) increase in the incidence of hepatocellular carcinomas was observed only in females at this dose. Squamous cell carcinomas of the lung, hard palate/nasal turbinate, or tongue were also observed and were statistically significant (Fisher's exact test). Ingestion of 210 ppt TCDD in the diet (0.01 ug/kg/day) induced less toxicity than the higher dose. Observed toxicological effects included increased urinary excretion of porphyrins in females, liver toxicity, increased hepatocellular nodules and increased focal hyperplasia in the lungs. At the lowest dose tested, 22 ppt TCDD in the diet (0.001 ug/kg/day), no effects related to TCDD administration were observed.

552. Kohli, J. D., Khanna, R. N., Gupta, B. N., Dhar, M. M., Tandon, J. S., and Sircar, K. P. (1974a) Absorption and excretion of 2,4-dichlorophenoxyacetic acid in man. Xenobiotica 4(2):97-100.

Plasma and urinary levels of 2,4-D and metabolites were analyzed in human subjects administered 2,4-D orally. Six 22-30 year old male volunteers were administered 5 mg/kg 2,4-D (99% purity) in a gelatin capsule with water. Blood and urine samples collected over the subsequent 7 days were analyzed by gas chromatography for 2,4-D and its metabolites. No adverse effects were reported by any of the subjects or detected by clinical laboratory tests. A mean of over 20 ug/ml of 2,4-D was detected in plasma 1 hour after administration; peak levels were reached in 7-24 hours. Within 96 hours, 75% of the dose had been excreted and no metabolites were detected in any samples. From kinetic analysis of the plasma data using a model with first order rates of absorption and clearance, constants were determined for the computer-generated curve that best fit the data. Rates for absorption and

clearance were 27 and 2×10^{-2} per hour, respectively; the plasma half-life was 33 hours and the volume of distribution was 10×10^2 l/kg. The authors concluded that 2,4-D was absorbed rapidly and excreted rapidly by the kidney and was not biotransformed.

553. Kohli, J. D., Khanna, R. N., Gupta, B. N., Dhar, M. M., Tandon, J. S., and Sircar, K. P. (1974b) Absorption and excretion of 2,4,5-trichlorophenoxy acetic acid in man. Arch. Int. Pharmacodyn. 210:250-255.

The kinetics of absorption and excretion of 2,4,5-T by humans was studied. Eight male volunteers ingested from 2 to 5 mg/kg 2,4,5-T. No clinical symptoms of toxicity were detected by the subjects. Urine samples collected over the subsequent 4 days and blood samples collected over the subsequent 7 days were analyzed for 2,4,5-T. The maximum blood levels were reached 7-24 hours after ingestion, although after 1 hour 2,4,5-T was detected in the blood. After 96 hours, urinary 2,4,5-T accounted for 63% of the 5 mg/kg dose, 79% of the 3 mg/kg dose, and 73% of the 2 mg/kg dose. The half-life of plasma clearance, calculated assuming that the rates of absorption and excretion followed first order kinetics, was reported as 18.8 hours and the volume of distribution was calculated as 15.8 liters/kg. Based on kinetic data presented in the present paper and by other investigators, the author predicted that tissue accumulation would be unlikely after repeated exposure of 2,4,5-T.

554. Kolberg, J., Helgeland, K., and Jonsen, J. (1972) The herbicide 2,4-D. II. Triglyceride accumulation in L cells. Acta. Pharmacol. Toxicol. 31(5-7):481-487.

Endogenous biosynthesis of fatty acids and uptake of fatty acids were evaluated in cultured fibroblasts exposed to 2,4-D. Mouse L929 fibroblasts were cultured in the presence of 500 ug 2,4-D (99+% purity) per ml culture medium for 24 hr. Fibroblasts were cultured in the presence of [3 H]-palmitic acid and [14 C]-acetate (together) for 24 hours. Lipids were extracted from whole cells and cell homogenates after lipid particles were separated by centrifugation. Lipid extracts were analyzed by thin-layer chromatography and triglycerides, cholesterol, and phosphorous by chemical procedures. The amount of lipid particles was maximum after 24 hr. of 2,4-D treatment, increasing to two times the pretreatment amount represented. Most of the additional lipid was triglycerides. Incorporation of palmitate and of acetate into lipid fraction increased threefold. Incorporation of only acetate into non-lipid fractions occurred. Both precursors were incorporated into triglyceride and 14 C was incorporated into cholesterol, as well. The specific activity of 3 H in triglycerides did not change and decreased for 14 C in triglycerides and for both isotopes in phospholipids. The authors concluded that increased incorporation of both fatty acid precursors may have reflected an effect of 2,4-D on plasma membrane permeability.

555. Kolberg, J., Helgeland, K., Jonsen, J., and Tjeltveit, O. (1971) The herbicide 2,4-dichlorophenoxyacetic acid. I: Effects on L cells. Acta. Pharmacol. Toxicol. 29:81-86.

The effects of 2,4-D on cell growth of L929 cells were studied in vitro. Strain L929 mouse fibroblasts were grown in monolayer cultures for 24 hours and then the medium was replaced with medium containing from 50 to 500 ug/ml 2,4-D (as the sodium and potassium salts). Culture media were renewed every 3 days and for some experiments cell layers were washed several times and control medium was used to replace 2,4-D treatment media. Cells were trypsinized and counted or analyzed for lipids cytochemically. 2,4-D produced a dose-related inhibition of cell growth without causing cytopathogenic changes. The effect on cell growth was reversible when 2,4-D-containing medium was replaced with fresh medium on day 3 or 12 of culture. Uniformly-sized lipid-staining vacuoles appeared in the cytoplasm after 3 hours of exposure to 350-500 ug/ml 2,4-D; increased in number during the next 21 hours; decreased subsequently and disappeared by day 4-5 of culture. Lower doses caused fewer or no vacuoles. The formation of vacuoles and absence of cell division by 2,4-D treatment was observed with time-lapse cinematography. The authors suggested that the presence of lipid vacuoles was not necessarily a sign of degeneration and did not attempt to relate their observations to 2,4-D toxicity in vivo.

556. Kolmodin-Hedman, B., and Erne, K. (1980) Estimation of occupational exposure to phenoxy acids (2,4-D and 2,4,5-T). Arch. Toxicol. Suppl. 4:318-321.

Concentrations of 2,4-D and 2,4,5-T in air from the breathing zone of workers applying these herbicides and urine and plasma levels for workers after exposure are presented. Four workers engaged in spraying a 2% emulsion of the 2 phenoxy acids in kerosene made up the study group. All workers were male, all were smokers, previous exposure to herbicides varied from 2-14 years for the group, and the mean age was 39 years. Two additional staff members served as the control group and had low, indirect exposure to the herbicides. No other information on the exposure levels or on other characteristics of this group were given. Air was sampled from the workers' breathing zone for 60 minute periods. The effective spraying time was 2-4 hours/day and urine and blood samples were collected before the start and at the end of the first work day of the week, at the end of a week of exposure and up to 36 hours after the exposure week ended. Levels of phenoxy acids in body fluids were determined by gas chromatography and in air by UV-spectrophotometry and thin-layer chromatography. The lowest level of detection was 0.05 ug/ml in urine and 0.02 ug/ml in plasma. Levels of phenoxy acids were 0.1-0.2 mg/m³ in air samples. Plasma levels were all below 0.2 ug/ml. Plasma levels were variable, did not show a rising trend during the exposure week and fell to the limit of detection 1 day after exposure ended. The mean peak urine levels were 10 ug/ml of 2,4-D and 3.5 ug/ml of 2,4,5-T and occurred in the afternoon of the day of exposure. Subsequent urine levels were substantially lower in 3 of 4 cases. No phenoxy acids were detected in urine from

the controls. The only symptom reported was slight eye irritation by 1 man from direct contact with the liquid. The authors concluded that the extent of occupational exposure to these phenoxy acids would be monitored most accurately from urine samples collected on the afternoon of the exposure day.

557. Kolmodin-Hedman, B., Erne, K., Hakansson, M., Engqvist, A. (1979) Occupational exposure to phenoxy acids (2,4-D and 2,4,5-T). Arbete. och. Halsa (2).

[Abstract, only.]

558. Konstantinova, T. K. (1974) Experiments on the effects of 2,4,5-T butyl ester on pregnant animals and on the development of their offspring. Gig. Sanit. 39(8):101-102.

The teratogenic effects of 2,4,5-T to offspring following maternal treatment and maternal toxicity are described. Albino rats (12-13 per group) were administered doses of 4.2, 0.42, 0.1 or 0.01 mg/kg 2,4,5-T butyl ester (purity and dioxin level not reported). The herbicide was administered orally, in water, throughout gestation. Maternal body weights, peripheral blood composition, histopathology, and central nervous depression (measured as a summation threshold index; no other explanation of the parameter was provided), and organ weights were determined. Fetal mortality, body weights, visceral malformations (determined by a microanatomical technique that was not described further) and skeletal malformation, visualized by alizarin stain, were determined. All doses, except the lowest dose, produced nervous system inhibition, and increased organ weight coefficients (calculations used to obtain this coefficient were not described); the organs with increased coefficients varied at different dose levels and included the liver at 4.2 and 0.1 mg/kg, brain and kidney at 4.2 and 0.42 mg/kg, the lung at 0.42 mg/kg and the heart at 0.1 mg/kg. The highest dose also produced hemodynamic disorders and dystrophic changes in maternal organs. Fetal changes from the highest dose included high fetal death, reduced body weights, malformations (hydrocephaly in 6 percent of fetuses, liver abnormalities in 19 percent and abdominal hemorrhage in 11 percent), plethora of internal organs and granular dystrophy in the liver and kidneys. At 0.42 mg/kg, 2,4,5-T produced hydrocephaly (4.2 percent), increased limb bone length and plethora and granular dystrophy. At 0.1 mg/kg increased fetal weight and limb bones and decreased litter sizes were observed and at 0.01 mg/kg, only increased weight and bone length occurred. The author concluded that 0.01 mg/kg was the threshold dosage for the toxic maternal and fetal effects of 2,4,5-T. The author's failure to analyze the dioxin content of 2,4,5-T or to describe methods or results adequately to compare them to other studies, the lack of dose-related effects or effects seen in rats by other investigators renders this paper useless for evaluating teratology of 2,4,5-T.

559. Konstantinova, T. K. (1967) Toxicology and some problems of the embryotropic action of the butyl ester of 2,4-D. In Proceedings of a Conference on Problems on Hygiene and Toxicology of Pesticides, Bygodchikov, ed., Moscow Meditsina.

[Not available.]

560. Konstantinova, T. K., Efimenko, L. P., Antonenko, T. H., Nechkina, M. A., and Shilov, V. N. (1978) Data for the hygienic standardization of 2,4-D herbicides in environmental substances. Gigiena i sanitariya. 9-13.

Reproductive effects of 2,4-D, 2,4-D sodium and amine salts, and 2,4-D butyl ester were studied in the rat. Groups of rats were administered 0.5-1.0 mg/kg 2,4-D, 2,4-D sodium or amine salt or 2,4-D butyl ester orally and the embryotropic, cytogenetic, and gonadotropic effects were studied. The explanations of the methods used to measure these parameters were given. Parameters were reported in a table as being altered or not altered by a dose of 0.5-1.0 mg/kg 2,4-D or by a dose between 0.1 to 0.01 mg/kg. The results were undecipherable. For example, under the heading "embryotropic effect" in the table of results were four subheadings, "mother," "fetus," "permeability of the placenta," and "progeny," which may have referred to toxicity in the mother, which is not an embryotropic effect. Under "cytogenetic effects in males" were subheadings of "one-time," "5 days," "45 days," "5-6 mo." but these time intervals were not explained and no alterations were noted from 2,4-D treatment (the only compound tested for these effects). All of the compounds studied produced an embryotropic effect in the fetus but not in the progeny and only 2,4-D amine salt and 2,4-D butyl ester caused an embryotropic effect in the mother, 2,4-D crossed the placenta and transport was not determined for other compounds. The authors concluded that regulation of occupational exposure to 2,4-D should be based on the levels that produce embryotropic effects.

561. Kontek, M., Jasinski, K., Marcinkowska, B., Tokarz, F., Pietraszek, Z., and Handschuh, R. (1973) Electroencephalographic study of farm workers exposed to derivatives of arylalcanocarboxylic acids. Polski Tygodnik Lekarski. 28(25):937-939.

The authors conducted electroencephalographic (EEG) examinations on 17 farmworkers, aged 19 to 42 years, who sprayed fields with various herbicides (including 2,4-D). EEG's were conducted on these individuals before and after the spraying season. Time intervals between the EEG examinations ranged from 4 to 28 days. A group of 41 farmworkers not involved with herbicide spraying was used as controls. Of the 17 sprayers examined prior to spraying activities, 7 exhibited slightly perturbed EEG activities in the form of irregular alpha waves and numerous, slow theta waves originating from all areas of both cerebral hemispheres. Seven individuals from the control group also exhibited irregular EEG patterns, but not to the same extent as the test group. EEG readings obtained from the 17 sprayers after the spraying season

had ended revealed that 5 of them exhibited distinct abnormalities in alpha wave frequencies and theta wave amplitudes. Four of these five were the same persons that displayed irregularities in their EEG's taken prior to the spraying season. The irregularities had apparently intensified in these individuals. An additional 8 persons from the study group also exhibited some irregular bioelectric activity following the period of spraying. Additional laboratory tests such as blood morphology, urinalysis and enzyme reactivity were conducted and no abnormalities in these parameters were evident. The authors concluded that EEG patterns were effected in persons that contacted arylalcano-carboxylic acid herbicides, concluding 2,4-D. The authors briefly correlated their findings with results of animal studies that demonstrated abnormal cerebral electrical activity caused by administration of 2,4-D.

562. Kopaczyk-Locke, K. (1973) Effects of paraquat and diquat on rat liver mitochondria. Fed. Proc. Fed. Am. Soc. Exp. Biol. 32(3):250.

[Abstract, only.]

563. Koschier, F. J., and Acara, M. (1979) Transport of 2,4,5-trichlorophenoxyacetate in the isolated, perfused rat kidney. J. Pharmacol. Exp. Ther. 208:287-293.

Characteristics of 2,4,5-T excretion by perfused rat kidneys are studied. Urine was collected from a urethral catheter of anesthetized male Sprague-Dawley rats and perfusate fluid which contained various test compounds was circulated through the kidneys in situ. The levels of radioactivity in the urine and blood were analyzed after radio-labeled compounds were introduced to determine the rate of renal clearance for each compound. The ratio of 2,4,5-T clearance to inulin clearance was below 1, with 99.5% of the 2,4,5-T in the perfusate bound to bovine serum albumin. This ratio increased to 6 after dextran, which bound less than 30% of the perfusate 2,4,5-T, was used instead of albumin. The presence of probenecid and of para-aminohippurate each decreased the clearance ratio and increased tubular reabsorption. The kinetics for clearance of 2,4,5-T from perfusate fluid involved 2 phases and the half lives and rate constants were calculated for perfusate fluids which contained albumin and dextran, and inhibitors. At 1 mM, 2,4,5-T inhibited the clearances of PAH and tetraethylammonium and an initial diuresis, followed by a progressive fall in glomerular filtration rate. This concentration of 2,4,5-T did not alter oxygen consumption, Na,k-ATPase, Mg-ATPase, or water distribution studied in renal slices but did alter electrolyte balance. The authors concluded that proximal tubular secretion of 2,4,5-T is limited by plasma protein binding of 2,4,5-T; 2,4,5-T was also reabsorbed to some extent and caused nephrotoxicity.

564. Koschier, F. J., and Berndt, W. O. (1977) Relationship between 2,4,5-trichlorophenoxyacetate and the renal organic base transport system. Biochem. Pharmacol. 26:1709-1713.

The interaction of 2,4,5-T with the renal transport of an organic cation, tetraethylammonium (TEA) was studied in rat renal cortical slices. Kidneys were removed from male Sprague-Dawley rats, sliced and incubated with ^{14}C -labeled organic ions including 2,4,5-T, TEA, N'-methyl-nicotinamide (NMN) and para-aminohippurate (PAH). Radioactivity in the tissue homogenates and media were determined as a measure of tissue uptake. Efflux of ^{14}C -organic ions preloaded in tissue slices was determined by transferring the tissue through a series of seven sequential one minute baths and measuring the radioactivity in each wash chamber. Oxygen consumption by renal slices was measured by means of an oxygen electrode. At 10^{-3}M , 2,4,5-T did not alter oxygen consumption by renal slices in the presence or absence of lactate. The rate constants for TEA efflux were calculated from the loss of radioactivity from kidney slices preloaded with ^{14}C -TEA and transferred through seven chambers of control medium and then through eight chambers with 2,4,5-T present in the medium. 2,4,5-T increased TEA efflux but not NMN efflux, which was studied in the same way. Cyanine, a competitive inhibitor of the base transport system, phenoxybenzamine, an irreversible inhibitor of this system and mepiperphenidol, a competitive inhibitor of the cation system, all failed to alter 2,4,5-T transport. The authors concluded that 2,4,5-T interacts with specific intracellular TEA binding sites to block TEA accumulation, rather than with a carrier-mediated aspect of base transport and proposed a two step kinetic model to explain this conclusion further. The high degree of 2,4,5-T renal tissue binding was considered to be related to its interference with TEA transport.

565. Koschier, F. J., and Berndt, W. O. (1976a) Specificity of 2,4,5-trichlorophenoxyacetate on tetraethylammonium transport. Toxicol. Appl. Pharmacol. 38:297-306.

The effect of 2,4,5-T on the uptake of organic anions and cations by rat kidney slices was evaluated. Male Sprague-Dawley rats were administered 90 mg/kg of 2,4,5-T (with less than 0.05 ppm TCDD contaminant) subcutaneously in 70% ethanol, 24 hours before the kidneys were removed. Control rats received ethanol only. Renal cortical slices were incubated in modified Krebs-Ringer phosphate buffer which contained the organic cation or anion labeled with [^{14}C]. After a 5 min. incubation, to study initial uptake, or a 60 min. incubation, isotope in the media and in the tissue was counted by liquid scintillation methods. Compounds were also added to the incubation media to assess their effect on the radioactive anions or cations. Data were usually presented as double reciprocal plots. Uptake of 2,4-D was inhibited in 2,4,5-T pretreated rat kidneys and showed competitive Michaelis-Menten kinetics. By increasing substrate concentration from 0.4 to 22 ug/ml, inhibition by 2,4,5-T fell from 28 to 2.5%. Unlabeled 2,4,5-T competed for TEA steady-state uptake by 2,4,5-T-pretreated kidney slices, but did not compete for the initial influx of TEA. TEA only minimally

inhibited 2,4,5-T uptake and hexamethonium had no effect on 2,4,5-T uptake. The presence of 2,4,5-T in the medium inhibited N'-methyl-nicotinamide (NMN) uptake minimally (by 6%). The presence of 10^{-4} M acetate in the medium augmented the effect of 2,4,5-T on NMN uptake. The authors concluded that their results were consistent with a two-compartment model system, in which the initial step of transmembrane influx of TEA is not susceptible to 2,4,5-T competition while the second step of accumulation of TEA in the proximal tubular cell is where 2,4,5-T competes. 2,4-D uptake also showed a biphasic double reciprocal plot, consistent with a two-compartment model.

566. Koschier, F. J., and Berndt, W. O. (1976b) The nature of the inhibition of renal transport caused by the acute administration of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Tox. Appl. Pharmacol. 37:169.

[Abstract, only.]

567. Koschier, F. J., and Berndt, W. O. (1976c) In vitro uptake of organic ions by renal cortical tissue of rats treated acutely with 2,4,5-trichlorophenoxyacetic acid. Toxicol. Appl. Pharmacol. 35:355-364.

The renal excretion of 2,4,5-T and the effect of 2,4,5-T pretreatment on transport of organic ions were studied in the rat. Male Sprague-Dawley rats were administered a dose of 45-135 mg/kg 2,4,5-T in 70% ethanol, subcutaneously. After 24 hours, the kidneys were removed, sliced and incubated with a radioactively labeled organic ion for 3 hours. Radioactivity of the tissue and media were then counted. In some rats, daily doses of [14 C]-2,4,5-T were administered and radioactivity excreted daily in the urine was determined. The daily rate of urinary excretion was 80% of the administered dose (20 mg/kg) for the first 6 days and 100% of the dose on the seventh day. By day 18, only 1.2% of the total daily dose remained in the rat. After 6 daily doses of 90 mg/kg, 35% remained in the rat, but by day 17, only 2.4% of the daily dose remained. Weight loss, seen during the first week, was reversed during the subsequent days. One day after an acute dose of 2,4,5-T, the renal concentration and urinary excretion of 2,4,5-T were determined to be dependent on the amount administered, with a lower proportion excreted and higher tissue levels after large doses. After 2,4,5-T pretreatment (45-90 mg/kg) uptake of 2,4-D, of 2,4,5-T and tetraethylammonium chloride were inhibited, but uptake of para-aminohippurate and alpha-aminoisobutyric acid were not altered. PAH (1200 mg/kg) pretreatment did not affect in vitro renal transport of any of the organic ions. At higher pretreatment doses of 2,4,5-T and PAH, sporadic alterations in organic ion transport were observed. The authors concluded that high doses of 2,4,5-T may prolong the half-life of the herbicide by blocking its renal uptake and the almost complete excretion of daily doses of 2,4,5-T explains its low chronic toxicity.

568. Koschier, I. J., Girard, P. R., and Hong, S. K. (1978) Transport of 2,4-dichlorophenoxyacetate by rat renal cortical slices. Toxicol. Appl. Pharmacol. 45:883-894.

The kinetic characteristics of the efflux of 2,4-D from rat renal slices was determined in the presence of various metabolic inhibitors. Renal slices were prepared from kidneys of male Sprague-Dawley rats and were preloaded by incubation with 7.65 ug of [¹⁴C]-2,4-D in 3 ml buffer for 1 hour at 25°C. The slices were then transferred through a series of 18 sequential 1 minute washes and the radioactivity transferred into each wash solution and the amount that remained in the tissue was counted. The rate constant for efflux was then calculated. Tissue extracts were analyzed by paper electrophoresis for radioactive metabolites of 2,4-D. The kinetics of efflux was comprised of a fast and a slow component. Compounds that significantly increased the slow phase when added to the efflux medium were probenecid, dinitrophenol, stilbene derivative, iodoacetamide, succinate; succinate and lactate both increased the rate for the fast phase. The rate of 2,4-D efflux was temperature-dependent, with faster rates for 15°C and 35°C than at 25°C. Efflux was significantly increased (slow phase only) in potassium-free buffer, but was unaltered in calcium-free buffer. Uptake of 2,4-D was linear for 7 hours and transported 2 times more 2,4-D into the kidney than was transported out by the efflux mechanism. At 20 uM, 2,4-D produced a 50% inhibition of para-aminohippurate (PAH) uptake by renal slices. No metabolites of 2,4-D were detected in renal tissue extracts. The authors compared the renal transport of 2,4-D with that of PAH. They concluded that the in vitro influx of 2,4-D was rapid and 2,4-D was a potent inhibitor of PAH transport; although in vivo renal secretion of PAH was more rapid than for 2,4-D, other factors were proposed that could mediate this effect.

569. Koschier, F. J., Hong, S. K., and Berndt, W. O. (1979) Serum protein and renal tissue binding of 2,4,5-trichlorophenoxyacetic acid. Toxicol. Appl. Pharmacol. 49:237-244.

Binding of 2,4-D and 2,4,5-T to bovine serum albumin (BSA) was assessed as well as the capacity of 2,4,5-T to bind several sites of rabbit and rat kidney. BSA and between 10⁻⁵ to 10⁻⁶M ¹⁴C-2,4-D or ¹⁴C-2,4,5-T were incubated at 25°C for 120 minutes and then centrifuged in an ultra-filter cone membrane. The radioactivity in the filtrate and retentate were determined to calculate the proportion of bound radioactivity. At 0.5 mg/ml BSA, 20% of 2,4-D and 40% of 2,4,5-T were bound and at 5.0 and 50 mg/ml BSA, over 80% of each herbicide was bound to BSA. When rat kidney cortex homogenates were used instead of BSA, about 30% of the 2,4,5-T and 25% of 2,4-D were bound to the tissue. Binding of 2,4,5-T to microsomes of rabbit renal cortex was complete in 15 seconds, and was high, with a binding capacity of 17.5 umol/g protein and an association constant of 2.4 x 10³ M⁻¹. About 25% of 2,4-D and 2,4,5-T bound to rat and rabbit cytosol fractions of the renal cortex. These values were substantially higher than binding of the other organic ions studied. Two 2,4,5-T binding sites were identified in the

rabbit cytosol fraction, with association constants of $1.5 \times 10^4 M^{-1}$ and $0.04 \times 10^3 M^{-1}$ and corresponding binding capacities of 4.5 and 630 $\mu\text{mol/g}$ protein, respectively. 2,4,5-T inhibited tetraethylammonium binding to the cortex cytosol but not N¹-methylnicotinamide binding. The authors concluded that the ability of 2,4,5-T to bind to tissue components at least partially accounts for its renal accumulation after acute and chronic administration, and affects its nephrotoxicity by concentrating 2,4,5-T in proximal tubular cells or protecting the cell by lowering the free 2,4,5-T concentration within the cell.

570. Kouri, R. E., Ratrie, H., Atlas, S. A., Niwa, A., Nebert, D. W. (1975) Aryl hydrocarbon hydroxylase induction in human lymphocyte cultures by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Life Sciences 15(9):1585-1595.

The induction of aryl hydrocarbon hydroxylase (AHH) activity by TCDD was determined in cultured human lymphocytes. Lymphocytes were isolated from 19 healthy volunteers and cultured in the presence of mitogens. TCDD, added to the culture medium at a concentration of 33 ng per ml, produced a 1.7-2.4 fold increase in AHH activities of cells from 6 individuals. At 10 ng/ml, TCDD induced AHH activity from 1.7-2.7 times in cultures of cells from 13 individuals. Maximum induction, with a 2.9 fold increase in enzyme activity, was produced in 1 culture by TCDD at 100 ng/ml. Induction of AHH activity by 3-methylcholanthrene was maximal at 1.5 μM , compared with a maximally effective dose of TCDD of 30 nM, indicating that TCDD is about 50 times more potent than 3-MC in this culture system. Individuals whose cells showed low basal enzyme activity also showed the lowest increases in activity in the presence of inducers. The authors discuss the implications of their results as they relate to long-term risk potential of TCDD as a synergistic agent with other chemical carcinogens and they draw inferences related to the genetic mode of regulation of AHH induction, suggesting that several non-linked genetic loci may be involved.

571. Kouri, R. E., Rude, T. H., Joglekar, R., Dansette, P. M., Jerina, D. M., Atlas, S. A., Owens, I. S., and Nebert, D. W. (1978) 2,3,7,8-Tetrachlorodibenzo-p-dioxin as cocarcinogen causing 3-methylcholanthrene-initiated subcutaneous tumors in mice genetically "nonresponsive" at Ah locus. Can. Res. 38(9):2777-2783.

The authors studied the effects of intraperitoneal and subcutaneous injections of TCDD on the induction of hepatic, skin, and subcutaneous tissue aryl hydrocarbon hydroxylase (AHH). They also studied the related effects of intraperitoneal and subcutaneous pretreatment with TCDD on the tumorigenesis of 3-methylcholanthrene (3-mc). Two strains of mice were tested: C57BL/6Cum(B6), which is genetically susceptible to 3-mc-induced tumors; and DBA/2Cum(D2), which is a resistant strain. Treatment began at 4 to 6 weeks of age. Mice received a single intraperitoneal dose of TCDD in p-dioxane, varying from .05 to 100 $\mu\text{g/kg}$. Three days after administration, hepatic AHH was measured. The enzyme

assay was not described in this report. In the B6 strain, 1 ug TCDD/kg induced hepatic AHH to 70 percent of the maximum inducible level; in D2 no increase was noted. At levels of 60-100 ug TCDD/kg, AHH was induced in D2 to 80 percent of that in B6. Mice received TCDD subcutaneously in a single dose of either 1 or 100 ug/kg, and skin microsomal fractions were tested daily for AHH induction. Skin and subcutaneous tissue AHH was induced in B6 by the high dose at only a slightly higher level and more rapidly than in D2. At the low dose, AHH was induced in B6 at a much higher rate than in D2. Skin and subcutaneous tissue AHH was increased more than 20- to 50- fold in both strains by subcutaneous administration of TCDD. Hepatic AHH was increased more than 8- to 10-fold in both strains by intraperitoneal administration. Skin and subcutaneous tissue AHH was not measured after intraperitoneal treatment. Other hepatic enzymes were also monitored after TCDD treatment, specifically epoxide hydrase, GSH S-transferase, and glucuronosyltransferase, but only AHH activity was found to increase at a significant level following TCDD treatment. Tumorigenesis was studied by pretreating mice intraperitoneally or subcutaneously with either 1 or 100 ug TCDD in p-dioxane per kg; pretreatment was done either 48 hours prior to or at the same time that application of 3-mc was made. Mice were treated with 3-mc in 0.5 ml trioctanoin in subcutaneous doses of 150 mg. They were checked weekly by palpation for tumors. When a subcutaneous tumor grew to 1.0 cm in diameter, latency was calculated. After the eight month observation time, the carcinogenic index (CI) was calculated as the percentage of tumor incidence divided by the average latency in days, multiplied by 100. Several control groups were also used and consisted of mice treated with either 150 mg 3-MC, 0.05 ml trioctanoin, or TCDD (1 mg or 100mg). No tumors developed from treatment with the trioctanoin vehicle nor from the maximum dose of TCDD. When either dose of TCDD was given i.p. 2 days before 3-mc initiation, neither D2 nor B6 mice showed any significant change in the level of sensitivity to 3-mc. When TCDD was given i.p. at the same time as 3-mc, B6 mice did not show any change at either dosage. D2 mice showed a significant increase in sensitivity to 3-mc only when both doses of TCDD were given s.c. simultaneously with 3-mc, or the high dose of TCDD was given s.c. simultaneously with 3-mc. The C.I. increased from 5 to 38 when the high dose of s.c. TCDD was used, and to about 14 when either the low dose of s.c. TCDD or high dose of i.p. TCDD was used. The authors concluded that the AHH inducibility of the B6 strain was already at a maximal level following 3-mc treatment, and that the change in sensitivity of the D2 mice is evidence that TCDD is a cocarcinogen. The TCDD cocarcinogenic activity thus appears to be highly dependent on species, dosage, dose route, and pretreatment time employed in animal testing.

572. Kouri, R. E., Salerno, R. A., and Whitmire, G. E. (1973) Relationships between aryl hydrocarbon hydroxylase inducibility and sensitivity to chemically induced subcutaneous sarcomas in various strains of mice. J. Natl. Cancer Inst. 50(2):363-368.

The authors studied the relationship between susceptibility to 3-methylcholanthrene (3-mc) - induced sarcomas and hereditary hepatic

inducibility of aryl hydrocarbon hydroxylase (AHH) activity in 14 strains of mice. A carcinogenic index (CI) was established for weanlings treated subcutaneously with 150 ug 3-mc and observed for 8 months; the CI applied accounted for both tumor incidence and latency. The most sensitive strains used were C3H/fMai (CI 100); Blo.Br/J(83); C57BL/6Cum(78); C3HHe(77); C58/J(77); BALB/cCr;(74); and C57BL/10ScSn(64). The least sensitive strains were SWR/J(31); Snell/Mail(24); 129/J(25); SJL/J(24); DBA/2J924); AKR/J(12); and DBA/1J(9). All the mice tested were female except BALB/cCr. At 4 weeks of age mice were inoculated intraperitoneally with 100 mg 3-mc/kg in a trioctanoin solution at .05 mg/dose. Twenty-four hours after inoculation, the hepatic levels of AHH in the mice were measured using the procedure of Nebert and Belboin (1969) as modified by Thomas et al. (1972). The authors determined AHH inducibility as the ratio of average AHH activity /g wet liver weight of 3-mc treated mice divided by the average in untreated mice. They also tested for group-specific (gs) antigen (primarily the gs-1 murine virus) in mouse spleens, using microfilter complement fixation tests with pools of sera from Fischer rats with induced sarcomas from the Moloney murine virus. The author observed a direct relationship between 3-mc sensitivity and AHH inducibility. Mice with high CI for 3-mc sensitivity were inducible; those with a low CI were not. There was some indication of a link between virogene expression (measured by gs antigen expression) and low CI/AHH noninducibility. The authors suggest that this could be related to the fact that certain strains have a tendency to spontaneous tumors (associated with high virogene expression) while being simultaneously resistant to chemically induced tumors. One strain tested, however, C58/J, was sensitive to both spontaneous and 3-mc-induced tumors. It was the only strain tested that was both AHH inducible and consistently positive for virogene expression. Varying the dosage of 3-mc confirmed this correlation. Inducible mice receiving 37.5 ug 3-mc had CIs equivalent to those in noninducible mice receiving 300 ug 3-mc. The authors compared the effects of 3-mc with those produced in similar testing with 150 ug DMBA or BP, and did not obtain the same differential response between inducible and noninducible strains. They postulate that this was partially due to the fact that these two chemicals are less effective carcinogens when given subcutaneously at doses equivalent to that of the 3-mc administered. The C3H/fMai strain (males) showed extreme sensitivity to all three compounds, which could not be explained. The authors did indicate that this showed that AHH inducibility is not the only factor in determining absolute sensitivity in this strain.

573. Kramer, C. G. (1974) Health of employees exposed to 2,4,5-T. Findings of the DOW Chemical Company, Corporate Medical Department, 2030 DOW Center, Midland, Mich. 19 pp.

Dow chemical performed a morbidity analysis of 126 workers exposed to 2,4,5-T. This population was divided into 3 non-exclusive cohort groups which were compared separately to a control population of 4,600. Data were obtained from personnel records to determine length and extent of exposure. Data on selected health indices were obtained from

the company preventive health records. Information from health records included both clinical lab tests and questionnaire data. Data were analyzed in a 2 step process. First step analyses were the Students T-Tests on 50 clinical parameters and comparative analyses of observed and expected frequencies on questionnaire response assuming a Poisson approximation to the underlying binomial distribution. Second step analyses performed were linear correlation between the log of total career exposure to the base 10 and the log of total exposure days. Dichotomous health inventory measures between exposure and responses were determined by the Wilcoxon and unpaired Rank Test. Data showed no statistically significant difference between the exposed and control groups at the 0.5 level.

574. Krieger, R. I., Lee, P. W., Black, A., and Fukuto, T. R. (1973) Inhibition of microsomal aldrin epoxidation by diquat and several related bipyridylum compounds. Bull. Environ. Contam. Toxicol. 9(1):1-3.

The capability of diquat to inhibit microsomal oxidation was studied in rat hepatic microsomes in vitro. Microsomes were prepared and suspended in buffer containing diquat and aldrin epoxidation was analyzed. At a concentration of 6.6×10^{-6} M, 50% inhibition of aldrin epoxidation activity was attained. A higher dose (amount was not reported) inhibited enzyme activity completely. The inhibitory capacity of paraquat and of a series of agents with redox potentials between -0.18 and -0.55 v were tested in this microsomal epoxidation system. The authors concluded that diquat inhibits microsomal oxidation by disrupting the microsomal electron transport system. The brevity of descriptions of experimental procedure and the indirect method of correlating the redox-potential of a compound with its ability to inhibit microsomal aldrin epoxidation provide weak support for this tenuous conclusion.

575. Kuhn, E., and Stein, W. (1966) Model myotonia toward 2,4-D. Calcium absorption of the vesicles, its sarcoplasmatic reticulum among 2,4-D. Klin. Wschr. 44:700-702.

[Foreign language.]

576. Kuhn, E., and Stein, W. (1964) Experimental myotonia with 2,4-D administration in the rat. Klin. Wschr. 42:1215-1216.

[Foreign language.]

577. Kumaki, K., Jensen, N. M., Shire, J. G. M., and Nebert, D. W. (1977) Genetic differences in induction of cytosol reduced-NAD(P): Menadione oxidoreductase and microsomal aryl hydrocarbon hydroxylase in the mouse. J. Biol. Chem. 252(1):157-165.

The induction of activities by TCDD was studied in two strains of mice. A dose of 80 ug/kg of TCDD in para-dioxane was given to C57BL/6 mice and DBA/2N mice. Ten days later, the mice were killed and liver enzyme activities were assayed in TCDD-treated and dioxane-treated mice. Reduced-NAD(P):menadione oxidoreductase activity in the cytosol was assayed spectrophotometrically and aryl hydrocarbon (benzo[a]pyrene) hydroxylase activity in microsomes was assayed by measuring fluorescence of the hydroxylated product. An eightfold increase in hydroxylase activity was produced by TCDD in each strain as well as in hybrid mice from a cross of the two strains. Oxidoreductase activity was induced 375% in C57BL mice, 150% in DBA mice and 365% in hybrids. Oxidoreductase activity in TCDD-treated mice of both strains were inhibited by alpha-naphthoflavone and metyrapone to the same extent as activity of dioxane treated mice. Induction of both enzyme activities followed the same time course, with activities of both enzymes returned to normal within 70-90 days after TCDD treatment. A backcross of hybrid mice with DBA mice was made and responsive (heterozygous for the Ah allele) and nonresponsive (homozygous for the Ah allele from DBA mice). Of 40 backcross mice, 27 had oxidoreductase activity that was not inducible by TCDD. The responsive mice were more susceptible to the toxic effects of TCDD than were non-responsive mice. Data were presented for enzyme inductions by other agents in several species of mouse. The authors concluded that two genes were involved with induction of these enzymes and only one, hydroxylase induction, involved the Ah locus.

578. Kurisaki, E., and Sato, H. (1979) Tissue distribution of paraquat and diquat after oral administration in rats. Forensic Sci. Internat. 14(3):165-170.

The tissue distribution and histopathology of diquat administered orally to rats is described. Male Wistar rats were administered 231 mg(cation)/kg or 116 mg/kg diquat (as the herbicide formulation, Reglox, containing 30% diquat) in water, orally and after 2 hours to 9 days were killed and tissues were removed, homogenized in acid and analyzed for diquat spectrophotometrically after separation on ion exchange chromatography. Other tissue samples were prepared for light microscopy. Within 2 hours of treatment, distension of the stomach was observed. At later times, slight congestion of all organs was observed and hemorrhages of the lung and intestines were observed. Two hours after exposure, diquat concentrations were highest in the lung and heart. At 24 hours diquat levels had decreased in the liver, heart, lung, and brain and were the same as at 2 hours in the kidney. At 48 hours the only change in tissue diquat levels was an increase in kidney levels. At 5 and 9 days diquat levels were constant or decreased for all tissues. Paraquat retention was also studied. The authors concluded that diquat was more rapidly excreted than paraquat and did not accumulate or cause toxicity in the lung.

579. Kutz, F. W., Murphy, R. S., and Strassman, S. C. (1978) Survey of pesticide residues and their metabolites in urine from the general population. Environ. Sci. Res. 12:363-369.

The levels of 2,4-D and 2,4,5-T in human urine samples were analyzed. Preliminary data were presented from a study of pesticide residues in fat, urine and blood of 28,000 civilian Americans between 6 months and 74 years of age, selected scientifically to include groups with high risk of poor nutrition. The study was incomplete at the time of the report and the composition of the group from which the preliminary data was obtained was not given. 416-418 urine samples were analyzed for 2,4-D and 2,4,5-T. The specific analytic methods used for 2,4-D or 2,4,5-T analyses were not indicated. The limit of detection was between 5 and 30 ppb for both residues. No 2,4-D or 2,4,5-T was detected in any urine sample. The samples were also analyzed for 18 other pesticides. The authors emphasized that their results were preliminary and should not be assumed to represent the general population.

580. Kutz, F., and Strassman, S. (1979) Survey of pesticide residues and their metabolites in the general population of the United States. In: Commission of the European Communities Off., For. Off. Publ. POB1033 Luxembourg 1, Lux. 8 pp.

The results of analyses of 267 human urine samples for 2,4,5-T is presented. Samples were collected through a human monitoring survey system which obtained patient samples from pathologists in hospitals and private practice and samples from normal volunteers with documented herbicide exposure (no other details about type of herbicide, extent of exposure or number exposed were given). Samples were collected from hospital patients who were treated on an out-patient basis or were admitted for less than 24 hours. Analyses of each sample were performed by a multi-phenol method that was not described further. Of the samples analyzed, 1.5% had detectable levels of 2,4,5-T and all samples had less than 0.1 ppm 2,4,5-T. The levels of other herbicides in human adipose tissue, milk and urine samples were also presented. The authors did not present any conclusions pertaining to the 2,4,5-T levels they reported.

581. Kuz'minskaya, U. H., and Bersan, L. V. (1975) The effects of the sodium salt of 2,4-dichlorophenoxy-acetic acid on the glycolysis, ATP-ase and transketolase activity of the erythrocytes. Farmakologiya i toksikologiya. 38(1):102-104.

The effects of 2,4-D on enzymes involved in cell metabolism were studied in rats. Male rats were administered 1 g/kg 2,4-D sodium salt in water orally and blood was removed after 10 minutes, 1 hour, and 1, 5, and 15 days. Other groups of male rats were orally administered a dose of 20 mg/kg daily and blood was removed after 3 and 6 months. Erythrocytes were isolated and assayed for glycolysis activity as the

rate of lactate production, and for transketolase and ATPase activities. Significantly increased glycolysis occurred 10 minutes, 1 hour, and 5 days after treatment, a 31% increase in transketolase occurred after 10 minutes but was not sustained at later times, and peak ATPase activity occurred at 5 days and was 40% above controls. Three months after chronic administration significantly decreased glycolysis and increased enzyme activities occurred and at 6 months these effects were abolished or reversed. At 3 months, toxicity was evidenced by clinical signs that included apathy, weight loss and hemuresis. Clinical signs improved by 6 months. The authors concluded that the pentose-phosphate pathway was the predominant source of erythrocyte energy production after acute 2,4-D exposure and effects observed after chronic exposure reflected the toxic state of the animals.

582. Lam, H. F., Takezawa, J., and Van Stee, E. W. (1979a) The effects of paraquat and diquat on lung function measurements in rats. Ann. Rev. Resp. Dis. 119:327.

[Not available.]

583. Lam, H. F., Takezawa, J., and Van Stee, E. W. (1979) Evaluation the effects of paraquat and diquat in rats using pulmonary function tests. Environ. Health Perspec. 33:338.

[Abstract only.]

584. Lamb, J. C., Moore, J. A., and Marks, T. A. Evaluation of 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) toxicity in C57BL/6 mice: Reproduction and fertility in treated male mice and evaluation of congenital malformations in their offspring. National Toxicology Program, Research Triangle Institute, Research Triangle Park, NC. Report No. NTP-80-44. 57 pp.

Mixtures of compounds present in Agent Orange were administered to male mice and reproductive toxicity was assessed. Mice were treated for 8 weeks with daily diets containing 40 mg/kg 2,4-D plus 40 mg/kg 2,4,5-T plus 2.4 ug/kg TCDD (group II), or 20 mg/kg 2,4-D plus 20 mg/kg 2,4,5-T plus 1.2 ug/kg TCDD (group III), or 40 mg/kg 2,4-D plus 40 mg/kg 2,4,5-T plus 0.16 ug/kg TCDD (group IV). After the exposure period, treated males were mated with untreated females. No changes were reported in any reproductive parameters for treated groups compared to the control group given a diet supplemented with the corn oil vehicle. Parameters measured were mating frequency, average fertility, percent implantation and resorption sites, percent fetal malformations, germ cell toxicity, sperm concentration, sperm motility, percent sperm abnormalities, survival of offspring, and neonatal development. Toxicity of the liver and thymus and decreased body weights were observed in treated males. These effects were dose-related and were reversible after treatment ceased.

585. Langer, H. G., Brady, T. P., and Briggs, P. R. (1973) Formation of dibenzodioxins and other condensation products from chlorinated phenols and derivatives. Environ. Health Perspect. 5:3-7.

[Background material.]

586. Laporte, J. R. (1978) Multinationals and health: Reflections on the Seveso catastrophe. Intern. J. Health Serv. 8(4)619-632.

[Review article.]

587. Laporte, J. R. (1977) Effects of dioxin exposure. Lancet: 82-83.

[Editorial.]

588. Larsson, B., Oskarsson, A., and Tjalve, H. (1977) Binding of paraquat and diquat on melanin. Exp. Eye Res. 25(4):353-359.

Binding of diquat to melanin in vivo in mice and in vitro to bovine melanin are described. Pigmented C57-B1 mice and albino NMRI mice were administered 3.3 mg/kg [¹⁴C]-diquat in saline, intravenously. Mice were killed 5 min. to 4 days after treatment and were prepared for whole body autoradiography. Pigment granules were separated from bovine uveal tissue and were combined with [¹⁴C]-diquat and maintained at room temperature for 45 min. Non-bound diquat was separated by centrifugation and counted. A substantial amount of radioactivity was associated with melanin-containing tissues of C57-B1 mice and was absent from the same structures in albino mice. A concentration-dependent uptake of diquat occurred in vitro, with at least 97% of diquat bound to melanin when doses of up to 1 umol of diquat were added to 10 mg of pigment granule suspension. Binding of paraquat was also studied. The authors concluded that diquat was strongly bound to melanin and suggested the binding may be ionic and may potentiate the toxic effect of cataract formation in non-albino animals by diquat by sequestering the compound in the organ where the lesion occurs.

589. Lavy, T. L. (1978) Measurement of 2,4,5-T exposure of forest workers. Project Completion Report to the National Forest Products Association.

The levels of 2,4,5-T exposure of forest workers were measured and body burdens were estimated. The subjects were workers in 4 work crews who applied 2,4,5-T propylene glycol butyl ether ester (Esteron 245) by mist blower or helicopter at the rate of 2 lb. per acre or by backpack at the rate of 1.6 lb. per acre. Workers were experienced in their particular tasks in the spray operations and were instructed not to alter their normal work habits during the study. Workers did not wear gloves or protective clothing. Data were collected for 2 exposures of each worker, which were separated by at least 6 days. Air samples collected by each worker with air monitors, urine samples collected for 6 days (beginning 1 day prior to exposure) from each worker and 10 x 10 cm gauze patches, attached to the workers' clothing (arms, chest and back) during the spraying operations (but not during the mixing operations) were all monitored for 2,4,5-T acid by gas chromatography. (Recovery by this method was 78% in air samples, 79-120% in patches and 64-99% in urine). The 2 patch samples and the urine sample highest in 2,4,5-T were also analyzed for TCDD content by gas chromatography-mass spectrometry (50-100% recovery of TCDD by this method). Urinary creatine excretion rates were determined for the workers. Urinary excretion of 2,4,5-T ranged from 0.002 mg/kg body weight for helicopter crew members to 0.072 mg/kg for formulation mixers. Dermal exposures were calculated from the patch levels of 2,4,5-T, bare skin areas (estimated from photographs of workers) and body weights. Dermal

exposures ranged from no detectable exposure of helicopter crewmember to 1.7 mg/kg for mist blower workers. Air residues ranged from undetectable (below 0.15 ug/liter air) for helicopter crewmembers to 0.007 mg/kg for one of the backpack sprayers and 0.007 mg/kg for other backpack sprayers. In general, dermal exposure values correlated poorly with urine excretion levels. The ratio of TCDD to 2,4,5-T in the urine and patch samples analyzed was below the 0.04 ppm level in the original formulation. The authors concluded that 2,4,5-T analyses in urine were the most reliable indicator of exposure and that exposure levels were highest for workers that mixed the herbicides, followed by backpack sprayers, lowest for helicopter flagmen, followed by supervisors.

590. Lavy, T. L., Shepard, J. S., and Bouchard, D. C. (1980) Field worker exposure and helicopter spray pattern of 2,4,5-T. Bull. Environ. Contam. Toxicol. 24(1):90-96.

The amounts of 2,4,5-T deposited on workers applying the herbicide by several methods is reported. Six 100cm² gauze patches were applied to specified areas of clothing of workers applying 2,4,5-T. These workers included a five-man helicopter team, a tractor driver spraying 2,4,5-T on rice levees, a four-man tracker mist-blower team applying 2,4,5-T in a forest, and 12 forest backpack spray workers. The 2,4,5-T preparations applied were Thompson Hayward's Ded Weed LV-6 and Dow's ESTERON 245 (the 2,4,5-T esters in these preparations and other ingredients were not specified). The rate of application was 2 pounds of active ingredients per acre and the herbicide was applied in water. All spray operations except on rice levees were conducted in the morning with wind velocity of less than 5 mph. The time for each operation was 116 min. for the helicopter team, 65 min. for the rice tractor driver, 245 min. for the forest mist blower team and 180 min. for the backpack workers. In addition to the workers, one person (with gauze patches fixed to his clothing) stood under the helicopter spray and another walked through the spray area 2 hours after ESTERON 245 was applied. Three sets of mylar sheets (9 x 9 in) were placed 80, 160, 320, 640, and 960 feet from the helicopter spray path and were analyzed for 2,4,5-T after the herbicide was applied, to determine the extent of drift. All 2,4,5-T analyses were done by gas chromatography with a lower limit of detection of 10 micrograms per gauze patch or sheet. The mean levels of 2,4,5-T on the patches of workers were 0.043, 0.12, 0.94, and 5.43 mg/100 cm² for the helicopter crew, tractor driver, mist blower crew, and backpack sprayers, respectively. For 8 of the 22 workers, the patch on the thigh area contained over half of the total amount of 2,4,5-T recovered from the 6 patches. The patches on the individual under the helicopter spray contained from 0.01 to 6.48 mg and the total (maximum) potential exposure was calculated to be 0.86 mg/kg body weight. The authors further estimated that 0.034 mg/kg 2,4,5-T would be absorbed and excreted by an individual from this exposure. No 2,4,5-T was detected in patches from the individual exposed to the spray area 2 hours after the application was made. Over 84% of the 2,4,5-T sprayed by helicopter was deposited within 80 feet of the spray path and 99%, within 160 feet. Slight drift occurred,

with trace levels of 2,4,5-T detected in 2 of 12 samples taken beyond 320 feet from the spray path. The authors concluded that workers who applied 2,4,5-T were unlikely to receive doses of 20 mg/kg or more of 2,4,5-T, the amounts reported by others to cause fetotoxicity in mice. This report is important in providing an estimate of human exposure potential from normal herbicide application, unfortunately several important points were not included in the report and include the composition of the herbicides and the ester applied, the amount of time the two people who were exposed to helicopter spray remained in the sprayed area, the recovery of 2,4,5-T from gauze pads for the analytical method used, and urine levels for the exposed workers.

591. Lawson, E. R. (1976) 2,4,5-T residues in storm runoff from small watersheds. J. Soil Water Conserv. 31:217-219.

In this study, the author measures levels of 2,4,5-T in storm runoff from sprayed forest areas. Two watersheds were chosen for the study: one 1.5 acre area on which all woody vegetation had been removed, and another 1.5 acre area which contained about 60 square feet of pine. Both watersheds were sprayed on Sept. 1971, June 1972, and July 1973; the cleared watershed was sprayed with 4 lb acid equivalent 2,4,5-T isooctyl ester/acre and the partially cut watershed with 21 lbs/acre. A control watershed a similar size was not sprayed. Storm runoff samples were collected and analyzed by gas chromatography. Only runoff samples taken 3 weeks after the first application showed any detectable 2,4,5-T. Samples from the cleared watershed had an average of 2.1 ppm 2,4,5-T, while samples from the partially cut watershed averaged 1.0 ppm. Storm runoff 7 and 11 weeks after the first spraying, and after the second and third spraying did not yield detectable levels of 2,4,5-T. The author concluded that none of the concentrations of 2,4,5-T found in this study were a hazard to wildlife or man.

592. Leahey, J. P., and Hemingway, R. J. (1975) The metabolism of diquat on hens and residues in eggs and tissues. Environ. Qual. Saf. [Suppl.] 3:157-162.

The tissue distribution, biotransformation, and excretion of diquat is described for the chicken. One hen each was administered and five daily doses of 4-5 ug/g [¹⁴C]-diquat ion (99.7 + % purity) in feed, and one hen was given 14 daily doses of 0.4-0.5 ug/g [¹⁴C]-diquat in feed. The feces, tissues, egg yolk, and egg albumin were analyzed for radioactivity. Metabolites were separated by paper chromatography. Three days after a single dose of diquat, 98.5% of the dose had been excreted in the feces and 5 days after five doses were administered, 94.5% was excreted in the feces. Eggs contained less than 0.1% of the dose. The yolk of eggs collected from the hen given 14 doses contained increasing amounts of diquat to day 9, and lower levels, subsequently. The pellets given to the hen were found to have less diquat available 9 days after they were prepared than fresh pellets given on day 1 of the experiment. Diquat (unmetabolized) accounted for 72-80% of the radioactivity in fecal samples collected on days 1-5 from the hen that

was being administered five daily doses, and 18-22% on days 6 and 7. Two metabolites, comprising 4 and 2% of the fecal radioactivity from day 5 samples were identified as the 9-oxo-6,7-dihydro-8H-dipyrido [1,2-a:2',1'-c]-5-pyrazinium ion(I) and 1-oxo-1,2,3,4-tetrahydro-2H-pyrido-[1,2-a]-pyrazinium ion (II), respectively. The radioactive composition of egg yolks from hens after subacute exposure identified by isotope dilution analysis, was 54-85% metabolite I, 26-39% diquat and 2-10% metabolite II. Seven days after 5 doses of diquat and 4 hours after 14 doses were given, hens were killed and kidney, liver, lung, muscle, fat, and blood were analyzed for diquat. Kidneys contained 0.004 ug/g diquat and all other tissue had less than 0.001 ug/g diquat. The authors concluded that the amount of diquat that remained in eggs after a dose 40 times larger than expected from normal commercial use was toxicologically insignificant, as were the tissue levels in hens after subacute exposure and levels of the two less toxic metabolites (based on oral LD₅₀ values in rats).

593. Lee, C. (1979) Dioxin (Dilemma)? Med. J. Australia 602-603.

[Editorial.]

594. Lee, I. P., and Dixon, R. L. (1978) Factors influencing reproduction and genetic toxic effects on male gonads. Environ. Health Perspec. 24:117-127.

The authors review metabolic parameters that modulate the function of the male gonad. Data are presented substantiating the ability of TCDD to induce gonadal mixed function oxidase activity. Aryl hydrocarbon hydroxylase (AHH) activity of the rat testis was induced twofold by one PO treatment of 10 ug/kg TCDD while AHH activity of the rat prostate was induced 150 times by this dose. Testicular AHH activity returned to normal within 72 hours, while prostate activity remained elevated after 3 weeks. The authors concluded that TCDD, by inducing enzymes that activate other chemicals, can modulate mutagenicity of germ cells and oncogenicity of prostate glands.

595. Lehman, A. J., and Habermann, R. T. (1964) Pathological changes in rats fed 2,4-D for 2 yrs. Unpublished report: FDA.

[Not available.]

596. Lehn, P. J., Pate, B. D., Kim, D. R., and Voight, R. C. Evaluation of the effects of defoliant on the animal communities of Test Area C-52A, Eglin Air Force Base, Florida. In Abstracts, 1972 Meeting of the Weed Science Society of America p. 90.

[Not available.]

597. Leistra, M., Smelt, J. H., and Zandvoort, R. (1975) Persistence and mobility of bromacil in orchard soils. Weed Res. 15:243-247.

The authors studied the movement of bromacil through 1 m of orchard soil. Two study sites were used which had received bromacil applications annually for 6 to 7 years prior to the experiment. One orchard contained sandy loam soil (Groesbeek), while the other had silty clay loam soil (Est). Bromacil was applied at 1.6 and 2.4 kg/ha at Groesbeek and at 1.2 and 2.4 kg/ha at Est. Soil samples from the top 150 cm of soil were collected and analyzed for bromacil by gas chromatography. There appeared to be differences in bromacil movement depending on the soil type. One year after application, the amounts of bromacil remaining in the Groesbeek soil were 0.91 and 1.75 kg/ha for the 1.6 and 2.4 kg/ha dose, respectively. At Est, the soil residues were 0.39 and 1.32 kg/ha for 1.2 and 2.4 kg/ha, respectively. The highest concentrations of bromacil occurred at 10-20 cm in Groesbeek soil and in the 5-10 cm and 10-20 cm layers for the Est samples. For both soils, the average residual bromacil was 54% of the applied herbicide. The authors calculated the half-life of bromacil to be approximately 8 months. The authors also calculated the amount of bromacil leaching from the top 1 m of soil as 1.8-2.8%, depending on dose and soil type. The effect of repeated applications to the test sites was not discussed by the authors. In light of the 8-month half-life of bromacil, it would be expected that some accumulation of bromacil would occur after repeated application.

598. Leng, M. L. Comparative metabolism of phenoxy herbicides in animals. In Fate of pesticides in large animals. G. W. Ivie and H. W. Dorough, eds., (New York: Academic Press, 1977) pp 53-76.

[Review article.]

599. Leng, M. L. (1978) 2,4,5-T RPAR - Review of EPA's rationale and their calculations for exposure to 2,4,5-T and TCDD. [Unpublished paper] DOW. 7 p.

[Background material.]

600. Leonard, A., and Lauwerys, R. R. (1980) Carcinogenicity, teratogenicity, and mutagenicity of arsenic. Muta. Res. 75:49-62.

[Review article.]

601. Levina, M. M. (1973) Industrial hygiene in monuron production. Gig. Tr. Prom. Sanit. Proizvod. Pestits. 178-192.

[Not available.]

602. Lewis, H. J. (1972) Herbicide Study. [letter to the editor] Science 177:745.

[Editorial.]

603. Leyland, A. (1973) A case of 2,4,5-T poisoning in the dog. Vet. Rec. 92(6):149-150.

The autopsy findings of a dog that ingested a lethal dose of 2,4,5-T is reported. Stomach contents contained 2,000 ppm 2,4,5-T and exposure was suspected to be from 2,4,5-T contaminated water or food from a farm that had been treated with a formulation of esters of 2,4,5-T.

Hemorrhages were observed in the gastrointestinal tract, kidneys, and diaphragm. Severe gastritis and congestion of the renal cortex were also evident at autopsy. The liver was not examined because it had undergone extensive autolysis in the 2 days after death which preceded the examination. The authors concluded that death was caused by ingestion of a dose of 2,4,5-T approximately 3-4 times larger than the LD₅₀.

604. Lindahl, R., Roper, M., and Deitrich, R. A. (1978) Rat liver aldehyde dehydrogenase - immunochemical identity of 2,3,7,8-tetrachloro-dibenzo-p-dioxin inducible normal liver and 2-acetylaminofluorene inducible hepatoma isozymes. Biochem. Pharmacol. 27:2463-2465.

Rat liver aldehyde dehydrogenases, induced by different conditions including TCDD treatment, were differentiated from each other by immunochemical techniques. Three aldehyde dehydrogenase isozymes were prepared from rat liver cytosol. Tau isozyme was induced by TCDD administration to rats phi isozyme was induced by phenobarbital; alpha isozymes were induced by chemical carcinogens (2-acetylaminofluorene, for example) and are hepatoma-specific isozymes. Rabbit antiserum against alpha and tau isozymes as well as the 3 isozyme antigens were obtained from methods described in other publications and were studied by Ouchterlony 2-dimensional immunodiffusion. Anti-alpha serum reacted with the tau isozyme forming a precipitin line. Anti-tau serum reacted with alpha isozyme but not with normal (uninduced) liver preparations. Neither serum reacted with phi isozyme. The authors described some previously published kinetic and physical properties of the enzymes and concluded that the TCDD-induced enzyme is immunochemically identical to the hepatoma isozymes, but distinct from the basal and phenobarbital induced isozymes.

605. Lindquist, N. G., and Ullberg, S. (1971) Distribution of the herbicides 2,3,5-T and 2,4-D in pregnant mice: accumulation in the yolk sac epithelium. Experientia 27:1439-1441.

The uptake and distribution of [¹⁴C]-2,4-D and [¹⁴C]-2,4,5-T were followed in the pregnant mouse by autoradiography. NMRI mice were given a single intravenous injection of 10 ul of either compound in 50%

ethyl alcohol. Mice were killed 5 minutes to 4 days after the injection and whole body autoradiography was performed. Radioactivity accumulated in the visceral yolk sac epithelium in mice given 2,4,5-T early (day 8-9) or late (day not indicated) in pregnancy. In early pregnancy no isotope was associated with the fetus, whereas at the later stage, the concentrations of isotope in the fetal blood, abdomen, pericardium, and ocular fluid reached that of the maternal blood. Radioactivity in ^{14}C -2,4-D treated mice accumulated only slightly in the yolk sac, reached the fetus, and was eliminated from all tissues within 24 hours. Both compounds were evenly distributed in tissues other than the yolk sac. The data in this report were presented as a brief summary without any other details of the results provided.

606. Lisk, D. J., Gutenmann, W. H., Bache, C. A., Warner, R. G., and Wagner, D. G. (1963) Elimination of 2,4-D in the urine of steers fed 4-(2,4-DB) or 2,4-D. J. Dairy Sci. 46:1435-1437.

The amount of 2,4-D excreted in urine and feces after one dose was administered to steer was determined. One Holstein steer was fed a diet that contained 5 ppm 2,4-D for 1 day. On the subsequent 7 days, urine and feces were collected and analyzed for 2,4-D by gas chromatography. The limit of sensitivity of this method was 0.1 ppm and recovery was 82%. Urine collected on day 1 contained 85.3 mg (17.6 ppm), on day 2 contained 15.4 mg (1.0 ppm) and on day 3 contained 0.1 ppm. Of the total dose of 113.5 mg administered, 100.7 mg were recovered in urine within 2 days of administration. Another compound, 4-2,4-dichlorophenoxybutyric acid, administered to one steer was recovered in the urine as 2,4-D only. The authors concluded that 2,4-D was excreted unchanged primarily in the urine.

607. Litchfield, M. H., Daniel, J. W., and Longshaw, S. (1973) The tissue distribution of the bipyridylum herbicides diquat and paraquat in rats and mice. Toxicology 1(2):155-165.

The distribution and accumulation of diquat into mouse tissues is presented. Male Alderley Park mice were administered 50 mg(cation)/kg [^{14}C]-diquat in saline intravenously. After 10 min. to 72 hr., mice were killed and prepared for whole-body autoradiography. A group of 40 Alderley Park rats were administered a diet that contained 250 ppm diquat ion as diquat dibromate monohydrate for up to 8 weeks. Food intake and body weights were recorded weekly and at 2, 4, 8, and 9 weeks 10 treated rats and 5 control rats were killed and tissues were weighed, homogenized, extracted with trichloroacetic acid, and diquat was separated by ion-exchange chromatography and analyzed colorimetrically. Ten minutes after injection, diquat was distributed to all tissues and was concentrated in cartilage and gall bladder and low in the brain and spinal cord. After 1 hr., the levels and radioactivity were lowered in all tissues (especially in cartilage) than at 10 min. except the urine and intestinal epithelium, which had higher levels. At 24 hr. diquat was present in the intestine and bladder, and at 72 hr. in the gastrointestinal tract, only. In 7 days only 5.5% of a single

dose of [^{14}C]-diquat was excreted in the urine by rats and by 14 days only 5.8% was excreted in the urine. No alterations in food consumption or body or organ weights were observed for rats on a diet with 250 ppm diquat, compared to controls. Diquat concentrations were highest in the kidney and in large intestine at 1.2 ppm by 8 weeks in both tissues. No diquat was detected in blood or muscle and low levels were detected in the brain. One week after exposure ended, no diquat was detected in any tissues (lower limit of detection, 0.05 ug/g). The authors concluded that diquat was rapidly distributed and eliminated and was not accumulated. The authors suggested that secretion of diquat into the intestine occurred in the absence of biliary excretion.

608. Lloyd, J. W., Thomas, J. H., and Mawhinney, M. G. (1973) 2,4,5-T and the metabolism of testosterone -1, 2- $^3\text{H}_2$ by mouse prostate glands. Arch. Environ. Health 26(4):217-220.

The effects of 2,4,5-T on fructose levels, testosterone accumulation, and metabolite formation in the prostate gland and on weights of male sex organs were studied in mice. Male Swiss-Webster mice were administered a daily dose of 6.25 to 25 mg/kg of 2,4,5-T for 10 consecutive days. On the eleventh day, 10 ug/kg of [^3H]-testosterone was administered intraperitoneally and 5 minutes later, the prostate glands were removed and radioactive testosterone and metabolites were extracted and analyzed by thin layer chromatography. In some groups of rats, no radioactivity was administered; instead, on the eleventh day, the prostate gland was removed and analyzed for fructose, or the liver was removed, sliced, or homogenized and incubated in the presence of [^3H]-testosterone. Radioactive metabolites were analyzed chromatographically. In 2,4,5-T-treated rats, a 35% reduction in prostate uptake of testosterone was observed. The size of the effect was the same for all 3 doses of 2,4,5-T. The relative proportion of radioactivity associated with each metabolite in the prostate or in liver tissue was unaltered after 2,4,5-T treatment. Fructose levels were unaltered in treated mice and the weights of the seminal vesicles, prostate glands, and testes all were the same for 2,4,5-T treated mice as those of vehicle controls. The authors concluded that 2,4,5-T could have produced a direct effect on the prostate gland, interfering with uptake of testosterone, and suggested inhibition of androgen binding to target tissue as a possible mechanism.

609. Lock, E. A. (1979) The effect of paraquat and diquat on renal function in the rat. Toxicol. Appl. Pharmacol. 48(2):327-336.

The effects of diquat on renal clearance of several compounds and of plasma and red cell volumes were studied in the rat. Male Alderley Park (Wistar derived) rats were administered 540-900 umol per kg of diquat perorally, followed in 24 hours by administration subcutaneously of [^{14}C]- or [^3H]-labeled inulin, para-amino hippurate (PAH), N'-methylnicotinamide (NMN), or diquat. Water equal to 5% body weight was then given intraperitoneally. Blood samples and urine samples were collected subsequently and analyzed for urea content and radioactivity.

Plasma and cell volumes were determined in rats injected with ^{51}Cr -tagged red cells in plasma containing ^{125}I -labeled human serum albumin. ^{51}Cr and ^{125}I radioactivities were determined in blood samples and in the excised kidneys and volumes were calculated from the extent of dilution of the administered isotopes. One day after diquat was administered, renal clearances of urea, inulin, PAH, NMN, and diquat were all significantly reduced to 17 to 55% of vehicle control levels. Urine flow was also reduced and the filtration fraction was double the values in saline-treated controls. Doses of 680 and 900 $\mu\text{g}/\text{kg}$ of diquat produced a significant decrease of 14-20% in plasma volume, no change in red cell volume and a significant increase in hematocrit 24 hours after treatment. The higher dose also produced a significant decrease of 18% in renal plasma volume. The slightly higher clearance of diquat than inulin indicated that active secretion of inulin occurs. The renal toxicity of paraquat was also studied. The authors concluded that the renal changes produced by diquat occur secondary to hemodynamic changes which involve water redistribution to the lumen of the gastrointestinal tract and resultant decreases in plasma volume and renal blood flow. In man, the reduced renal excretion of diquat was proposed to enhance the systemic toxicity of diquat.

610. Lock, E. A., and Ishmael, J. (1979) The acute toxic effects of paraquat and diquat on the rat kidney. Toxicol. Appl. Pharmacol. 50(1):67-76.

The effects of diquat on renal function were studied in the rat in vivo and in vitro. Male Alderley Park (Wistar-derived) rats were administered a single dose of 680 $\mu\text{mol}/\text{kg}$ or 900 $\mu\text{mol}/\text{kg}$ diquat in saline perorally. Some rats were also administered 50 $\mu\text{Ci}/\text{kg}$ [^{14}C]-diquat. Urine and plasma biochemical analyses and cell counts were performed and urinary enzymes were assayed fluorimetrically with 4-methyl-umbelliferyl substrates. Uptake of ^3H -para-aminohippuric acid (PAH) and [^{14}C]-N'-methylnicotinamide (NMN) from incubation medium into renal cortical slices was determined with diquat in the medium [1- ^{14}C]-glucose or [6- ^{14}C]-glucose was included with diquat in the medium of some renal slices and [^{14}C]-carbon monoxide. CO_2 production was determined by trapping [^{14}C]- CO_2 on potassium hydroxide saturated filters and then determining radioactivity of the filters. Some renal slices were incubated in medium containing [^{14}C]-acetate, then homogenized and the radioactivity in fatty acid extracts of tissue homogenates was determined. A dose of 680 $\mu\text{g}/\text{kg}$ of diquat produced proteinuria and glucosuria during the next 24 hours. The urinary albumin to total protein ratio was not altered by this dose but an increased number of cells was excreted in the urine. After 24 hours, 7.5% of the oral dose of diquat was excreted in the urine. Mild focal hydropic degeneration was observed in the proximal tubules after diquat treatment. At 1mM in the medium, diquat inhibited the accumulation of NMN (by 55%), but not of PAH into renal slices. Slices of kidneys removed from diquat-treated rats when in vivo clearance was reduced did not show reduced NMN or PAH accumulation, compared to control kidney slices. Oxidation of [1- ^{14}C]-glucose, but not of [6- ^{14}C]-glucose was

stimulated 30% by 0.1 and 55% by 1mM diquat but oxygen consumption was unaltered by these doses. Incorporation of [¹⁴C]-acetate by fatty acids was increased by 40% by 0.1 or 1mM diquat added to culture medium but not in renal slices from rats treated in vivo 24 hours earlier. The effects of paraquat and mercuric chloride on renal function were also studied. The authors concluded from their metabolic studies that orally administered diquat had not entered renal cells sufficiently within 24 hours to alter the redox state of the cell. From the PAH and NMN accumulation studies in vitro, they concluded that in vivo alterations in NMN and PAH clearances reported by others were the result of altered renal hemodynamics.

611. Loos, M. Phenoxyalkanoic acids. In Herbicides: Chemistry, degradation, and mode of action. Vol. 1, P. C. Kearney and D. D. Kuvman, eds., (New York: Marcel Dekker, Inc., 1975) pp 1-128.

[Review article.]

612. Lucier, G. W., Lui, E. M. K., and Lamartiniere, C. A. (1979) Metabolic activation/deactivation reactions during perinatal development. Environ. Health Perspec. 29:7-16.

[Review article.]

613. Lucier, G. W., McDaniel, O. S., and Hook, G. E. R. (1975) Nature of the enhancement of hepatic uridine diphosphate glucuronyltransferase activity by 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats. Biochem. Pharmacol. 24:325-334.

The characteristics of hepatic enzyme induction by TCDD were investigated in the rat. CD rats were administered a single oral dose of TCDD in acetone-corn oil (1:9) and actinomycin D was administered intraperitoneally immediately after TCDD to some rats. Hepatic microsomes were prepared from treated and vehicle-control rats. Glucuronyltransferase activity was solubilized from microsomes with deoxycholate, and then purified by ammonium sulfate precipitation, dialysis, and molecular sieve chromatography. Glucuronidation of 1-naphthol and paranitrophenol (PNP) were assayed colorimetrically and of testosterone and estrone were assayed using radioactive substrates. Product formation was quantified for all substrates by separation of radiolabeled substrates and products on ion exchange chromatography. The activity of beta-glucuronidase was determined by measuring hydrolysis of PNP-beta-D-glucuronide. Maximum (sixfold) induction of PNP glucuronidation was observed between 9 and 16 days after a dose 25 ug/kg of TCDD was given to male rats and the enzyme levels remained elevated (by 160%) after 73 days. A lower dose produced smaller increases in enzyme activity with the same time course for induction. Glucuronidation of 1-naphthol showed the same induction pattern by TCDD as PNP, while no induction of testosterone or estrone glucuronidations or beta-glucuronidase occurred. The identity and amount of each

glucuronidation product was confirmed by the results of ion exchange chromatography. The magnitude of induction of PNP glucuronidation was the same in rats aged 17, 38, 80, and 335 days when TCDD was administered. Addition of $10^{-6}M$ TCDD directly to the microsomal incubation mixture did not alter rates of glucuronidation for any of the 4 substrates tested. Addition of magnesium or detergent (triton X-100) stimulated enzyme activity from TCDD-treated rats and controls equally. Microsomal cholesterol and phospholipid contents and kinetic parameters for glucuronidation activity from TCDD-treated and control rats were the same, except for an increase in the maximum velocity of the reaction by enzyme from treated rats. Actinomycin D seemed to block the TCDD inductive effect, although it also decreased total microsomal protein, complicating the interpretation of this result. Purified glucuronidation enzymes from treated and control rats eluted in the same positions chromatographically, with far more activity associated with peaks from TCDD-treated preparation. The pH optimum of 7.2 and temperature optimum of 40°C for TCDD treated and control DNP glucuronidation was established. The authors concluded that TCDD exerts its effect on glucuronidation indirectly, probably by increasing synthesis or decreasing degradation of the enzyme. The conclusions are supported by well-designed experiments that considered many aspects of enzyme induction and used the most technically appropriate methodology to test each aspect.

614. Lucier, G. W., McDaniel, O. S., Hook, G. E. R., Fowler, B. A., Sonawane, B. R., and Faeder, E. (1973) TCDD-induced changes in rat liver microsomal enzymes. Environ. Health Persp. 5:199-209.

The effects of TCDD on hepatic microsomal enzyme activities and liver function was studied in the rat. The time-course of enzyme induction was studied in male Charles River rats (3 per group) who were administered a single oral dose of 5 or 25 ug/kg TCDD in acetone-corn oil and were killed 1-38 days later. Hepatic microsomes were prepared and analyzed for microsomal enzyme activities and serum ornithine transcarbamylase activities were measured. Dose-response relationships were examined in another series of experiments in which male and female rats were administered a single dose of 0.2, 1.0, 5.0 or 25 ug/kg TCDD and livers were removed 3 days after treatment. Hepatic microsomes and mitochondria were isolated. Microsomal enzyme activities were assayed and mitochondrial oxidative phosphorylation rates were measured polarographically with an oxygen electrode. No rats died in any treatment group. In the time-course study, aniline hydroxylation activity increased by 100%, UDP glucuronyltransferase activity by 600%, cytochrome P-450 and b_5 levels by 60% and aminopyrine demethylation decreased by 30%. These changes were maximal 3-16 days after treatment and were still different from control values after 38 days. Liver function was unaltered, as were NADPH cytochrome c reductase and beta-glucuronidase activities. Microsomal protein contents increased significantly 28 days after treatment, by 60%. For the dose-response study, dose-related elevations in glucuronyltransferase (to 500%, maximum) and benzpyrene (to 1400%) were observed, with larger increases in female than male microsomes. Increased cytochrome P-450 and b_5

levels and aniline hydroxylation activity of up to 100% occurred, while oxidative demethylation of aminopyrine, ethylmorphine and benzphetamine decreased and NADPH cytochrome C reductase activities were unchanged. The ratio of smooth to rough endoplasmic reticular enzyme and protein levels were decreased by TCDD. No alterations in mitochondrial state 3 or state 4 oxidative phosphorylation rates were observed after TCDD treatment. The authors concluded that hepatic microsomal enzymes are extremely sensitive to TCDD body burdens and recommended 8 areas for future research.

615. Lucier, G. W., Sonawane, B. R., McDaniel, O. S., and Hook, G. E. R. (1975) Postnatal stimulation of hepatic microsomal enzymes following administration of TCDD to pregnant rats. Chem. Biol. Interact. 11:15-26.

The effect of TCDD administration to pregnant rats on maternal, fetal and neonatal enzyme levels is described. Pregnant Charles River rats were administered 3 ug/kg TCDD in acetone-corn oil (1:9) on day 5, 10 or 16 of gestation. At various stages of gestation or after birth, maternal livers, livers of offspring, and placenta were removed and microsomes were prepared and assayed for benzyrene hydroxylase (BPH) and UDP gluouronyltransferase (UDPGT) activities. For 1 experiment newborn rats of mothers treated on day 5 of gestation were fostered by control mothers from birth to day 8 and control newborns were likewise fostered by TCDD-treated mothers. TCDD treatment did not alter fetal, placental or fetal liver weights, litter sizes, fetal microsomal protein contents or the incidence of gross malformations. Both maternal BPH and UDPGT (para-nitrophenol, substrate) activities were induced about 15 fold during pregnancy and remained elevated by 3 fold, 3 weeks after birth. Prenatal TCDD treatment resulted in elevated UDPGT levels in 4-day old or older offspring. Treatment on day 5 with TCDD did not alter BPH levels of fetuses on day 20 of gestation, but caused elevated levels in neonates. Treatment on day 10 or 16 of gestation resulted in elevated fetal and neonatal BPH levels. Neonatal cytochrome P-450 and b₅ levels were also elevated following TCDD treatment on day 10 of gestation. Placental BPH and UDPGT levels were induced 20 and 3 fold respectively from TCDD treatment on day 16. Testosterone glucuronidation activity was not induced in any offspring by TCDD treatment during gestation. Newborn rats exposed to TCDD only by consumption of milk of treated foster mothers and newborn rats exposed only in utero exhibited elevated enzyme levels, comparable and intermediate between control and continuously exposed rats, respectively. The authors concluded that multiple factors contribute to TCDD-induction of neonatal enzymes.

616. Luning, K. G., and Ryman, N. (1975) Comments on tests of dominant lethals in mice. Mutat. Res. 31:127-128.

[Editorial.]

617. Luster, M. I., Albro, P. W., Faith, R. E., and Lawson, L. D. (1978) Inability of passive antibodies to reverse the effects of dioxin toxicity. Chemosphere 7(1):29-34.

Immunization with antibodies raised against protein-bound TCDD was studied as a means of protection against the acute effects of TCDD in the mouse. 1-Amino-3,7,8-trichlorodibenzo-p-dioxin (TriCDD-NH₂) conjugated to bovine serum albumin or bovine thyroglobulin and 1-amino-TCDD diazo-linked to human serum albumin were injected into rabbits. Sera were assayed by immunodiffusion analysis for antibodies and complement-inactivated. Gamma globulin was then isolated by ion exchange chromatography, dialyzed, ultrafiltered, and administered intramuscularly to male C57Bl mice. A single dose of 250 ug/kg TCDD (99+% purity) in acetone-corn oil (1:6) by gavage. The immune rabbit globulin (IRG) administered had the capacity to bind 44% of the total TCDD administered and TCDD competed as well as triCDD-NH₂ for displacement of binding of radioactive antigen with antibody. Mortality rates and body weights were recorded for all mice. Both of these parameters were the same for mice treated with IRG 1 day prior to TCDD IRG 1 day after TCDD, saline plus TCDD, or normal rabbit globulin plus TCDD. Substantial weight loss and the same time course for lethal effects occurred in each group. Control mice treated with normal globulin plus corn oil survived and gained weight. The authors proposed specific technical difficulties as well as tight TCDD-receptor binding as explanations for the failure of immune globulins to protect against TCDD toxicity. Less than half of the dose of TCDD maximally could bind the administered antibody and inactivation by metabolism during the 24 hours between administration of antigen and antibody could have caused the negative effect, invalidating any conclusions regarding mechanisms of TCDD toxicity.

618. Luster, M. I., Clark, G., Lawson, L. D., and Faith, R. E. (1979a) Effects of brief in vitro exposure to 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) on mouse lymphocytes. J. Environ. Pathol. Toxicol. 2:965-977.

The effects of in vitro TCDD exposure of mouse spleens on lymphocyte functions are described. Female B₆C₃F₁ mouse spleens were submerged in a solution of TCDD (99+% purity), [¹⁴C]-TCDD (99% purity), amino-3,7,8-trichlorodibenzo-p-dioxin (Tri CDD-NH₂) or 3,4,3', 4'-tetrachlorobiphenyl (TCB) in dimethylsulfoxide (DMSO) for 10 sec. Cell cultures were prepared from the treated tissue and cell viability was monitored by a trypan blue exclusion technique. Lymphocyte responses to mitogens (phytohemagglutinin, concanavalin A and lyopolysaccharide mitogen) were assessed by determining the treated to control ratio of radioactive precursor incorporation into RNA and protein. Tissue incorporation of radioactivity from [¹⁴C]-TCDD was determined in some spleens and binding of [³H]-concanavalin A (Con A) to TCDD-treated splenic lymphocytes was assessed in vitro. Total uptake of TCDD ranged from 0.29 ng per spleen after 5-10 sec. of immersion to 0.66 ng after 60 min. Compared to non-treated controls, DMSO treatment reduced thymidine, leucine and uridine incorporation in response to mitogens. Immersion of spleen in

5-50 ng TCDD/ml DMSO reduced these rates of incorporation below vehicle controls for all 3 mitogen treatments. A decrease in cell viability was observed in TCDD-treated cultures after 48 hours, but the effect was not dose-dependent. Neither Tri CCD-NH₂ nor TCB produced statistically significant decreases in DNA synthesis of Con A-treated lymphocytes. No decrease in Con A binding or histological changes by light or electron microscopy were observed in TCDD-treated spleens. The authors concluded that DMSO-solubilized TCDD produced a direct effect on splenic lymphocyte proliferative response to mitogens, especially T-lymphocyte mitogens.

619. Luster, M. I., and Faith, R. E. (1979) Assessment of immunologic alterations caused by halogenated aromatic hydrocarbons. Ann. N.Y. Acad. Sci. 320:572-578.

[Review article.]

620. Luster, M. I., Faith, R. E., and Clark, G. (1979b) Laboratory studies on the immune effects of halogenated aromatics. Ann. N.Y. Acad. Sci. 320:473-486.

[Review article.]

621. Lutz, H., and Lutz-Ostertag, Y. (1973) Pesticides, teratogenesis and survival in birds. Arch. Anat. Histol. Embryol. 56:65-78.

The effects of spraying 2,4-D on fetal mortality and hatchability of several species of birds is described. Three preparations of 2,4-D (amino salt) were used in this study. Lot H (purity and other ingredients not reported) in water was sprayed at the rate of 880 g/hectare on red partridge and gray partridge eggs before incubation and on pheasant and quail eggs before, or 3 or 7 days after incubation started. Lot W (purity and other ingredients not reported) in water was sprayed on group of quail eggs before incubation at 2 rates, 880 and 110 g/hectare. A purified lot (99.1% purity) of 2,4-D dimethylamine salt in water was sprayed on pheasant and red partridge eggs and before incubation at the rate of 1200 g/hectare. Impurities in this preparation included other phenoxyalkanoic acids and 400 ppm 2,4-dichlorophenol. Control quail and pheasant eggs were sprayed with water. Mortality prior to hatching and the proportions and types of external malformations in chicks at hatching were recorded. Treatment and control groups contained 100-350 eggs, except for 1 group of 632 pheasant eggs sprayed on day 0 with lot H. Lot H produced 74-75% mortality of pheasant eggs and 43-77% mortality of all other eggs prior to hatching, compared to 6% for control quail eggs and 46% for control pheasant eggs. From 36-42% of the quail and pheasant eggs treated before incubation and 22-30% of eggs treated on day 3 or 7 with lot H hatched normally. Malformations included lordosis, shriveled feet, and paralyzed toes and occurred in 12-13% of all quail groups and up to 4% of the other groups treated with lot H. Mortality of quail embryos

sprayed with the higher dose of lot W was 17% 2 days before hatching and 13% for the lower dose 3 days before hatching. The rate of hatching was 71-72% for both groups and cell hatched chicks were normal. The purified preparation resulted in 26-30% mortality before hatching. The authors presented a review of a previous study from their laboratory on 2,4,5-T and studies on other pesticides. The authors concluded that only lot H was teratogenic and did not attributed this toxicity to 2,4-D because the other lots were unable to produce teratogenicity.

622. Lutz, J. F., Byers, G. E., and Sheets, T. J. (1973) The persistence and movement of picloram and 2,4,5-T in soils. J. Environ. Qual. 2(4):485-488.

The authors describe the persistence and movement of 2,4,5-T and picloram in three soil types: clay loam (Fannin), fine sandy loam (Chandler), and loam (Chester). Experimental plots on each of the three soil types were sprayed with 2.24 kg/ha 2,4,5-T plus picloram. Four additional plots received 4.48 kg/ha herbicide. Plots were 0.04 acre in area, with an average slope of 27%, and vegetation consisted of orchard grass. Soil core samples (20 per plot) were collected immediately after spraying and 15, 50, and 100 days after spraying. Samples were analyzed by gas chromatography (detection limit 1 ppb picloram, 3 ppb 2,4,5-T). Approximately 90% of the 2,4,5-T and 60% of the picloram disappeared 15 days after application. By 100 days, 2,4,5-T had essentially disappeared, while about 10% picloram remained. Results were similar for both spraying levels. For all soils treated with 2.24 kg/ha, 68% or more of the total picloram remaining could be found in the top 7.5 cm of soil. Less than 10 ppb 2,4,5-T was detected at soil depths greater than 7.5 cm. At 4.48 kg/ha, more picloram but not more 2,4,5-T penetrated to the 15-45 cm depths than at 2.24 kg/ha. Both herbicides disappeared most rapidly from Fannin soil and least rapidly from Chandler soil. Little downslope movement of herbicide was observed, possibly, according to the authors, because of the high hydraulic conductivity of the soil resulting in very low runoff. The authors made no conclusion about the overall effects of these herbicides on the ecosystem under study. However, it appears that neither herbicide is very persistent or mobile in soil.

623. Lynn, G. E. (1965) A review of toxicological information on TORDON herbicides. Down to Earth 20(4):pp. 6-8.

[Review article.]

624. MacKay, D., and Leinonen, P. J. (1975) Rate of evaporation of low-solubility contaminants from water bodies to atmosphere. Environ. Sci. and Technol. 9:1178-1180.

[Background material.]

625. Maddy, K. T., and Edmiston, S. (1976) Summary of occupational illnesses and injuries reported by physicians as due to exposure to pesticides of workers cleaning and/or repairing pesticide handling machinery. California Dept. of Food and Agriculture, Report No. ACF 59-300. 5 pp.

[Background material.]

626. Maddy, K. T., Peoples, S. A., and Tochilin, S. J. (1976) Occupational illnesses due to exposure to pesticides or their residues as reported by physicians in California in 1975. California Dept. of Food and Agriculture, Report No. ACF 59-202. 17 pp.

[Background material.]

627. Madge, D. S. (1977) Effects of trichlorophenoxyacetic acid and chlorodioxins on small intestinal function. General Pharmacology 8(5-6):319-324.

Absorption of D-glucose was studied in everted gut sacs of mice treated with TCDD or 2,4,5-T in vivo. CD-1 mice were administered a single dose of 2,4,5-T methyl ester (99.6% purity) or TCDD in corn oil by oral intubation or vehicle only. From 1-28 days later the small intestine was removed and everted sacs were prepared and incubated with test sugars or amino acids. Small intestines were examined histologically 7 days after treatment with TCDD, 2,4,5-T or vehicle. TCDD elicited slight histopathological damage while no other compounds produced any change in gut histology. 2,4,5-T at doses of 25-250 mg/kg and TCDD from 10-75 ug/kg produced dose-dependent decreases in D-glucose absorption. Changes in fluid transfer by all treatments were minor. Maximum decrease in glucose absorption occurred 14 days after 2,4,5-T treatment. Neither TCDD nor 2,4,5-T treatment altered galactose, L-histidine or L-arginine transfer 7 days later. The decreases in D-glucose transport were eliminated by adding an exogenous energy source, D-mannose to serosal fluid or by adding D-maltose which metabolizes glucose. D-glucose metabolism by the gut was high from the levels of accumulated D-glucose in treated intestine. The author concluded that 2,4,5-T or TCDD treatment enhanced D-glucose metabolism by the gut during transfer, without affecting the transfer mechanism, itself.

628. Magnusson, J., Ramel, C., and Eriksson, A. (1977) Mutagenic effects of chlorinated phenoxy acids in Drosophila melanogaster. Hereditas 87:121-123.

The authors investigated the induction of sex linked recessive lethals, nondisjunction, and chromosome loss with 2,4,5-T and 2,4-D. 2,4,5-T was tested in two different forms: purified 2,4,5-trichlorophenoxyacetic acid containing less than 0.1 ppm TCDD and 2,4,5-T butoxyethyl ester containing 1 ppm TCDD dissolved in petroleum ether and with an unspecified emulsifier. A purified preparation (unspecified) of 2,4-D was also tested. In all experiments, each compound was dissolved in ethanol (amount unspecified) and mixed in the usual corn agar growth medium. In the chromosome loss and nondisjunction tests, males and females were treated during their entire larval period with 250 ppm 2,4,5-T butoxyethyl ester or 100 ppm 2,4-D. No increases in chromosome loss or nondisjunction were induced by 2,4,5-T or 2,4-D. However, only 1 dose of each of these compounds was tested. For the recessive lethal test both the dose of each herbicide and type of 2,4,5-T were changed. Therefore, no comparison can be made between the two phases of the experiment. In the recessive lethal test adult wild type Karsnas 60 strain males were treated with 1,000 ppm 2,4-D or 2,4,5-T containing less than 0.1 ppm TCDD. After 2 weeks of treatment males were mated to Muller 5 strain females (age and number unspecified). No effects of these chemicals on successive broods were studied. A total of approximately 13,000 individuals in the combined F₁ and F₂ generations were observed per chemical tested; 10,000 individuals from the control group were scored. When F₁ and F₂ results were combined both 2,4-D and 2,4,5-T induce significant increases (p less than 0.01) in recessive lethals. However, when data from each generation were tabulated separately only 2,4-D induced a statistically significant increase in recessive lethals in F₂. This is an indication that 2,4-D gives rise to lethal mosaics.

629. Mahle, N. H., Higgins, H. S., and Getzendaner, M. E. (1977) Search for the presence of 2,3,7,8-tetrachlorodibenzo-p-dioxin in bovine milk. Bull. Environ. Contam. Toxicol. 18(2):123-130.

The authors analyzed milk samples from dairy and beef cows for TCDD contamination. Twenty-five milk samples were obtained from cows in Missouri, Arkansas, and Oklahoma grazing on pasture that was treated with 2,4,5-T for brush control. Sample collections occurred 5 days to 48 months after spraying had occurred. Exact spray formulations and quantities could not be documented. Milk was analyzed by GC-MS (detection limit 1 ppt). Control samples were obtained from a local supermarket. No TCDD was detected in any of the samples.

630. Maiback, H. I., and Feldman, R. (1974) Appendix R: Systemic absorption of pesticides through the skin of man. In: Occupational Exposures to Pesticides, A Report to the Federal Working Group on Pest Management from the Task Group on Occupational Exposure to Pesticides, Washington, DC. pp. 120-127.

The extent of absorption of 2,4-D and diquat were studied in human male volunteers. ^{14}C -2,4-D and ^{14}C -diquat were applied to the forearms of groups of six men and absorption was calculated from the urinary excretion of radioactivity over the 5 day period following exposure. The extent of urinary excretion of the compounds, estimated from excretion of intravenously administered ^{14}C -2,4-D and ^{14}C -diquat, was also used to calculate absorption. (The urinary excretion rates after intravenous administration of the compounds were not reported). A mean of 5.8% of ^{14}C -2,4-D and 0.4% of ^{14}C -diquat was absorbed. Occlusion of the exposed skin for 24 hours, increased penetration of ^{14}C -2,4-D to 14.7% of the dose and of ^{14}C -diquat to 1.4% of the dose, based on 5 day urinary excretion rates. Skin damaged by removing the stratum corneum with cellophane tape absorbed 33.8% of the dose of ^{14}C -2,4-D and 3.8% of the dose of ^{14}C -diquat. Penetration of ^{14}C -2,4-D was decreased by washing the forearm for 2 minutes with soap and water to 0.5% of the dose when washed one minute after 2,4-D was administered and 3.7% when washed after 4 hours. Absorption of 10 other pesticides was also studied under various experimental conditions.

631. Mailman, R. B., and Hodgson, E. (1972) The cytochrome P-450 substrate optical difference spectra of pesticides with mouse hepatic microsomes. Bull. Environ. Contam. Toxicol. 8:(3):186-192.

The types of difference spectra produced from binding of pesticides with microsomal enzymes was identified. Hepatic microsomes from inbred mice (from the North Carolina Department of Health) were prepared and incubated with each herbicide. The optical difference spectra of the pesticide-exposed microsomes relative to a control sample of microsomes without pesticide added was recorded and characterized as type I with the absorption minimum at 416-420 nm and maximum at 385-390 nm or type II, with the minimum at 390-410 nm and maximum at 425-435 nm. The spectral size reported for each pesticide was calculated as the difference in optical density between the peak and trough of the spectrum, and was reported as a proportion of the P-450 optical density. Diuron, bromacil, picloram, and 2,4-D all produced type I difference spectra with relative spectral sizes of .126, .004, .016, and .024, respectively. The absorbance of diquat (or a metabolite) obscured its binding with microsomal enzymes. The binding of a total of 51 compounds were characterized in this system. The authors concluded that aromatic compounds generally cause type I binding while compounds with an unhindered nitrogen atom produce type II binding.

632. Majumdar, S. K., and Golia, J. K. (1974) Mutation test of 2,4,5-trichlorophenoxyacetic acid on Drosophila melanogaster. Can. J. Genet. Cytol. 16:465-466.

The authors studied the ability of 2,4,5-T to induce recessive lethals in Drosophila melanogaster. Recessive lethals were detected by the Muller-5 technique (BASC-method) in which two-day-old Oregon R male flies were fed 250 or 1,000 ppm 2,4,5-T for 15 days. Negative control flies received only their usual diet while positive controls received 250 ppm ethylmethane sulfonate. The stock of 2,4,5-T used in this study contained no detectable dioxin (detection limits not specified). After treatment males were mated with BASC females and kept in vials containing medium without 2,4,5-T. Three 4-day matings were conducted to determine the effects of 2,4,5-T on the spermatogenic cycle of the males. At the end of each 4-day mating period, males were placed in bottles containing new females. The absence of wild type males in the F₂ generation of flies was used as the end point for induction of recessive lethals. The authors reported that 2,4,5-T induced a statistically significant increase in recessive lethals at 1,000 ppm but not at 250 ppm, according to the significance test of Kastenbaum and Bowman. Results seemed to be pooled for all three matings, so that no conclusions can be drawn on the sensitivity to 2,4,5-T of various portions of the spermatogenic cycle in Drosophila.

633. Majumdar, S. K., and Hall, R. C. (1973) Cytogenetic effects of 2,4,5-T on in vivo bone marrow cells of Mongolian gerbils. J. Hered. 64:213-216.

The authors studied the effects of 2,4,5-T on chromosomes of Mongolian gerbil bone marrow cells. Male and female 50 to 80-day-old animals received 5 daily intraperitoneal injections of 10, 30, 50, 70, or 100 mg/kg 2,4,5-T dissolved in dimethyl sulfoxide, a total dose of 50, 150, 350, and 500 mg/kg respectively. The authors reported that the 2,4,5-T used in these experiments contained no measurable amount of TCDD but did not report the detection limits of their chemical analysis. Ten animals were treated at each dose level except at 100 mg/kg where 17 animals were treated because of low mitotic index. Water and DMSO negative control groups were included in the study, but no positive control group was reported. Twenty four hours after the last injection and 2 hours after an intraperitoneal injection of 0.2 ml of a 0.4 mg/ml solution of Colcemid, the animals were killed and chromosome spreads prepared from bone marrow cells. Metaphases (500 per treatment group) were scored for chromatid gaps, chromatid breaks, and fragments. Percent abnormal metaphases were analyzed statistically by the t-test. The authors did not state whether scoring took place by a double-blind technique to minimize bias on scoring. No increases in chromosomal aberrations were observed up to 150 mg/kg dose level. At 250 mg/kg, chromosome damage (chromatid gaps and breaks) increased significantly. No significant differences in fragments were observed. No comparison of the 500 mg/kg dose and controls was reported. The authors also statistically analyzed using the t-test the total number of abnormal metaphases per dose group. Up to 150 mg/kg, no significant differences

were observed between control and treated groups. Groups receiving 250 mg/kg, 350 mg/kg, or 500 mg/kg showed statistically significant increases in abnormal metaphases. No analysis of aberrations based on the sex of the animals was presented.

634. Malcolm, G. N. (1977) 2,4,5-T and human birth defects. New Zealand Institute of Chemistry, Bulletin No. 11, Sept. 29, 1977.

[Editorial.]

635. Malizia, E., Andreucci, G., Chiavarelli, M., Amato, A., and Gagliardi, L. (1979) A follow up of 20 months in Seveso, an environmental calamity. Vet. Hum. Toxicol. 21(Supplement):139-140.

[Review article.]

636. Malling, H. V., and Wassom, J. S. Action of Mutagenic Agents. In Handbook of Teratology, Wilson, J. G., Fraser, F., eds. (New York: Plenum Press, 1977) pp. 99-152.

[Review article.]

637. Manis, J., and Apap, R. (1979) Intestinal organic anion transport, glutathione transferase and aryl hydrocarbon hydroxylase activity: Effect of dioxin. Life Sciences 24(15):1373-1380.

The effects of TCDD on intestinal transport of iron penicillin, and para-amino-hippurate and hepatic and intestinal glutathione-S-transferase (GSH) and aryl hydrocarbon hydroxylase activities were studied in the rat. Male Sprague-Dawley rats were administered 17 ug/kg TCDD in dioxane orally 2 days before sacrifice or intraperitoneally 1-14 days before sacrifice. Everted gut sacs were prepared from the duodenum to measure iron transport and from the mid-intestine to measure penicillin transport. $^{59}\text{FeSO}_4$ or [^{14}C]-benzyl penicillin or [^{14}C]-aminohippurate transport was evaluated by introducing the radiolabeled chemical to one side of the gut and measuring the radioactivity on each side after a 2 hour incubation period at 37°C. GSH-T activity was measured in supernatants from 100,000 xg centrifugation of tissue homogenates. AHH activity was measured in crude liver homogenates with benzo(a)pyrene as substrate. Serosal transfer of iron was increased by 38% in gut sacs from rats given TCDD orally, compared to vehicle controls, while mucosal transport of iron, transport of penicillin, and intestinal GSH-T activity remained unchanged. Hepatic GSH-T activity increased by 70% and AHH activity by 20-fold from TCDD treatment. Intestinal AHH activity increased 100-fold after oral and intraperitoneal treatments with TCDD. No change in penicillin or para-aminohippurate uptake, or GSH-T activity were observed in intestines of rats pretreated with TCDD 1-14 days previously. Hepatic GSH-T levels were elevated 1.5-2.0-fold

4 and 11 days after pretreatment but no significant elevation in activity was observed 1 or 14 days after pretreatment. The authors concluded that TCDD selectively inhibited 2 intestinal mechanisms (iron transport and AHH activity), without altering other systems, as GSH-T activity which is sensitive to stimulation by other inducers.

638. Manis, J., and Kim, G. (1979a) Stimulation of iron absorption by various polyhalogenated aromatic hydrocarbon environmental contaminants. Bioch. Pharmacol. 28(18):2841-2843.

The effect of 2,4,5-T on in vitro intestinal iron transport was studied in the rat. The report also included results of 14 other polyhalogenated aromatic hydrocarbons on in vitro and in vivo iron transport. Male Sprague-Dawley rats were administered 100 mg/kg 2,4,5-T in dioxane by gastric intubation 1 day prior to excising the intestine. Everted duodenal sacs were prepared and incubated in medium containing $^{59}\text{FeSO}_4$. The radioactivity inside and outside of the sac and associated with gut tissue were determined at the end of the incubation period and net serosal transfer and mucosal transfer of iron was calculated. Mucosal uptake of iron was increased significantly in gut sacs from 2,4,5-T treated rats compared to transfer in sacs from vehicle treated controls, while no alteration in serosal transfer was observed. The authors suggested that stimulated iron transport from exposure to certain polyhalogenated hydrocarbons, including 2,4,5-T, could pose health hazards related to changes in iron absorption and metabolism.

639. Manis, J., and Kim, G. (1979b) Stimulation of iron absorption by polychlorinated aromatic hydrocarbons. Am. J. Physiol. 236(6):E763-E768.

The effects of TCDD on intestinal transport of iron, calcium, galactose and proline and hepatic and intestinal aryl hydrocarbon hydroxylase (AHH) were studied in the rat and mouse. Male Sprague-Dawley rats were pretreated with TCDD in dioxane by intraperitoneal injection or gastric intubation. Pretreated rats showed normal weight gain (compared to controls) and none of the pretreated rats died from TCDD treatment. Iron absorption was studied in vivo by anesthetizing the rat and tying off a loop of the duodenum. The loop was filled with medium containing $^{59}\text{FeSO}_4$ and after 30 minutes the radioactivity in the luminal fluid gut and in the gut wall was determined. Transfer of iron from the lumen into the mucosa (mucosal uptake) was calculated as the amount of isotope lost from the lumen during the 30 minute experimental period and the amount of iron transferred to the bloodstream was calculated as the difference between mucosal uptake and the isotope in the excised gut wall. Everted gut sacs were also prepared from sections of duodenum and were used to study in vitro iron transport. AHH activity was assayed in liver and intestine from rats and mice. Transfer of iron to the blood was increased 41%-67% by pretreatment of rats with 33 ug/kg of TCDD by either route. Mucosal uptake was affected minimally. Serosal transport was increased 100-108% by everted guts of rats

pretreated 2 days previously with 22-42 ug/kg TCDD by either route, while mucosal transport was nonaffected. The effect was diminished when pretreatment occurred longer than 2 days prior to removing the gut. Serosal transfer of calcium was decreased 26% and mucosal uptake by 11% in TCDD in everted sacs from pre-treated rats, while galactose and proline transport were not effected. Everted gut sacs from TCDD-pretreated mice showed 78-156% increases in iron transport over control levels. AHH activity was induced 20-fold in mouse liver, and 100-1000-fold in mouse intestine by doses of 31-124 ug/kg TCDD. In the rat a 200-fold increase in intestinal AHH levels occurred at 10 ug/kg TCDD, a dose that failed to alter iron transport. The authors concluded that TCDD selectively affects discrete intestinal mechanisms for transport of specific compounds and is not a general metabolic effect. The gut transport effect of TCDD is not mediated by enzyme induction solely because these 2 effects occur at different doses.

640. Manis, J., and Kim, G. (1979c) Introduction of iron transport by a potent inducer of aryl hydrocarbon hydroxylase, 2,3,7,8-tetrachlorodibenzo-p-dioxin. Arch. Environ. Health 34(3):141-145.

The effects of TCDD on intestinal iron transport and aryl hydrocarbon hydroxylase (AHH) activity were studied in the rat. Male Sprague-Dawley rats were given 67 ug/kg of TCDD in dioxine by gastric intubation 2-4 days prior to excising various sections of the gut. Everted gut sacs were incubated at 37°C in the presence of ⁵⁹Fe-iron sulfate and the transfer of radioactivity into or out of the sacs were determined by liquid scintillation counting of the bath medium and fluid within the sac. The AHH activity of the mucosal layer was measured using benzo(a)pyrene as substrate. In everted duodenal sacs from TCDD-pretreated rats, 50% more iron was transferred serosally than by sacs from either non-treated controls or vehicle controls. This TCDD effect did not occur in gut sacs prepared from more distal segments of the intestine. AHH activity was significantly elevated in the duodenum as well as the adjacent distal section of TCDD-treated rats compared with the vehicle controls. After a dose of 17 ug/kg of TCDD, the increase in serosal transfer of iron fell to 35% above controls 2 days after TCDD treatment and was not significantly increased statistically after 4 days. Hepatic GSH-transferase levels were significantly elevated to 70% and 102%, 2 and 4 days after 17 ug/kg of TCDD was given orally, compared to vehicle controls. Two to 4 days after an intraperitoneal dose of 17 ug/kg TCDD was given, no changes in iron transport occurred, while hepatic GSH-transferase remained elevated to 80% above control levels for 2 weeks after TCDD was injected. The authors concluded that the locus for iron transport stimulation is more accessible to TCDD from the mucosal surface than the serosal surface of intestinal cells.

641. Mantovani, A., Vecchi, A., Luini, W., Sironi, M., Candioni, G. P., Spreafico, and Garratini, S. (1979) Effect of 2,3,7,8 tetrachloro-dibenzo-p-dioxin on macrophage and natural killer cell mediated cytotoxicity in mice. Istituto di Ricerche Farmacologiche "Mario Negri" via Enitrea 62-2, Milano, Italy.

The effects of TCDD on cellular immunity was studied in mice. Target cells were prelabeled with ³H-thymidine and the ability of peritoneal macrophages from TCDD-treated mice to lyse these cells was determined. The effect of TCDD treatment on the cytostatic and cytotoxic activity of macrophages against tumor cells after endotoxin stimulation was also measured. TCDD stimulation of peritoneal exudate cells was determined by measuring the number of nucleated cells and the proportion of polymorphs in this cell population over 47 days after treatment. The effect of spleen natural killer cells in producing lymphoid cytotoxicity was evaluated in vitro. The effect of TCDD on ³H-thymidine uptake of lymphoma TCDD did not affect the functional status of macrophages or natural killer spleen cells in any of these studies. In TCDD-treated mice, the total numbers of spleen cells and macrophages recovered were diminished. The authors suggested that TCDD effects on cells that are important in in vivo resistance against infection and neoplasia may lead to impairment in these functions.

642. Manzo, L., Gregotti, C., Di Nucci, A., and Richelmi, P. (1979) Toxicology of paraquat and related bipyridyls: biochemical, clinical and therapeutic aspects. Vet. Hum. Toxicol. 21(6):404-410.

[Review article.]

643. Marriage, P. B., Saidak, W. J., and Von Stryk, F. G. (1975) Residues of atrazine, simazine, linuron, and diuron after repeated annual applications in a peach orchard. Weed Res. 15(6):373-379.

[Not available.]

644. Martin, J. V. (1979) 2,4,5-T. Lancet p. 1243.

[Editorial.]

645. Martinez-Cabruja, R. (1976) Liver response and other effects of tetrachlorodibenzo-p-dioxins. Arch. Farmacol. Toxicol. 2(3):211-234.

[Not available.]

646. Mason, R. W. (1975) Binding of some phenoxyalkanoic acids to bovine serum albumin in vitro. Pharmacology 13(2):177-186.

A high affinity hydrophobic binding site on bovine serum albumin and several sites with lower affinity for phenoxy-acids are described. Bovine serum albumin (BSA) was combined with 2,4-D or 2,4,5-T in vitro at 37°C or 23°C and the extent of binding was assessed by ultrafiltration and presented as Scatchard plots. Affinity constants were calculated from the Scatchard plots. Absorption peaks were determined for difference spectra of mixtures of 2,4-D or 2,4,5-T with cetriride or BSA. The differential spectral constant for 2,4,5-T was greater than for 2,4-D. The binding properties of other phenoxy acids were studied. The author concluded that BSA contained a hydrophobic binding site with high affinity for phenoxy acids and compared this site to a binding site on human serum albumin implicated in drug binding.

647. Matsumura, A. (1970) The fate of 2,4,5-trichlorophenoxyacetic acid in man. Jap. J. Environ. Health 12:20-25.

The pharmacokinetics of 2,4,5-T was described. Three male volunteers ingested 100-150 mg of 2,4,5-T and either blood or urine was collected from each subject periodically during the subsequent 72 hours. Levels of 2,4,5-T in urine of workers of a chemical manure factory and in the workplace were assayed. Plasma levels of 2,4,5-T, plotted as a function of time, showed a biphasic pattern and each of the 2 components, which reflected absorption and excretion, showed first order kinetics. Absorption was rapid, since the maximum plasma concentration was reached 4 hrs. after a single oral administration. The half-life for plasma clearance was 11 hr. About 80% of the dose ingested by volunteers was excreted in the urine in 72 hrs. No metabolites were detected in gas chromatograms of urine samples. Workers excreted up to 3.6 mg of 2,4,5-T in urine daily; the breathing zone in the factory contained 0.2-0.7 mg/m³ 2,4,5-T, while components of the factory apparatus contained up to 15 mg/m³ of 2,4,5-T. The volume of distribution for 2,4,5-T, 8% of the body weight, approximated the total blood volume. The authors concluded that intake of 2,4,5-T by humans can be established by urinalysis, because almost all of the dose is gradually excreted into the urine unchanged.

648. Matsumura, F., and Benezet, H. J. (1973) Studies on the bioaccumulation and microbial degradation of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Environ. Health Perspec. 5:253-258.

The authors measured the bioaccumulation of TCDD and three other pesticides in three model aquatic ecosystems and its microbial degradation in 100 species of microorganisms known to degrade persistent pesticides. Bioaccumulation of ring-labeled ¹⁴C-TCDD was measured in Ostracoda (algae) species, brine shrimp, mosquito larvae, and northern brook silverside fish. Numbers of organisms used were not specified. In the first model ecosystem 5-10 pmole TCDD was added directly to water, food, and primary organisms, and this mixture was

then added to the aquarium containing invertebrate test organisms. Final concentrations in the test organisms of 1,592, 1,956, and 7,069 ppb TCDD, were observed in daphnids, brine shrimp, and algae respectively. In the second model ecosystem, TCDD (20 pmole ppb) was applied to the inner surface of a container containing the test organism food source. Twenty-four hours later the primary food organisms were transferred to an aquarium containing the test invertebrates. Final concentrations of 279 and 879 ppb TCDD were found in algae and daphnids, respectively. Flaws in these two models are extensive: the compounds were used above the limit of water solubility, and the extent of direct pick-up from partitioning and food intake is uncertain. To correct for the solubility problem, a third model ecosystem was used. TCDD (1.62 (u)g) was added to 1g sand; the solvent was evaporated, and the sand was added to a test aquarium containing invertebrates and fish. Final concentrations of TCDD were 2, 157, and 4,150 ppb in fish, brine shrimp, and mosquito larvae, respectively. In a two-step bioaccumulation experiment, mosquito larvae were exposed to TCDD (1.62 (u)g) and were then added to an aquarium containing fish. The TCDD concentration in fish increased to 708 ppb but in the mosquito larvae TCDD remained nearly the same as the previous experiment. The length of time of the study was not reported. In additional testing, TCDD was microbially degraded by only 5 of the 100 species tested, and TCDD (0.1 (u)mole) translocation from sand to organic soil or water was observed to be nearly nonexistent in a 10 x 1.5 cm glass column.

649. Matsunaka, S., and Kuwatsuka, S. (1975) Environmental problems related to herbicidal use in Japan. Environ. Qual. Saf. 4:149-159.

[Review article.]

650. Matsushita, T., Arimatsu, Y., Misumi, J., Tomio, T., and Nomura, S. (1975) Skin disorders caused by herbicides sodium chlorate and sodium 2,2-dichloropropionate. Kumamoto Med. J. 28(4):164-169.

One case report and a field study of 76 workers exposed to the herbicide 2,2-dichloropropionate are described. A 42-year-old man had been engaged in spraying sodium chlorate and phenoxy herbicides for 5 years when he began to spray the herbicide, sodium 2,2-dichloropropionate. Within a few days he developed contact dermatitis on the face and hands and nausea. A patch test of 0.1% sodium 2,2-dichloropropionate showed mild erythema after 24 hours. A moderately high blood sugar level was the only notable clinical finding (but other tests that were conducted were not reported). A group of 76 forest workers (mean age, 40.8 years) who sprayed herbicides about 10-20 days per year and were employed in this occupation for 6-9 years (mode of work period of the group) were compared with 16 control workers from the same area. The number of men in the group who were exposed to sodium 2,2-dichloropropionate was not specified, although this herbicide and sodium chlorate were used by at least some workers in the group. Clinical blood chemistry tests, hematology tests, urinalyses, and dermatologic tests were performed and reports of other symptoms

were obtained by questionnaire and by interview. Nausea, skin lesions, and copious sweating were reported significantly more frequently by exposed workers than controls. The symptoms that were reported to be associated with sodium 2,2-dichloropropionate exposure were nausea, skin lesions, anorexia, and pain in the throat; the size of the group or incidences of these symptoms were not given. No differences in the results of patch tests for 2,2-dichloropropionate or in clinical findings were observed in the exposed group, compared to the control group. Contact dermatitis was diagnosed in 6 cases out of the group of 76 workers and symptoms were described as mild. A case of leucomelanoderma in a worker exposed to sodium chlorate was also described. The authors concluded that an allergic reaction to 2,2-dichloropropionate occurred only in the single case report presented, and warranted precautions in handling this herbicide.

651. Matthews, H. B., and Kato, S. (1979) The metabolism and disposition of halogenated aromatics. Ann. N.Y. Acad. Sci. 320:131-137.

[Review article.]

652. May, G. (1973) Chloracne from the accidental production of tetrachlorodibenzodioxin. Br. J. Ind. Med. 30:276-283.

This report describes the health effects in workers at a factory in Derbyshire, England, that manufactured 2,4,5-T from trichlorophenol. In 1968, the building was closed after an exothermic reaction caused an explosion which killed one worker. Eleven of the 14 workers in the building at the time of the explosion were affected, as evidenced by abnormal liver function tests or abnormal white cell counts. Within 10 days these test results were within normal limits, and the factory was reopened, with the damaged areas sealed off. During the next 8 months, 79 cases of chloracne developed, primarily in maintenance workers, who did not use gloves to handle factory equipment. TCDD was identified as the causative agent. The most severe cases started as malar erythema. All cases involved the face; the extremities and trunk were also involved in some cases. Conjunctivitis accompanied the early stages of the condition. Most cases resolved within 4 to 6 months of treatment with steam, ultraviolet light, and lotions. No systemic symptoms developed in these workers. The building was finally closed, and heavy equipment was dismantled and buried or repeatedly cleaned with steam. An incident in Germany, involving dioxin exposure and an explosion, was also described. Many of the exposed workers developed chloracne and severe liver damage.

653. Mazarean, H. H., Dux, L., and Guba, F. (1979a) Changes of metabolism during experimentally induced myotonia of rats I. Alterations in lactate and malate dehydrogenase isoenzyme activities. Biochem. Med. 22(3):350-358.

Alterations in several enzymes involved in energy production were measured in several muscles of rats after 2,4-D administration. Doses

of 100 mg/kg of 2,4-D in aqueous sodium bicarbonate solution were administered intraperitoneally to rats daily for 14 days. Controls were given sodium acetate. Myocardium, soleus (red muscle), and semimembranosus (white muscle) samples were removed, homogenized, and the supernatant after centrifugation (8,000 g for 20 minutes) was assayed spectrophotometrically for lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) activities. Isoenzymes were separated by polyacrylamide gel electrophoresis and the stained bands were quantitated by densitometry. The total LDH activity was not altered in any muscles by 2,4-D treatment. The density of the isoenzyme band, LDH 5 was elevated significantly in the semimembranosus of treated rats while LDH 4 was decreased. The LDH 5 band for the m. soleus muscle was increased in density while the LDH 1, 3, and 4 bands were decreased after treatment. Myocardial LDH 4 and 5 decreased while LDH 1 increased after treatment. The ratio of H subunits, calculated from the proportions of each LDH isoenzyme were significantly elevated in myocardium and decreased in the other two muscles after 2,4-D treatment. MDH activity decreased in all three myotonic muscles, with the largest change in the myocardium. The proportion of MDH associated with cytoplasm of the soleus and semimembranosus muscles was significantly decreased. The authors concluded that the elevation of LDH 5 isoenzyme during myotonia in muscles that favor anaerobic glycolysis represents an alternate pathway that is not active when the oxygen supply is normal. The decrease of H-related isoenzymes was less in soleus than in semimembranosus because the red muscle has a higher oxidative capacity from its extensive capillarization. The increased LDH 1 activity was suggested to be a secondary effect from the high circulating lactate level. The decreased MDH levels were attributed to the myotonic, the limitations of mitochondrial oxidative processes during hypoxia. The actual activities of isoenzymes have not been measured in these studies and are assumed to correlate with densitometry results from polyacrylamide gels to enable the authors to draw their conclusions that a shift to anaerobic energy production occurs in muscles after 2,4-D treatment.

654. Mazarean, H. H., Dux, L., and Guba, F. (1979b) Changes of metabolism during experimentally induced myotonia of rats II. Alterations in creatine kinase and acid phosphatase activities: a possible mechanism in the development of muscle damage. Biochem. Med. 22(3):359-364.

Enzyme changes in several types of muscles after 2,4-D administration were measured. Myotonia was induced in rats by 2,4-D administration (described in another report, Mazarean; 1979a). Muscle homogenates of m. soleus, m. semimembranosus, and myocardium were assayed for acid phosphatase and creatin kinase activities. After 2 weeks of treatment, the acid phosphatase activity was increased substantially in all tissues and the creatine kinase activity was about one-half of the original values for all three muscles. The authors concluded that the decrease in creatine kinase was proportional to the loss of muscle tissue through cellular damage and the increase in acid phosphatase reflects an increase in lysosomal damage accompanying induced myotonia.

The authors proposed that prolonged muscle contraction and hypoxia of skeletal muscles damage the oxidative energy-producing system which stimulates the rate of anaerobic glycolysis.

655. Mazzocchi, P. H., and Rao, M. P. (1972) Photolysis of 3-(p-chlorophenyl)-1,1-dimethylurea (monuron) and 3-phenyl-1,1-dimethylurea (fenuron). J. Agric. Food Chem. 20(5):957-959.

The authors report on the photolysis of monuron under anaerobic conditions. A solution of 9 g monuron in 450 ml ethanol was irradiated for 84 hours in a bank of GE-G15 low pressure mercury lamps (2537A). Formation of photolysis products was measured by gas-liquid chromatography and thin layer chromatography. The following products were identified: 3-phenyl-1,1 dimethyl urea and methyl p-chlorophenyl carbamate. The anaerobic conditions of the experiment prevents extrapolation of these results to a field situation.

656. McCarthy, F. (1980) Statement of the Agent Orange Victims International before the Interagency Work Group to Study the Possible Long-Term Health Effects of Phenoxy Herbicides and Contaminants, Sept. 22, 1980. 3 pp.

[Testimony.]

657. McCollister, D. D., and Leng, M. L. (1969) Toxicology of picloram and safety evaluation of TORDON herbicides. Down to Earth 25(2):5-10.

[Review article.]

658. McConnell, E. E., and McKinney, J. D. (1978) Exquisite toxicity in the guinea pig to structurally similar halogenated dioxins, furans, biphenyls, and naphthalenes. Toxicol. Appl. Pharmacol. 45:298.

[Abstract, only.]

659. McConnell, E. E., and Moore, J. A. (1977) The toxicopathology of TCDD. Manuscript provided at Workshop TCDD, Milan, Oct. 23-24.

[Not available.]

660. McConnell, E. E., Moore, J. A., and Dalgard, D. W. (1978a) Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in Rhesus monkeys (Macaca mulatta) following a single oral dose. Toxicol. Appl. Pharmacol. 43(1):175-87.

The acute toxicity of TCDD was studied in monkeys. Female juvenile Rhesus monkeys (three per group) were administered by oral gavage a single dose of 70(u)g/kg or 350(u)g/kg TCDD (99+ percent purity) in

corn oil or vehicle only. Body weights, blood chemistries, hematology and general appearance, appetite and behavior were monitored until death or until animals became moribund and were killed. Histological examination of an extensive number of tissues was performed, and major organ weights were recorded. Hepatic porphyrin levels were assessed quantitatively and qualitatively (by examining tissue for fluorescence under ultraviolet light). Monkeys in the high dose group died or were killed between days 28 and 34, and in the low dose group on days 14, 42, and 47. Monkeys in both treatment groups showed the same pattern of toxicity. TCDD-treated monkeys lost from 13 to 38 percent of their body weight. This loss began by day 3, recovered slightly (days 7-9), then fell continuously until death. Appetite was variable throughout the experiment, and water consumption fell at the end of the study. Blepharitis, loss of fingernails and eyelashes, and facial alopecia with acneiform eruptions progressed in treated monkeys. Hematologic changes included a trend toward increased neutrophils, and in the high dose group, lymphopenia and decreased platelet counts. Blood chemistry tests showed progressively decreased cholesterol levels to half of control values for the high dose group, decreased glucose concentrations starting on day 14, and decreased albumin levels by day 30. Elevated glutamic oxaloacetic transaminase, aldolase, and terminal blood urea nitrogen values were reported. The organ to body-weight ratios of the liver, adrenals, and kidneys, and the absolute heart and spleen weights were significantly elevated in both treatment groups. The thymus weights, both absolute and relative, were lower in treated groups than in controls. The treated monkeys had no body fat, the bile ducts were distended with hyperplasia of the mucosal lining, and congestion of the intestine and depletion of lymphoid tissue, especially of the thymus, were observed. Multinucleated hepatocytes were observed in the liver of one monkey. No elevation of hepatic porphyrins was detected. Half of the monkeys showed epithelial hyperplasia of the renal pelvis. Gastrointestinal lesions, degranulation of the exocrine pancreas, and a mild loss of cellularity and increase in myeloid to erythroid cell ratio in sternal bone marrow were observed. The authors concluded that death was caused by starvation, which resulted from an inability to use consumed nutrients. No other lesions appeared to be severe enough to be lethal.

661. McConnell, E. E., Moore, J. A., Haseman, J. K., and Harris, M. W. (1978b) The comparative toxicity of chlorinated dibenzo-p-dioxins in mice and guinea pigs. Toxicol. Appl. Pharmacol. 44(2):335-356.

The acute toxicities of various dioxins, including 2,3,7,8-TCDD were studied in the mouse and guinea pig. TCDD (98% purity) was administered by oral intubation in corn oil to male C57Bl/6 mice and male Hartley guinea pigs. Controls were given corn oil. Treatment groups were comprised of 8 mice or 6 guinea pigs. After one animal died in each group, the remaining animals were killed after they became moribund. Serum proteins were fractionated by cellulose acetate electrophoresis. Histopathologic examination was performed on a number of tissues and the weights of major organs were determined. Porphyrins

were detected by fluorescence of liver, skull, and teeth under ultraviolet light. The symptoms produced by the various dioxins were described together because all compounds produced the same effects in the same species and only the doses needed to elicit effects were different for the various dioxins. The LD₅₀₋₃₀ for 2,3,7,8-TCDD was 2 ug/kg in the guinea pig and 284 ug/kg in the mouse. A dose-related decrease in weight gain or weight loss occurred in both species. In guinea pigs, failure of individual weight stabilization within 7-10 days of treatment was associated with a poor prognosis. Dehydration and, near death, decreased food consumption were described in guinea pigs and death occurred within 17-20 days and by 12 days from higher doses. Exophthalmus and distension of the abdomen occurred in mice and death occurred within 22-25 days and by 18 days from higher doses. Decreased thymus, testicle, and heart weights were reported, while the liver weights were higher after treatment in both species. Significantly decreased serum alpha-globulin levels occurred in the mouse only and fluorescence was observed in mouse tissues only. Hemolysis and hyperproteinemia were present in samples from moribund animals of both species. Histological changes in guinea pigs included a reduction of fat depots and muscle mass, sternal bone marrow hypocellularity, hypoplasia of the renal pelvis, and occasionally of the mucosa of the urinary bladder, adrenal hemorrhages and lesions, loss of seminiferous components of the testicles. In mice, changes included ascites and subcutaneous edema, hydrothorax, loss of the body fat, retro-orbital hemorrhage, and hepatic and testicular lesions. Intestinal hemorrhage and microcysts and thymic and splenic lesions occurred in both species. Depletion of lymphoid tissue was apparent in several tissues. Death was attributed to depletion of energy reserves and the reason why the usual metabolic sources did not replace the energy reserved was not known.

662. McCorkle, F. M., Chambers, J. E., and Yarbrough, J. D. (1977) Acute toxicities of selected herbicides to fingerling channel catfish, Ictalurus punctatus. Bull. Environ. Contam. Toxicol. 18(3)267-270.

This paper describes the acute toxicity of 2,4-D and 2,4-D dimethylamine salt, dalapon, diuron, monuron, and 2,4,5-T to one-year-old channel catfish fingerlings. Static bioassays were conducted in 76 liter tanks containing 5 fish (average weight 14g). Each compound (purity and solvent unspecified) was tested for 48 hours at 1 and 10 ppm. However, no analysis was made to verify herbicide concentrations in the treated water. All of the above herbicides produced less than 10 percent toxicity in 48 hours. No controls were reported. The authors concluded that these herbicides were not toxic to catfish at 10 ppm and could be used around ponds without causing harm to the catfish.

663. McCormack, K. M., Gibson, J. E., and Hook, J. B. (1976) Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on renal function in rats in vivo. Toxicol. Appl. Pharmacol. 37(1):177.

[Abstract, only.]

664. McKinney, J. D. (1978) Analysis of 2,3,7,8-tetrachlorodibenzo-para-dioxin in environmental samples. In: Chlorinated Phenoxy Acids and Their Dioxins. ed. C. Ramel, (Ecol. Bull. no. 27 Stockholm: Swedish Natural Science Research Council, pp. 53-66.
- [Background material.]
665. McLennan, M. W. (1974) 2,4-D toxicity in dairy cattle. Pestic. Abstr. 9(2):88.
- [Abstract, only.]
666. McLeod, J. G. (1971) Peripheral neuropathy caused by drugs and toxic substances. Aust. N.Z. J. Med. 3:268-269.
- [Review article.]
667. McNulty, W. P. (1977) Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin for Rhesus monkeys: Brief report. Bull. Environ. Contam. Toxicol. 18(1):108-109.
- The toxicity of subchronically administered TCDD was evaluated in two Rhesus monkeys. Male monkeys, 1 year of age, were fed diets containing either 2 or 20 ppb TCDD. Histological examination of tissues was performed when death occurred. The monkey given the higher dosage showed a loss of appetite and decreased activity. Vomiting occurred and on the twelfth day when death occurred, the animal lost 30% of its body weight. The monkey given the low dosage diet retained its appetite. The eyelids became swollen and the face was edematous after 9 weeks, when the monkey underwent surgery to remove a biopsy sample of the stomach, and was given a control diet subsequently. Two weeks later when the animal died, the body weight loss was 33%. Mucous metaplasia of the gastric mucosa was observed in biopsy sections and at autopsy of both animals. Other changes observed in both monkeys were cachexia, hyperplasia, and ulceration of the gastric mucous, squamous metaplasia of sebaceous glands and thymic atrophy. Total intake was estimated to be well below 10 ug/kg TCDD. The author concluded that young male Rhesus monkeys show high susceptibility to TCDD toxicity, compared to other laboratory animals.
668. McNutt, W. S., and Robins, H. I. (1974) The temperature sensitive adenylation of tRNA in carcodylate and other buffers. Bioch. Biophys. Acta. 366(4):411-423.

669. McQueen, E. G., Veale, A M. O., Alexander, W. S., and Bates, M. N. (1977) 2,4,5-T and human birth defects. Report prepared by Dept. of Health, New Zealand. 41 pp.

The causal relationship between 2,4,5-T exposure and 3 separate instances of clusters of neural tube defects that occurred in New Zealand is evaluated. For each of the 3 clusters of 4-8 neural tube defects maternal exposure to pesticides and drugs during early pregnancy, (when the neurospores close) family history of neural tube defects, illnesses during pregnancy, date of birth of afflicted child, and occupation of the father and other relevant information are described. The doses of 2,4,5-T and of TCDD required to cause defects were extracted from animal studies and maximum maternal exposure levels from agricultural use of these chemicals was estimated. The first cluster of cases occurred in Northland. One case of spina bifida occurred in 1975; in 1976, 2 cases of anencephaly and 4 of spina bifida were reported. This incidence was not considered statistically different from the expected incidence of 4-5 cases. Two cases had family histories of spina bifida and 2,4,5-T exposure in early pregnancy could not be confirmed in any of the 7 cases. Clomiphene was administered to 1 of the mothers just before conception. One case of spina bifida and 3 of anencephaly occurred in Taranaki between 1974 and 1977. The probability of the occurrence of this incidence by chance alone was calculated to be 1 in 80. The mother of one of the cases of anencephaly and the father of another case were siblings. The mother a third case had another child with spina bifida. Exposure of 1 mother to 2,4,5-T sprayed in the vicinity of her house 2 weeks after the date of her last menstruation was documented; water sources included both roof and main water supplies in this case. Pregamal (folic acid and ferrous sulphate) was the only drug consumed by more than 1 mother in this group. In a 4-month period (December, 1975 to January, 1976) 7 cases of spina bifida and 1 of anencephaly were born in Waikato. The probability of this incidence of occurring by chance was calculated at 1 in 140. Two of the involved families had histories of neural tube defects, and a third family history included other congenital deformities. Two cases involved 2,4,5-T exposure of the fathers, but the temporal relationship of exposure to conception could not be established. One mother had influenza in early pregnancy and one mother took iron tablets. The authors determined that a woman would have to drink 42,840 liters of roof water contaminated with 2,4,5-T from the spraying rates and TCDD contamination used in New Zealand agriculture to approach a teratogenic dose of 2,4,5-T or TCDD. The committee concluded that no evidence of an association between herbicide exposure and neural tube defects could be established.

670. Meselson, M., O'Keefe, P., and Baughman, R. (1978) The evaluation of possible health hazards from TCDD in the environment. Presentation for Symposium on the Use of Herbicides in Forestry, Arlington, Va. Feb. 21-22, 1978. 11 pp.

[Background material.]

671. Midwest Research Institute and Criteria and Evaluation Division, OPP, EPA. (1975a) Substitute chemical program--initial scientific and minieconomic review of bromacil. Criteria and Evaluation Division, OPP, EPA. PB-241 801. 79 pp.

[Review article.]

672. Midwest Research Institute. (1975b) Substitute chemical program initial scientific and minieconomic review of monuron. Environmental Protection Agency. PB248 110. 120 pp.

[Review article.]

673. Midwest Research Institute. (1975c) Substitute chemical program. Initial scientific review of cacodylic acid. Prepared for Environmental Protection Agency, PB-251 541. 130 pp.

[Review article.]

674. Military operations: herbicide operations. MACV Dir. 525-1. (1969) U.S. Military Assistance Command, Vietnam. DTIC No. AD 779794. 130 pp.

[Background material.]

675. Mill, T. (1980) Data need to predict the environmental fate of organic chemicals. In Dynamics, exposure and hazard assessment of toxic chemicals. Bizuanual Haque, ed. (Ann Arbor: Ann Arbor Science), pp. 297-322.

[Background material.]

676. Miller, A. S. (1979) 2,4,5-T: Chemical time bomb. Environment 21(5):2-4.

677. Miller, R. A., Norris, L. A., and Hawkes, C. L. (1973) Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in aquatic organisms. Environ. Health Persp. 5:177-186.

The authors describe the toxicity of TCDD to fish (guppies, coho salmon, and rainbow trout) and aquatic invertebrates (snail, oligochaete worm, and mosquito larvae). The effects of 24-96h exposure of young salmon to greater than 23 ng/g body weight results in death in 10-80 days. Fish exposed to toxic levels of TCDD had a declining interest in food after 5 (guppies) to 8 (salmon) days. Skin discoloration and fin necrosis appeared in 15 days in guppies and in 30 days in salmon. Erosion of the upper jaw was also observed in guppies

surviving 30-60 days. Exposure of salmon to TCDD levels of 0.054 ng/g for 24 hours or longer resulted in 12 percent mortality in 60 days, to 5.4 ng/g resulted in 55 percent mortality during 60 days and to 54 ng/g resulted in 100 percent mortality in 40 days. In all cases, duration of exposure seemed to be less important than levels of exposure. In both salmon and guppies, larger fish survived longer periods of TCDD exposure than smaller fish. In rainbow trout, fed diets containing 2.3 ppm TCDD for 4 weeks, no deaths or signs of toxicity were observed at 6.3 pg/tank/week or 6.3 ng/tank/week. At 6.3 ug/tank, decreased appetite in fish was observed at 10 days, and fin necrosis at 14 days. The authors indicated that deaths began occurring at 33 days, but no numerical data were presented. TCDD at 0.2 ppb had no effect on pupation of mosquito larvae during a 30 day test period. However, the same dose of TCDD for 36 days inhibited reproduction but no juvenile or adult survival, in pulmonate snails. Exposure of adult oligochaete worms to 2 ppb TCDD in water for 55 days resulted in a decrease in reproduction of worms.

678. Mitchell, J. W. Hodgson, R. E., and Gaetjens, C. F. (1946) Tolerance of farm animals to feed containing 2,4-D. J. Animal Sci. 5:226-232.

The toxicity of 2,4-D sprayed on pastures was assessed in sheep and cows that grazed on the treated pasture. An 0.15% aqueous solution of purified 2,4-D was sprayed at the rate of 215 gallons per acre and starting on the subsequent day 2 cows and 2 sheep grazed on the exposed field for 48 and 12 days, respectively. Autopsies were performed on the cows at the end of the exposure period. A lactating Holstein cow was fed 5.5 gms of 2,4-D daily for 106 days. During the last 38 days of exposure, milk was collected and fed to a calf. 2,4-D levels were estimated by a bioassay; the sera of the cow and calf and in cow tissues removed at autopsy at the end of the exposure period were analyzed for 2,4-D. Levels were determined by measuring growth stimulatory effects. Aqueous extracts of the tissues were applied to bean seedlings and internodal elongation was measured after 24 hours. No adverse effects were observed in sheep and cows that grazed on sprayed fields or a cow fed 2,4-D or calf exposed to 2,4-D in milk, although no controls were studied to compare the data for milk production, food consumption, or body weights in the lactating cow. Milk and cow serum, but no tissues, gave positive reactions in the bioassay used to measure 2,4-D. The authors concluded that purified 2,4-D was not toxic. The methods used in this study are outdated and recent studies provide more information on the extent of 2,4-D toxicity.

679. Miura, H., Omori, A., and Shibue, M. (1975) The effect of chlorophenols on the excretion of porphyrins in urine. Pestic. Abstr. 5(7):456-457.

[Abstract, only.]

680. Moilanen, K. W., and Crosby, D. G. (1974) The photodecomposition of bromacil. Arch. Environ. Contam. Toxicol. 2(1):3-8.

The authors studied the photodecomposition of bromacil under simulated sunlight conditions. An aerated 10 ppm aqueous solution of bromacil was irradiated using a sunlight simulator for 6 days. An identical solution was aerated in the dark as a control. Both solutions were analyzed by gas-liquid chromatography and thin layer chromatography to identify photodecomposition products. Only 1 photoproduct was formed, 5-bromo-6-methyluracil. Decomposition of the herbicide proceeded slowly; 96% of the herbicide remained after 6 days irradiation. The authors concluded that bromacil is stable in sunlight and that photo decomposition probably contributes only a minor role to the environmental disappearance of bromacil.

681. Monarca, G., and Di Vito, G. (1961) Folia Med. 44:480-485.

A case report of accidental inhalation exposure to a toxic dose of 2,4-D is described. A 57-year-old man sprayed a 40% aqueous solution of 2,4-D by a manual pump under windy conditions. During the evening and day after the work was performed, the farmer experienced generalized asthenia, profuse perspiration, vomiting and oliguria and was admitted to the hospital. Central and peripheral effects that were experienced in the early phase of illness included dizziness, change of reflexes and uncertain walking. The only alterations in routine laboratory test results was a transitory albuminuria. The clinical condition of the patient gradually improved, until 18 days after the accident, when symptoms related to the nervous system worsened and a serious form of hemorrhagic enterocolitis developed. Neural effects included uncertain walking, sluggish palellar and achilles tendon reflexes, positive Romberg sign and dysmetria. Laboratory tests did not provide additional information at this stage. By 5 months, all symptoms except hyporeflexia had receded. The authors suggested that the acid form of 2,4-D produced the early effects and the phenol metabolite elicited enterocloitis by an irritative-caustic action on the mucous membranes of the gastrointestinal tract.

682. Montgomery, M. L., and Norris, L. A. (1972) A preliminary evaluation of the hazards of 2,4,5-T in the forest environment. Ind. Veg. Manage. 4(1):19-22.

[Review article.]

683. Moore, J. A. (1980) Statement at the Meeting of the Interagency Work Group to Study the Possible Long-Term Health Effects of Phenoxy Herbicides and Contaminants, Sept. 22, 1980. 7 pp.

[Testimony.]

684. Moore, J. A. (1978) Toxicity of 2,3,7,8-tetrachlorodibenzo-para-dioxin. In Chlorinated Phenoxy Acids and Their Dioxins. ed. C. Ramel (Ecol. Bull, No. 27, Stockholm: Swedish Natural Science Research Council, 1978) pp. 133-144.

[Review article.]

685. Moore, J. A., Gupta, B. N., Zinkl, J. G., and Vos, J. G. (1973) Postnatal effects of maternal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Environ. Health Perspec. 5:81-85.

The effect of TCDD on the development of the kidney in fetal and neonatal mice is studied. C57Bl/6 strain mice were administered TCDD orally in acetone-corn oil or the vehicle alone. On day 18 of gestation fetuses were removed and tissues were examined histologically. When 3 ug/kg of TCDD was administered on days 10 through 13 of gestation, a mean of 55.4% of the fetuses of each litter had cleft palate, 95.1% had kidney anomalies, and 83.1% had bilateral kidney anomalies. At 1 ug/kg, TCDD administered on days 10-13 caused 1.9% cleft palate, 58.9% kidney anomalies, and 36.3% bilateral kidney anomalies. The same dose administered on day 10 only caused no cleft palates, 34.3% and 8.8% kidney anomalies, and bilateral kidney anomalies, respectively. None of these malformations were observed in vehicle controls. The renal anomaly observed in treated animals was hydronephrosis, characterized by a reduced or absent renal papilla in an enlarged renal pelvis. A reciprocal cross-fostering study was conducted in which mice exposed to TCDD in utero nursed untreated mothers and mice born to control mothers nursed treated mothers. The highest incidence of hydronephrosis, 34%, occurred in mice exposed to TCDD before and after parturition. The incidences of hydronephrosis were 2-2.1% for those exposed in utero, 8.5% for those exposed after birth, and 1.7% for unexposed mice. When mothers were treated on the day of parturition with 3 or 10 ug/kg TCDD, 71-75% of the pups per litter had hydronephrosis, while none of the controls and 12% of the 1 ug/kg dose group (given at parturition) were afflicted. A dose-related decrease in thymic weight of pups in the 3 and 10 ug/kg dose groups was mentioned. The authors concluded that the renal disorder was progressive hydronephrosis which involved the right kidney in 90% of the unilateral cases. Complete atrophy of the right kidney was observed in 55-day-old mice from the highest treatment group. The dose and length of exposure to TCDD determine the severity of the condition, although the authors postulated that pathogenesis of lesions developed in utero or postnatally were the same. The strain and species-specific nature of this striking defect should be kept in mind in extrapolating these results to human exposures.

686. Moore, J. A., Harris, M. W., and Albro, P. W. (1976) Tissue distribution of [¹⁴C]tetrachlorodibenzo-p-dioxin in pregnant and neonatal rats. Toxicol. Appl. Pharmacol. 37(1):146-147.

[Abstract, only.]

687. "More on Agent Orange". (1980) Congressional Record - U.S. Senate, Sept. 30. S 14160-S 14162.

[Testimony.]

688. Morgan, D. P. (1976) Recognition and management of pesticide poisonings. U. S. Environmental Protection Agency Report No. EPA-540/9-011. pp.13-30.

[Review article.]

689. Morton, H. L., Moffett, J. O., and Martin, R. D. (1974) Influence of water treated artificially with herbicides on honey bee colonies. Environ. Ent. 3(5):808-812.

In this paper, the authors study the effects of 2,4,5-T ingestion on honey bees. Ten colonies of bees were placed in 2 apiaries at least 2 miles from the nearest water source and at least 3 miles from areas treated with pesticides. One apiary was used as a control and received untreated water, the other apiary had water available that contained 1,000 ppm 2,4,5-T triethylamine salt (purity unspecified). The experiment was initiated in the dry season when the only available water was that which was supplied to the bees. Treatment continued for 12 weeks; water was changed every 2 days. Numbers of dead bees and brood production was measured in each colony. Adult worker bees, honey, and wax were also removed at intervals over a 24-month period and analyzed for 2,4,5-T content by gas chromatography. The number of bees dying in traps on colonies treated with 2,4,5-T did not differ significantly from controls at the end of the 12-week treatment period. However, a large number of bees were drowned in the 2,4,5-T-treated water, possibly, according to the authors, because of the change in surface tension for the treated water. Treated colonies examined for brood development had less capped brood than controls 1 month after the study began. There was little brood production during the second month of treatment. One month after the rainy season started the bees had access to an untreated water source, and brood production increased. No differences in brood production were seen during the second year of the study. The concentration of 2,4,5-T in adult worker bees reached a peak of about 150 ppm during treatment. When treatment was terminated, 2,4,5-T levels decreased to about 5 ppm. Detectable levels of 2,4,5-T were found in bees during most of the 2-year study. In honey, 2,4,5-T reached a peak of 50 ppm. Upon removal of treatment, 2,4,5-T levels in honey decreased to about 10 ppm. 2,4,5-T was last detected in honey 480 days after initiation of the experiment. The compound was detected in wax as long as 650 days after initiation of the experiment. The authors concluded that 2,4,5-T could be transferred to honeybee colonies, honey, and wax if the bees were exposed to contaminated water sources. This can potentially be another source of human exposure to 2,4,5-T. The effects of paraquat on honey bees was also discussed in this paper.

690. Morton, W. E., Crawford, E. D., Maricle, R. A., Douglas, D. D., and Freed, V. H. (1975) Hypertension in Oregon pesticide-formulating workers. J. Occ. Med. 17(3):182-185.

The incidence of hypertension was determined for chlorophenoxy herbicide workers, other pesticide workers and non-pesticide-exposed workers. Workers at 28 plants who were engaged in the formulation of pesticides were requested to complete a questionnaire indicating clinical symptoms, family history of hypertension, and hazardous product exposure. Blood pressures were measured (number of examiners taking blood pressure was not reported). Of 3 sets of readings, the set of readings with the lowest systolic value was used for the study. The control group was comprised of workers from a wood products plant, as well as employees of the State health department and from the medical school. All chlorophenoxy herbicides workers were employed at the same plant. Compliance for the herbicide, pesticide and control groups were 85%, 85%, and 10%, respectively and the sizes of the study groups ranged from 69-84. The groups were similar in terms of sex ratio, age range and body size. Tobacco use was highest and alcohol use lowest for herbicide workers. Exposure to phenoxy-herbicides was indicated by 68% of the herbicide workers and to phenoxy herbicide intermediates, by 78%. Over half of the herbicide group was exposed occupationally to pesticides for more than 10 years, while 2/3 of the pesticide workers were exposed for less than 10 years. Herbicide workers indicated experiencing higher incidences of symptoms than the other groups (specific symptoms for each organ system or statistical analyses of any results were not reported). The mean systolic and diastolic blood pressures were the same for all 3 groups, although 39% of the herbicide groups, 29% of the pesticide workers and 30% of the control group were diagnosed with hypertension (systolic pressure, at least 150 and/or diastolic, at least 90). Family histories of hypertension were most frequent among the herbicide group and age-standardization of results did not alter the trends. The authors concluded that the modest excess in the prevalence of hypertension among herbicide workers was probably attributed to higher predisposition, evidenced by data on family histories. The authors indicated that the size and selection bias of their control group was unsatisfactory and that exposure to corrosive chemicals and solvents by both experimental groups was probably a significant confounding factor in the study.

691. Moses, M. (1979) Effects of TCDD on human health. Testimony before the House Subcommittee on Oversight and Investigations, June 26, 1979. 6 pp.

[Testimony.]

692. Mosier, A. R., and Guenzi, W. D. (1973) Picloram photolytic decomposition. J. Agric. Food Chem. 21(5):835-837.

The authors report on the photolytic decomposition of picloram under laboratory conditions. An aqueous solution of 2.08×10^{-3} M picloram sodium salt was irradiated with 300-380 nm ultraviolet light. Within 72 hours, picloram was 99% degraded. After 34 hours of irradiation, 11 degradation products were observed by thin layer chromatography. Attempts to characterize these compounds were unsuccessful. The authors also discussed the possible mechanisms of picloram degradation.

693. Muller, R. O. (1980) Statement of Executive Director of Vietnam Veterans of America before the Interagency Work Group to Study the Possible Long-Term Health Effects of Phenoxy Herbicides and Contaminants, Sept. 22, 1980. 11 pp.

[Testimony.]

694. Mullison, W. R. (1980) 2,4,5-T update -- January 1980. Presented at the 29th Conference of the Texas Agricultural Aviation Association, January 15-17, San Antonio, Texas.

[Editorial.]

695. Mullison, W. R. (1980) Some public concerns about 2,4,5-T. Presentation to the 33rd meeting, Western Society of Weed Science, Salt Lake City, Utah, March 18-20, 1980.

[Editorial.]

696. Murai, Y., and Kuroiwa, Y. (1971) Peripheral neuropathy in chlorobiphenyl poisoning. Neurology 21:1173-1176.

Results of clinical examinations and nerve conduction velocity tests in patients that had been exposed to chlorobiphenyl (kanachlor 400) are described. Twenty-one patients, ranging from 7 to 60 years of age, were examined. Neurologic symptoms in most of the patients included numbness, muscular aches, hypoesthesia and areflexia. No symptoms were found that suggested the involvement of the central nervous system. Nerve conduction velocities were measured in both motor (tibial nerve) and sensory nerves (radial and sural nerves). Results of these tests demonstrated that conduction velocities in motor nerves were within the normal range while conduction velocities in the sensory nerves were reduced in about half of the patients. There was one exception; conduction velocities in the motor nerve were slowed in one patient that exhibited areflexia in all four limbs. The authors concluded that accidental ingestion of chlorobiphenyl caused sensory neuropathy, and that this could be an early manifestation of mixed (sensory and motor) polyneuropathy.

697. Murakami, M., and Fukami, J. (1978) Persistent pesticides and environmental chemicals are taken up to a greater extent than non-persistent compounds by cultured human cells. Bull. Environ. Contam. Toxicol. 19(4):423-427.

Uptake of 2,4-D and 2,4,5-T by human embryonic lung cells is described. Confluent monolayer cultures of human embryonic lung diploid cells were cultured in the presence of 4×10^{-6} M [14 C]-2,4-D or [14 C]-2,4,5-T for 4, 24, and 48 hours. The cell layer was digested with sodium hydroxide and counted for radioactivity. Total cell protein was assayed to evaluate cell growth. For 2,4-D, uptake varied between 2.6 and 5 pmol/mg cell protein for the 3 time points and cell growth was 79% of control cell growth. For 2,4,5-T, uptake varied between 6.6 and 12 pmol/mg protein and cell growth was 83% of control growth. Uptake and cell growth were also tested for DDT, dieldrin, aldrin, PCB, HCB, carbaryl, malathion, parathion, and chlordimeform. Uptake of 2,4-D and 2,4,5-T were less than for the other compounds, but they were more toxic in terms of cell growth. The authors concluded that the test compounds that are persistent in the environment were taken up to a greater extent by cultured cells but cytotoxicity was not the result of greater uptake of the test compound.

698. Muranyi-Kovacs, I., Rudali, G., Imbert, J. (1977) Study on the carcinogenicity of 2,4,5-T in mice. (Meeting Abstract). Presented at the 4th Meeting of the European Association for Cancer Research, Lyon, France, Sept. 13-15. p. 64.

[Not available.]

699. Muranyi-Kovacs, I., Rudali, G., and Imbert, J. (1976) Bioassay of 2,4,5-trichlorophenoxyacetic acid for carcinogenicity in mice. Br. J. Cancer 33(6):626-633.

The authors evaluated the carcinogenicity of 2,4,5-T in a feeding study in mice. Male and female (6 weeks old) XVII/G and C3Hf mice were given water containing 2,4,5-T (100 mg/l) for 2 months after which the animals received 80 ppm 2,4,5-T in their diets up to 28 months or until death. The 2,4,5-T preparation used in this study had less than 0.05 dioxins by gas chromatographic analysis. The authors did not report any analysis of food or water treated with 2,4,5-T to confirm their formulations. In addition, they did not specify the length of time or temperature at which a particular water or diet formulation was stored. The authors also did not report the number of animals initially used in the experiment. However, 40-45 control animals per group and 20-25 treated animals per group were alive during the 11th to 12th month of the experiment. Tumors developing in the test animals were placed in one of two classes: 1) incidental tumors which were discovered at necropsy of an animal which died from other causes and 2) non-incidental tumors which were diagnosed during the animal's lifetime or caused the death of the animal. In XVII/G animals, there was no significant difference between the number of tumor bearing mice observed and the

number expected by Peto's method of statistical analysis. However, in C3Hf mice the differences between observed and expected non-incident tumors were significant in both male and female mice. When incidental and non-incident tumors were group together, a significant difference was observed in only females. Incidence of incidental tumors alone was not significantly different in either sex. When males and females were pooled, incidence of total tumors and non-incident tumors was statistically different between control and treated animals. From this information, the authors concluded that additional data are required to make an assessment of the carcinogenic potential of 2,4,5-D.

700. Murphy, J. M., Murfin, G. D., Jamieson, N. L., Rambo, A. T., Glenn, J. A., Jones, L. P., and Leighton, A. H. (1974) The effects of herbicides in South Vietnam. Part B. Working papers: Beliefs, Attitudes, and Behavior of Lowland Vietnamese. National Academy of Sciences-National Research Council. AD -779 030.

[Background material.]

701. Murray, F. J., Smith, F.F., Nitschke, K. D., Humiston, C. G., Kociba, R. J., and Schwetz, B. A. (1979) Three generation reproduction study of rats given 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the diet. Toxicol. Appl. Pharmacol. 50(2):241-252.

Reproductive toxicity was demonstrated in rats treated with TCDD in a three generation study. Rats were administered 0, 0.001, 0.01, or 0.1 ug TCDD/kg/day orally by monitoring consumption and adjusting dietary TCDD accordingly. For the f_0 parental generation, administration of the TCDD diet was initiated 90 days prior to mating (at about 7 weeks of age) and was continued for the duration of the experiment. The f_0 generation produced two litters, the (f_{1a}) and second (f_{1b}) generations. The f_{1b} generation was mated to produce an f_2 generation and the f_2 generation was mated to produce an f_3 generation. All animals, except the control group, were fed diets containing TCDD throughout the experiment. Litter size and body weights were recorded at parturition and 1, 7, 14, and 21 days after birth. Gross and histopathological examinations were performed on weanlings not selected for mating. Signs of toxicity were seen in only one f_0 adult in the high dosage group. Of the 4 litters delivered from f_0 females in the high dosage group, only one litter had viable pups (4/5) and no subsequent matings were made. For the other two treatment groups, the fertility indices, body weights, number of days from cohabitation to delivery, and postnatal survival were adversely affected in the f_1 and f_2 generations of the intermediate dosage group, compared to the control group, as were the sizes and gestational survival indices of their litters. The reproductive capacity of the f_0 rats of the middle dosage group and all of the low dosage groups resembled the controls. Dilated renal pelvis was observed in rats from the high and low dosage groups. A cross-mating study was performed on rats from the f_0 generation which had received the TCDD diet for 12 months. Male or female rats from the f_0 control group or the f_0 group given 0.1 ug TCDD/kg/day were mated with

younger, untreated rats. Females were killed 20-21 after sperm was detected in vaginal smears and numbers of viable, dead, and resorbed fetuses were recorded. Incidences of pregnancy from matings of f_0 males of the high dosage group with untreated females and of f_0 control males with untreated females were both 58-60% compared to 5% for matings of untreated males and f_0 control females and 15% for matings of untreated males and f_0 females from the highest dosage group. The 70% incidence of resorptions for the last group was significantly higher than for its control (15%). The decreased fertility of all f_0 rats was attributed to the age of the females when the cross-mating study was conducted.

702. Myers, P. W. (1981) Status of Herbicide Orange Studies. Presentation before the Veterans Affairs Committee, House of the Representatives. (Subcommittee on Oversight and Investigations) released by the Committee on Veterans Affairs, House of Representatives, 39 pp.

[Testimony.]

703. Nagy, Z. S., Mile, I., and Antoni, F. (1975) The mutagenic effect of pesticides on *Escherichia coli* WP2 try. (1975) Acta Microbiol. Acad. Sci. Hung. 22:309-314.

The authors evaluated the mutagenicity of 2,4-D and 29 other pesticides in *Escherichia coli* WP2 try. Two bacterial strains which revert from tryptophan requiring to non-requiring were used. One strain (hcr⁻) was repair deficient in addition to requiring tryptophan; the other strain (hcr⁺) was repair proficient. 2,4-D was tested in a spot test without metabolic activation either as 1-3 mg crystals or 20-25 ul liquid. Both positive and negative controls were included in the assay. Numerical values were not reported. According to the authors, 2,4-D was negative in this assay.

704. Nash, R. G. and Beall, Jr., M. L. (1980) Distribution of Silvex, 2,4-D and TCDD applied to turf in chambers and field plots. J. Agric. Food Chem. 28:614-623.

[Background material.]

705. Nash, R. G., and Beall, M. L., Jr. (1978) Environmental distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) applied with Silvex to turf in microagroecosystem. Final Report EPA-IAG-D6-0054, Agricultural Environmental Quality Institute, U.S. Department of Agriculture, Beltsville, Maryland.

[Background material.]

706. Nash, R. G., Kearney, P. C., Fertig, S. N., et al. (1981) Agricultural exposure to 2,4-D. [Abstract of paper presented at 181st Am. Chem. Soc. National Meeting, Atlanta, Ga., April 1981.]

[Abstract, only.]

707. Nash, R. G., Kearney, P. C., Maitlen, J. C., Sell, C. R., and Fertig, S. N. (1981) Agricultural applicator exposure to 2,4-D. (in press)

Urinary excretion data for 43 workers who applied 2,4-D were used to estimate 2,4-D exposure levels. A group of 17 aerial applicators supplied 24-hour urine samples on alternate days of a 2-week period of peak 2,4-D spraying. A group of 26 ground applicators provided six consecutive 24-hour samples following a single 2,4-D application. Urine samples were analyzed for 2,4-D by gas-liquid chromatography. An average of 0.4 ppm 2,4-D was detected in urine collected prior to the experimental period from both groups. The mean level of 2,4-D excretion for aerial applicators over the 2 week period was 0.11 mg/kg body weight per day. The highest levels were excreted by mixer/loaders (0.02 mg/kg/day) and the lowest by pilots (0.006 mg/kg/day). Ground applicators were divided into three groups by occupation. Applicators

excreted a total of 0.005 mg/kg of 2,4-D; mixer/loaders excreted 0.007 mg/kg; and mixer/loader/applicators, 0.018 mg/kg. The urinary levels from each group were not associated with age, weight, or type of clothing, and were associated with the type of job, length of time of exposure, and amount of 2,4-D applied. The half-lives for excretion of 2,4-D by ground applicators were between 35-48 hours. The average total exposure was estimated to be 12.7 ug/kg for all ground applicators. The authors concluded that exposure of an 80 kg man to 2,4-D during spraying 30 days per year for 30 years could result in absorption and excretion in an estimated 0.9 g of 2,4-D total, which is far below 2g, a dose that produced no adverse effects when administered intravenously to one man (reported elsewhere).

708. National Center for Toxicological Research. (1975) 2,4,5-T Teratology Study.
[Not available.]
709. National Forest Products Association. (1980a) Forest Industry Statement before the Interagency Work Group on Phenoxy Herbicides - Public Meeting Sept. 22, 1980: Proposed Outline. Sept. 11, 1980, 1 p.
[Testimony.]
710. National Forest Products Association. (1980b) Statement of the National Forest Products Association before the Interagency Working Group on Phenoxy Herbicides on Health Effects Information, Sept. 22, 1980. 19 pp.
[Testimony.]
711. National Research Council. (1974) The Effects of Herbicides in South Vietnam: Part A. Summary and Conclusions. National Academy of Sciences, Washington, D.C. AD-774-749.
[Review article.]
712. National Institute for Occupational Safety and Health. (1978) Criteria for a recommended standard: Occupational exposure during the manufacture and formulation of pesticides. DHEW (NIOSH) Publ. No. 78-174.
[Background material.]
713. National Veterans Law Center. (1980) Statement of the National Veterans Law Center before the Senate Veterans Affairs Committee, United States Senate, Sept. 10, 1980. 31 pp.
[Testimony.]

714. Neal, R. A., Beatty, P. W., and Gasiewicz, T. A. (1979) Studies of the mechanisms of toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Ann. N. Y. Acad. Sci. 320:204-213.

The authors proposed a series of mechanisms of action to account for the acute toxicity of TCDD and tested each hypothesis, experimentally. The hypothesis that TCDD inhibits mitosis was tested by examining the effect of TCDD on the growth of five mammalian cell lines in vitro. At 10^{-6} M, TCDD had no effect on any cells tested. The similarity of TCDD toxicity to the hypothyroid state and other reasons led to an experiment that tested the ability of triiodothyronine (T_3) administration to protect mice from TCDD toxicity. The survival time of these mice was prolonged, but T_3 did not decrease the mortality. The authors tested the effect of TCDD on Rana catesbeiana tadpole metamorphosis, a T_3 -dependent process and found no alteration, nor any toxicity in the tadpoles or adults of the species. The effect of TCDD on the oxidation-reduction state of the cell and on the ATP levels was examined. Rats were treated with 50 ug/kg TCDD intraperitoneally and 14 later the livers were excised. The ATP content of the liver and the ratio of the levels of oxidized to reduced pyridine nucleotides (NAD/NADH) were the same in TCDD treated and ad libitum-fed control rats. The total liver content of the several pyridine nucleotides were measured in another experiment and only the NAD levels of TCDD-treated rats were significantly lower than control values. Next, the hypothesis that TCDD produced toxicity by mimicking glucocorticoid compounds or stimulating their biosynthesis (through enzyme induction) was studied. At 7 and 14 days after 50 ug/kg of TCDD was administered to rats, plasma corticosterone levels were 2.5 times the levels of pair-fed controls. All adrenalectomized rats given 10, 20, 40, or 80 ug/kg TCDD died within 6 days, while the 20 day LD_{50} in the rat including sham-operated and vehicle controls was 50 ug/kg. Even at a 200 fold molar excess, TCDD was unable to compete with [3 H]-dexamethasone for the rat cytosol glucocorticoid receptor. TCDD was also unable to interfere with a peripheral action of glucocorticoids, the induction of rat liver tyrosine aminotransferase activity by dexamethasone. Although TCDD markedly affects lipid metabolism, the fatty acid compositions of the livers, plasma, and adipose tissue of guinea pigs were unaltered by TCDD treatment. Absorption of nutrients was not impaired by TCDD, as [14 C]-glucose, [14 C]-alanine and [14 C]-oleate administered orally to guinea pigs produced as much [14 C]- CO_2 by TCDD-treated animals as pair-fed controls. Other parameters that were studied but did not reveal further information on the mechanism of TCDD toxicity were increases in blood NH_3 , increased superoxide anion levels, changes in cyclic nucleotide metabolism and inhibition of riboflavin coenzyme activity. The authors concluded that TCDD produces stress an acute stress in rats that is combated by increased glucocorticoid output, TCDD does not interfere with thyroid hormone binding nor causes a pathologic alteration in the cellular energy state in the rat. The details of the experimental protocols were not provided, but the rationale and interpretation of results were adequately described and successful identification of the mode of action of TCDD would greatly enhance understanding TCDD toxicity.

715. Nebert, D. W., and Jensen, N. M. (1979) The Ah locus: genetic regulation of the metabolism of carcinogens, drugs, and other environmental chemicals by cytochrome p-450-mediated monooxygenases. CRC Crit. Rev. Biochem. 6:401-430.
- [Review.]
716. Nebert, D. W., Robinson, J. R., and Poland, A. P. (1973) Genetic expression of several drug-metabolizing enzyme activities inducible in the mouse by aromatic hydrocarbons. Genetics 74:S193.
- [Abstract, only.]
717. Neely, W. B., Branson, D. R., and Blau, G. E. (1974) Partition coefficient to measure bioconcentration potential of organic chemicals in fish. Environ. Sci. and Technol. 8(13):1113-1115.
- [Background material.]
718. Neilands, J. B. (1973) Survey of chemical and related weapons of war. Naturwissenschaften 60:177-183.
- [Review article.]
719. Nelson, B. (1969) Herbicides: Order on 2,4,5-T issued at unusually high level. Science 166: 977-979.
- [Editorial.]
720. Nelson, C. J., and Holson, J. F. (1978) Statistical analysis of teratologic data: problems and advancements. J. Environ. Pathol. Toxicol. 2:187-199.
- [Review article.]
721. Nelson, C. J., Holson, J. F., Green, H. G., and Gaylor, D. W. (1979) Retrospective study of the relationship between agricultural use of 2,4,5,-T and cleft palate occurrence in Arkansas. Teratology 19:377-384.

The author performed a retrospective study to determine a possible causal relationship between 2,4,5-T exposure and the teratogenic effect of cleft palate in Arkansas. The ecological model employed estimated the herbicide exposure level by the number of rice fields in a given area. Data were obtained from both the vital records and the files of the State Crippled Children's Services for the years 1943 to 1974 inclusive. Data were apportioned in six-year intervals with the

exception of the 2 final intervals which had seven years. Regression analyses were performed separately on cleft lip with and without cleft palate and cleft palate only, prevalence rates versus years by exposure group (high, medium or low) race and sex. A significant linear trend with time for cleft lip with or without cleft palate was found for high exposure black females and high and low exposure white males. Black females exhibited increased rates with increased exposure. For cleft palate only a significant linear trend with time was found for high exposure black females, low exposure black and white males and high and low exposure white females. The author concluded that since data were not available to compare individual exposures and the difficulty of determining if cases in the assigned groups actually received a high or low exposure that no inferences of cause and effect should be drawn.

722. Neubert, D., and Dillmann, I. (1972) Embryotoxic effects in mice treated with 2,4,5-trichlorophenoxy-acetic acid and 2,3,7,8-tetrachlorodibenzo-p-dioxin. Arch. Pharmacol. 272(17):243-264.

An extensive study designed to separate the teratogenic effects of 2,4,5-T from those of TCDD was conducted with NMRI mice. Three preparations of 2,4,5-T containing less than 0.02 ppm dioxin (A), 0.05 + 0.02 ppm dioxin (B), and (C) an unknown amount of dioxin, but with physical properties suggestive of higher dioxin contamination than (B). The butyl ester of 2,4,5-T, 2,4,5-trichlorophenol, and pure TCDD were also tested. Compounds were administered in rape seed oil by stomach tube to pregnant mice on various schedules. On day 18 of gestation the number of viable fetuses, resorbed fetuses and implantation sites were counted, placentae and fetuses were weighed, and the incidence of cleft palate was determined. A total of 700 pregnant mice and 7,000 fetuses were used in these experiments. The LD₅₀ for non-pregnant mice given 10 daily oral doses was 130 mg/kg 2,4,5-T. Non-pregnant mice given 120 mg/kg 2,4,5-T on this regimen gained significantly less weight than controls but no fatalities occurred. Pregnant mice given doses of 2,4,5-T above 30 mg/kg, or TCDD above 2 ug/kg showed decreased weight gains. At doses above 30 mg/kg, 2,4,5-T or 2,4,5-T butyl ester administered from day 6 through day 15 of gestation produced cleft palates in a dose-dependent manner. The purest preparation was significantly less potent than the other preparations. Administration of a single dose of 300 mg/kg 2,4,5-T was most effective in producing cleft palate when administered on day 12 compare to any other day between days 6 and 15. Doses higher than 45 mg/kg 2,4,5-T given on days 6 through 15 significantly increased the total number of resorptions and this increase occurred sporadically among litters. All doses of 2,4,5-T caused a reduction in fetal weight which was not dose-dependent and was not accompanied by a decrease in placental weights. TCDD administered on days 6-15 at doses above 2 ug/kg produced cleft palate and embryo-lethal effects. Single doses of TCDD were most effective in producing cleft palate when administered on day 8 or day 11 of gestation. Although potentiation of the effect of 2,4,5-T in producing cleft palate occurred when TCDD was administered simultaneously, contamination of less than 1 ppm TCDD would not lead to potentiation of the effect of 30-60 mg/kg 2,4,5-T in producing cleft

palate. Trichlorophenol at doses up to 9 mg/kg was not teratogenic but produced a slight increase in embryo mortality. The authors concluded that neither TCDD nor trichlorophenol were likely to be responsible for causing or potentiating the embryotoxicity produced by impure preparations of 2,4,5-T, although other unidentified contaminants may be active in this regard. The doses of 2,4,5-T shown to be active in causing embryotoxic effects were considered to be low compared to the application rates of 10 to 300 mg/m² used for the herbicide.

723. Neubert, D., Zens, P., Rothenwallner, A., and Merker, H. J. (1973) A survey of embryotoxic effects of TCDD in mammalian species. Environ. Health Persp. 5:67-79.

The embryotoxic effects of pure preparations of TCDD and of 2,4,5-T alone and in combination were studied in the mouse and data from other laboratories on the embryotoxicity of these compounds were reviewed. Pregnant NMRI mice were administered TCDD (98.6% pure) or 2,4,5-T (less than 0.02 ppm contaminant) or both in rape seed oil by stomach tube. On day 18 of gestation, fetuses were removed and dead fetuses, including resorptions, were counted. Probit plots of the incidences of cleft palate and of resorptions from daily doses of 2-10 ug/kg TCDD given during days 6-15 of pregnancy overlapped. The probit plot of cleft palate incidence in mice given 10-50 ug/kg TCDD on day 13 only paralleled the data from doses given on days 6-15, but no increase in resorptions occurred from any dose given on day 13, only. From 6 to 8 fetuses per litter had cleft palates after 9 ug/kg TCDD was administered on days 6-15 or on days 9-13. Mortality was increased only from treatment on days 6-15, with 9 fetuses per litter affected, compared to one dead fetus per litter in controls and in the group treated on days 9-13. The maximum incidence of cleft palates resulted from administration of TCDD on day 11, with half of the maximum incidence produced from administration on day 10 or day 12. Maximum incidence of cleft palate occurred from administration on day 12, with 60% of maximum incidence from treatment on day 13 and about 15% of maximum from day 11 treatment. (The doses used were not indicated.) The probit dose-response curves were compared for TCDD, 2,4,5-T, 6-aminonicotinamide (6-AN), endoxan, and dexamethasone with cleft palate incidence as the response. TCDD was the most potent and 2,4,5-T the least potent compound; 2,4,5-T showed the most shallow response and 6-AN showed the steepest response. A combination of 60 mg/kg 2,4,5-T and a non-teratogenic dose of 2 ug/kg TCDD or 0.2 ug/kg TCDD showed potentiation of the incidence of cleft palate. This combination responds to contamination of 2,4,5-T with 3.3 ppm TCDD. Non-teratogenic levels of 2,4,5-T of 30 mg/kg TCDD at more than 20 ppm can cause potentiation. TCDD also potentiated the incidences of cleft palate produced by 6-AN and by dexamethasone and by combinations of these compounds and 2,4,5-T. The authors concluded that the degree of potentiation by TCDD was related to the steepness of the dose-response curve of the other teratogen, assuming that TCDD did not alter the metabolism of the teratogen. This study presents a good review of the embryotoxicity of TCDD and pertinent data on the potentiation of combinations of relevant compounds which is essential to understand other studies that employed TCDD-contaminated preparations of 2,4,5-T.

724. Newmeyer, J. (1973) Herbicides. The Encyclopedia of Chemistry 3d ed. Hampel, C. A., and Hawley, G. G. eds. (Reinhold, NY: Van Nostrand) 526-529.

[Not available.]

725. Newton, M., and Snyder, S. P. (1978) Exposure of forest herbivores to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in areas sprayed with 2,4,5-T. Bull. Environ. Contam. Toxicol. 20(6):743-750.

The authors describe the analysis of livers of mountain beavers for TCDD contamination. Ten beavers were captured 45-60 days after aerial spraying of 2,4-D (2.2 kg/ha) and 2,4,5-T (2.2 kg/ha). Animal livers were analyzed by gas chromatography-mass spectrometry (detection limit 3-17 ppt). No TCDD contamination was detected in either the exposed animals or a control animal captured in an untreated area. The authors concluded that forest herbivores are unlikely to accumulate TCDD in their tissues at detectable levels.

726. Nicholson, S. A., and Clerman, R. J. (1974) Toxicity of diquat to the crustacean amphipod Hyaella from Chautauqua Lake. Environ. Letters 7(3):215-227.

The authors report on the toxicity of diquat to the crustacean Hyaella. Organisms were collected from Lake Chautauqua and tested in lake water. A commercial preparation of diquat (.3 - 4.02 ppm cation) was added to tanks containing 10 animals. Test animals were examined at 24, 48, 72, and 108 hours. The median 24 hour tolerance limit (TL_M) of Hyaella was determined to be 1.26 ppm (0.67 ppm cation). The authors concluded that Hyaella is killed by diquat levels commonly used for weed control.

727. Nielsen, K., Kaempe, B., and Jensen-Holm, J. (1965) Fatal poisoning in man by 2,4-dichlorophenoxyacetic acid (2,4-D): Determination of the agent in forensic materials. Acta Pharmacol. Toxicol. 22:224-234.

A case of suicide by ingestion of 80 mg/kg of the dimethylamine salt of 2,4-D by a 23-year-old man was reported. The formulation ingested also included dimethylamine which was considered to be of low toxicity. Tissue samples were analyzed for 2,4-D contents and were examined for histological changes. All organs showed acute congestion. Severe, degenerative changes were observed in the ganglion cells and acute pulmonary emphysema was noted in the lungs. The kidneys, liver, spleen and adrenals showed acute congestion, but no degenerative changes. The pancreas, prostate, and testes appeared normal on histological examination. All organs analyzed contained 2,4-D. The highest concentrations were detected in the stomach contents followed by the blood. The brain, on the other hand, had almost negligible amounts suggesting extreme sensitivity of the ganglion cells to 2,4-D or anoxia as the cause of the observed histopathology. The authors were unable to

identify the mechanism of 2,4-D poisoning, but a diabetogenic effect on the pancreas (suggested by others) was considered unlikely since no histological changes were observed in this organ.

728. Nienstedt, W., Parkki, M., Uotila, P., and Aitio, A. (1979) Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the hepatic metabolism of testosterone in the rat. Toxicology 13:233-236.

The metabolism of testosterone was studied in homogenates of livers from rats after pretreatment with TCDD. Adult male Wistar rats were administered 20 ug/kg of TCDD in acetone-corn oil (1:99) intragastrically 1 week before they were killed. Hepatic tissue homogenates were incubated with [4-¹⁴C]-testosterone for 2 min. No cofactors for conjugation reactions were added to the mixture. Radioactive metabolites were separated by thin-layer chromatography and quantified by liquid scintillation counting. Only non-conjugated steroids were identified in the digestion medium. The production of polar compounds was reduced significantly from 78% of the radioactivity by liver homogenates in vehicle controls to 56% by the homogenates from TCDD-treated rats. Increases in the proportions of the radioactivity associated with testosterone and with dihydrosteroid intermediates occurred with TCDD treatment. The authors concluded that TCDD exerted its effect by inhibiting the cytochrome P-450 dependent hydroxylation reaction that produces the polar metabolites of testosterone, without altering enzymatic reduction reactions that produce the radioactive intermediates isolated in this study. The importance of this alteration of testosterone metabolism on reproductive function in the male has yet to be demonstrated.

729. Nikolaev, A. Z., Subkhankulova, F. B., Geller, I. S. (1970) Immune reactions in methylmercaptos, phosphamide, aldrin and monuron poisoning. Farmakol. Toksikol. 33(6):737-741.

[Foreign language.]

730. Nisbet, I. C. T (1980) Direct testimony before the U. S. Environmental Protection Agency, FIFRA Docket Nos. 415, et al.

[Testimony.]

731. Nitro Workers' Deaths, Dioxin Link Not Found. The Charleston Gazette, October 10, 1980.

[Editorial.]

732. Niwa, A., Kumaki, K., and Nebert, D. W. (1975) Induction of aryl hydrocarbon hydroxylase activity in various cell cultures by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Molec. Pharmac. 11:399-408.

The characteristics of TCDD induction of aryl hydrocarbon hydroxylase (AHH) activity were compared to 3-methylcholanthrene (3-MC) induction in cultures of various types of cells. AHH activity was assayed in cultures of 10 established cell lines, of fetal primary cultures from 5 animal species, and of human lymphocytes. For each of four cell lines, the lag time from introduction of the inducer into cultures until induction occurred, the length of rapid increase in enzyme activity and the maximal specific activities for TCDD and 3-MC were similar; the magnitudes of the maximum responses varied for different cell lines. Induction by either agent was blocked by actinomycin D and by cycloheximide and maximally effective doses of both agents did not produce a greater induction than either compound administered alone. The cytotoxic effect of TCDD (in the range of 0.7-1.0 μ M TCDD) was not related to its AHH-inducing activity. The dose of TCDD producing a half maximal inductive response (ED_{50}) varied from 0.1 nM for C58BL/6N mouse cells to 8.0 nM for human lymphocytes and over 200 nM for some established cell lines. The ED_{50} for fetal cells from chick, hamster, rat, rabbit, and two mouse strains and for six established animal cell lines were below 2 nM, while the ED_{50} of 6.4 nM for one mouse strain (AKR/N) and 11 for a human liver established cell line approached the value for human lymphocytes. TCDD was consistently more effective than 3-MC as an inducer, ranging from 250-900 times more potent, depending on the cell line. The authors recommended the use of H-4-II-E cell cultures for assaying TCDD at levels of 10^{-14} moles/ml culture medium by monitoring AHH induction. The authors also proposed a difference in affinity or number of TCDD-"receptor" sites to account for the differences in responsiveness to TCDD of different cell lines.

733. Nolan, R. J., Smith, F. A., and Hefner, J. G. (1979) Elimination and tissue distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in female guinea pigs following a single oral dose. Toxicol. Appl. Pharmacol. 48(1)Pt. 2:A162.

[Abstract, only.]

734. Norback, D. H., and Allen, J. R. (1973) Biological responses of the nonhuman primate, chicken, and rat to chlorinated dibenzo-p-dioxin ingestion. Environ. Health Persp. 5:233-240.

[Review article.]

735. Norman, R. L., Johnson, E. F., and Muller-Eberhard, U. (1978) Identification of the major cytochrome P-450 form transplacentally induced in neonatal rabbits by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Fed. Proc., Fed. Am. Soc. Exp. Biol. 37(6):1720.

[Abstract, only.]

736. Norris, L. A. (1966) Degradation of 2,4-D and 2,4,5-T in forest litter. J. Forestry 64:475-476.

[Background material.]

737. Norris, L. A., Montgomery, M. L., and Johnson, E. R. (1977) The persistence of 2,4,5-T in a Pacific Northwest forest. Weed Sci. 25(5):417-422.

The authors report on a study of the persistence of 2,4,5-T in forest vegetation and soil after aerial applications of the herbicide. The study area consisted of 87 ha in a highly productive Douglas fir forest. 2,4,5-T isooctyl ester (2.24 kg/ha) in diesel oil was sprayed by helicopter over the area in March 1971. A second application on the same mixture was applied to 67 ha in March 1972. Samples of the current season's vegetation (vine maple, blackberry vines, composites of several grass species, 15 cm terminal branch segments of Douglas fir), forest floor material, and forest soil were collected within 2 hours of application and 1,3,6, and 12 months after treatment. Samples were analyzed by gas chromatography (detection limit 0.01 ppm). In forest vegetation, maximum concentrations of 2,4,5-T occurred immediately after application and ranged from 10.6 ppm in vine maple to 114.5 ppm in grass. Concentrations dropped sharply during the first 3 months. One year after spraying, 2,4,5-T levels ranged from 0.48 ppm in vine maple to 0.03 ppm in blackberry foliage. Therefore, months after application only vine maple contained detectable levels of 2,4,5-T. In the area which received 2 herbicide sprayings, vegetation concentrations were also highest immediately after spraying and declined rapidly. After 1 year, only vine maple had detectable levels of 2,4,5-T (0.02 ppm). Levels of 2,4,5-T in forest floors increased during the first month after herbicide spraying and then declined rapidly. About 0.3% of the original application remained after 24 months. Levels of residues were higher after the second application than after the first application probably as a result of reduced vegetation due to the first spraying. One year after the second application, 2,4,5-T residue levels were identical to levels remaining 1 year after the first application. 2,4,5-T residue levels in soil were very low compared to forest floor and vegetation samples. After the first spraying, 2,4,5-T residues in the 0-15 cm layer of soil peaked at 0.08 ppm 3 months after spraying. No 2,4,5-T was detected in the top layer (0-15 cm) of soil after 12 months. In addition, no 2,4,5-T was detected deeper than 0.15 cm. After the second spraying, residues were detected in the top layer immediately after spraying but not at any other time. The authors concluded that no accumulation of 2,4,5-T occurred in the forest sample tested and that the short persistence of this compound should not adversely affect the forest environment.

738. Norris, L. A., Montgomery, M. L., Webb, W. L., Schroeder, H. J. Jr., and Gross, F. (1976) Distribution of 2,4-D and picloram applied by a mist blower. Bull. Environ. Contam. Toxicol. 16(6):631-639.

[Background material.]

739. Norris, L. A., and Moore, D. G. (1970) The entry and fate of forest chemicals in streams. In Proc., Symp., Forest Land Uses and Streams Environment. [Corvallis, Oregon: Oregon State University PG:138-158.

[Background material.]

740. O'Connor, G. A., and Anderson, J. U. (1974) Soil factors affecting the adsorption of 2,4,5-T. Soil Sci. Soc. Am. Pro. 38(3):433-436.

The authors describe soil factors that affect the adsorption of 2,4,5-T. Four soil types were studied, three agricultural soils (Glendale, Palouse, Ephrata) and one forest soil (Ordinance). Each soil was handled in four different ways: 1) untreated; 2) organic matter removed, calcium saturated; 3) organic matter and oxide coatings removed, calcium saturated; 4) calcium saturated. ^{14}C -2,4,5-T labeled at the carboxyl carbon position (specific activity 4.93 c/mg) was added to soil samples, and adsorption was determined from the specific activity of the original solution and the activity of ^{14}C -2,4,5-T remaining. The amount of herbicide adsorbed tended to increase with increasing organic matter. However, herbicide adsorbed was not in proportion to the amount of organic matter. Adsorption of 2,4,5-T in the four soils ranged from 14-290 (μ g/g soil). Removal of organic matter reduced the adsorption of 2,4,5-T, while removal of oxides of Fe and Al had little effect on three of the four soils studied. No differences in 2,4,5-T adsorption were observed when calcium-saturated soil was compared to untreated soils. The authors concluded that organic matter may be the single most important factor influencing the adsorption of 2,4,5-T by soil.

741. Okey, A. B., Bondy, G. P., Mason, M. T., et al. (1978) Regulatory gene product of the Ah locus. J. Biol. Chem. 254(22):11636-11648.

The isolation and characterization of the cytosol receptor product of the Ah locus is described. The receptor was isolated from liver cytosol from 5 strains of mice and Sprague-Dawley rats, using sucrose density gradient analysis. The isolated receptor was characterized as to its affinity, capacity, specificity, thermolability and protease susceptibility. The isolated receptor showed high-affinity, low-capacity binding to TCDD. The receptor bound polycyclic aromatic inducers of cytochrome P₄₅₀ was thermolabile, and was primarily protein. The receptor was isolated in mouse strains that are responsive to enzyme inducers and not in non-responsive strains. The liver contained 60 fmol of receptor per mg of cytosolic protein, equivalent to 5,500 receptors per cell. Kidney and lung tissue contained receptor in the same concentration range as in liver. [^3H]-TCDD-receptor complex was isolated from the livers of responsive strains of mice after in vivo TCDD treatment. The authors concluded that cytochrome P₄₅₀ induction and other effects elicited under Ah locus control, required that the presence of the receptor and its translocation to the nucleus, bound to the inducer.

742. Okey, A. B., Bondy, G. P., Mason, M. E., et al. (1980) 1979 Temperature-dependent cytosol-to-nucleus translocation of the Ah receptor for 2,3,7,8-tetrachlorodibenzo-p-dioxin in continuous cell culture lines. J. Biol. Chem. 255(23):11415-11422.

The effect of TCDD on aryl hydrocarbon hydroxylase activity and characteristics of the TCDD-Ah receptor complex in various cell lines

are described. Doses that produced half-maximal stimulation of aryl hydrocarbon hydroxylase activity were determined in Hepa-1, H-4-II-E, HTC and VERO contiguous cell lines. The effect of temperature, and of proteases on the [³H]-TCDD-receptor complex and the time required for the complex to move from the cytosol to the nucleus were determined in vitro. About 1% TCDD taken up by the Hepa-1, H-4-II-E and HTC cell lines was bound to the receptor and a concentration of 0.28 nM TCDD produced half-saturation of nuclear binding sites in Hepa-1 cells. The receptor was more thermolabile in cytosol than in the nucleus and was susceptible to proteases, but not nucleases. The receptor was estimated to be 6S, from its movement in sucrose density gradients. The half-maximal TCDD doses for enzyme induction were 0.23, 0.45, 110 and greater than 200 nM for H-4-II-E, Hepa-1, VERO and HTC cells, respectively. Hepa-1 cells had twice as many receptors as H-4-II-E cells and four times as many as HTC cells, while VERO cells had no detectable receptors. The authors concluded that enzyme induction does not necessarily result in cell lines (such as HTC cells) in which cytosol receptors could be demonstrated and translocation to the nucleus takes place.

743. Okonek, S., and Hofmann, A. (1975) On the question of extra corporeal hemodialysis in diquat intoxication. Arch. Toxicol. 33(3):251-257.

A case report of diquat poisoning from ingestion in a suicide attempt is described. A 43-year-old woman ingested an unknown quantity of commercial diquat herbicide preparation (Reglone; 200 g/l diquat ion). After 2 days of decreased appetite and vitality, an unknown amount of the same preparation was ingested again. The patient went into shock, was anuric, and had hemorrhagic mucosal necrosis of the mouth, throat, and esophagus within 1 day. Forced diuresis and hemodialysis were instituted and blood samples were collected and analyzed for 2,4-D colorimetrically after separation of 2,4-D by ion-exchange chromatography. At the end of the 6.5 hr. hemodialysis period, the serum diquat level had fallen to 30% of the initial level. A second hemodialysis was started 10.5 hr. after the first period ended and lasted 5 hr. The serum 2,4-D level was reduced by 30% by the second hemodialysis. The patient died in protracted cardiovascular collapse 19 hours after the second hemodialysis. The authors concluded that hemodialysis was unable to remove a toxicologically significant amount of diquat from the circulation 1 day after ingestion.

744. Olds, K. L., (1976). Monitoring of pesticide disposal practices, Iowa Army Ammunition Plant, Burlington Iowa, Sept 74 - Jan 75. USNTIS Publication No. ADO30862. 18 pp.

745. Oliver, R. M. (1975) Toxic effects of 2,3,7,8- tetrachlorodibenzo 1,4-dioxin in laboratory workers. Br. J. Ind. Med. 32:49-53.

Three cases involving scientists who synthesized TCDD in a laboratory and exhibited symptoms of dioxin toxicity are described. Two scientists

prepared TCDD in a fume hood wearing gloves and an overalls. Eight weeks later, one man developed chloracne on the face and neck, which subsided in 18 months. This scientist, who had no other clinical changes, had severe acne as an adolescent. The second scientist was involved in two syntheses, one week apart. Six weeks after the first synthesis, he noticed excessive oiliness of the skin and chloracne developed in two waves, which simulated the two exposure incidents. A follicular rash that developed on his hands and arms when the chloracne appeared cleared rapidly. No other abnormal findings were evident at the time, and the chloracne healed within one year. Two years after the exposure, the second scientist developed abdominal pains, flatulence, headaches, excessive fatigue, decreased concentration, irritability, hirsutism, blurred vision, and decreased muscular coordination. All of these symptoms, except the abdominal pains, subsided in 6 months. The third scientist worked with the TCDD preparation synthesized by the other scientists. Three years after his exposure, he developed an inability to concentrate, indigestion, diarrhea, decreased sense of taste, visual problems, insomnia, thigh pains, excessive oiliness of the skin, and hirsutism. All symptoms except hirsutism subsided in 6 months. A blood examination 3 years after the synthesis revealed hypercholesterolemia in all three men and hyperlipoproteinemia in the latter two men. None of the patients showed evidence of acquired porphyria. The authors concluded that TCDD exposure was the likely cause of all of the symptoms described.

746. Olson, J. R., Holscher, M. A., and Neal, R. A. (1980) Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the golden Syrian hamster. Toxicol. Appl. Pharmacol. 55:67-78.

The acute toxicity of TCDD in the hamster is described. TCDD was administered orally or intraperitoneally to groups of male and female golden Syrian Hamsters and the mortality rate was determined 50 days after a single dose was administered. Body weight gain and chemical and enzyme analyses of blood were determined during the 50 day period after exposure. Histopathology and body weight gains were determined at autopsy. The 50 day intraperitoneal LD₅₀ was above 3,000 ug/kg of TCDD and the oral LD₅₀ was calculated at 1157 ug/kg. Weight gains were reduced after TCDD was administered by either route. Thymic atrophy was the most severe lesion noted at autopsy, while severe ileitis and peritonitis were observed in orally-treated animals. Chemical changes in treated animals included decreased albumin, chloride, urea nitrogen, and triglyceride levels and increased alkaline phosphatase, bilirubin, protein, iron and cholesterol levels. The authors concluded that the hamster is the most sensitive mammalian species to TCDD toxicity and that higher toxicity after oral, compared to peritoneal exposure was caused by ileitis and peritonitis, seen only after oral dosing.

747. Olson, J. R., Gasiewicz, T. A., and Neal, R. A. (1980) Tissue distribution, excretion, and metabolism of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the golden Syrian hamster. Toxicol. Appl. Pharmacol. 56:78-85.

The distribution, excretion, and metabolism of TCDD in the hamster is described. A single oral or intraperitoneal dose of 650 ug/kg of [³H]- or [¹⁴C]-TCDD was administered to groups of male golden Syrian hamsters. The tissue distribution and urinary, fecal and biliary excretion of radio-activity were determined during the subsequent 35 days. Excretory rates were analyzed pharmacokinetically. The presence of metabolites were assessed in various tissues and isolation of biliary metabolites was carried out by high pressure liquid chromatography. The liver, adipose tissue, and adrenals contained the highest concentrations of radioactivity which was present as unmetabolized TCDD. Elimination was probably first-order for both isotopes, with half-times of 11-12 days for intraperitoneal doses and of 15 days for oral doses. The urine and feces contained 35 and 50%, respectively, of the doses administered in 35 days. On day 7 after treatment, the 24-hour bile sample contained 1% of the dose, which was recovered as 1 major and several minor metabolites, but not as unmetabolized TCDD. No entero-hepatic cycling was observed. The authors concluded that once TCDD metabolites were formed, they were rapidly excreted in bile and the enhanced rate of TCDD biotransformation partially explains the low sensitivity of this species to TCDD toxicity.

748. Olson, R. J., Trumble, T. E., and Gamble, W. (1974) Alterations in cholesterol and fatty acid biosynthesis in rat liver homogenates by aryloxy acids. Biochem. J. 142(2):445-448.

The effects of 2,4-D and 2,4,5-T on fatty acid biosynthesis by rat liver homogenates in vitro was studied. Radioactive substrates of fatty acid synthesis were incubated with rat liver homogenates (whose preparation was not described) in the presence of inhibitors and cofactors. Non-saponifiable lipids were extracted and the radio-activity in the extracts as well as in the fatty acid fraction and cholesterol fraction were determined. Thin layer chromatography was used to separate and identify some radioactive metabolites. From 23-31% of the incorporated radioactivity from [2-¹⁴C]-mevalonate was recovered in the non-saponifiable lipid fraction, with 89% in cholesterol, 2% in squalene and 4% in farnesol. Both 2,4-D and 2,4,5-T (less than 1 ppm dioxin contaminant) produced dose-related decreases in the incorporation of [¹⁴C]-nevalonate into non-saponifiable lipids of 77% maximum inhibition by 2,4-D and 65% maximum by 2,4,5-T. The proportion of non-saponifiable lipid radioactivity recovered as cholesterol decreased to 37% with 9mM 2,4-D and to 5% with 4.5mM 2,4,5-T, while the squalene fraction comprised 35% of the radioactivity in 2,4-D treated incubates and 54% with 2,4,5-T present, and 20% of the radioactivity was in farnesol for 2,4-D and 2,4,5-T homogenates. Incorporation of [1-¹⁴C]-nevalonate into ¹⁴CO₂ was unaltered by phenoxyacid treatment and incorporation of [1-²¹⁴C] isopentenyl pyrophosphate into cholesterol resembled nevalonate incorporation in

treated and control incubations, 2,4-D produced 100% inhibition of [2-¹⁴C]-acetate into non-saponifiable lipids and 2,4,5-T produced 73% inhibition. At low concentrations, both compounds stimulated [2-¹⁴C] acetate incorporation into fatty acids and inhibited incorporation at the highest concentrations (4.5-9.0mM). The authors concluded that both compounds inhibited precursor incorporation into lipids but were not able to identify which step(s) were blocked and indicated that no implications related to acute toxicity were found from the data.

749. Orberg, J. (1980) Effects of low protein consumption on the renal clearance of 2,4-dichlorophenoxyacetic acid (2,4-D) in goats. Acta Pharmacol. Toxicol. 46(2):138-140.

The rate of 2,4-D clearance was determined in goats that were fed a low protein diet. Four female goats, including one 6-month-old kid, were administered a normal diet of 6.3% digestible protein for 3 of 5 periods of 4-6 weeks and a low protein diet of 1.4% digestible protein for the second and fourth period. At the end of each feeding period, urea, [¹⁴C]-2,4-D and [³H]-inulin were administered by intravenous infusion. The resultant plasma level for 2,4-D was 10 ng/ml. Both blood and urine samples were collected during the infusion period and analyzed for urea and for radioactivity. The urea and inulin clearances were 3 and 1.7 times lower during the periods of low protein consumption than during control periods; the 2,4-D clearance was about 1.7 times lower during the low protein than control period. The authors concluded that the small decrease in 2,4-D clearance by about 50% would not be likely to significantly enhance the toxicity of the compound in animals with low protein consumption.

750. Orberg, J. (1980) Observations on the 2,4-dichlorophenoxyacetic acid (2,4-D) excretion in the goat. Acta Pharmacol. Toxicol. 46(1):78-80.

The rate of renal clearance of 2,4-D and the proportion of 2,4-D bound to plasma proteins was determined in goats. Three female goats were administered 2,4-D by continuous venous infusion at the rate of 0.5-32 mg [¹⁴C]-2,4-D per minute. [³H]-inulin was also infused and urine and blood samples, collected for six consecutive 10 minute periods, were counted by radioactivity. Protein binding was determined in blood samples by equilibrium dialysis from the amount of radioactivity accessible to both sides of a cellulose membrane. Urinary metabolites were analyzed by thin-layer chromatography. About 97% of the plasma 2,4-D was bound to protein for 2,4-D plasma concentrations less than 20 ug/ml and gradually decreased at higher 2,4-D plasma concentrations. The ratio of 2,4-D clearance to inulin clearance and the glomerular filtration rates were constant for plasma concentrations below 40 ug/ml of 2,4-D; both parameters steadily decreased at higher plasma concentrations. 2,4-D was excreted much more rapidly than inulin at low 2,4-D plasma levels, despite high protein binding; at high levels, inulin is transported more rapidly than 2,4-D. The authors concluded that 2,4-D was transported by tubular secretion and 2,4-D altered renal blood flow and/or renal blood pressure (because it decreased the rate of glomerular filtration).

751. Oreopoulos, D. G., and McEvoy, J. (1969) Diquat poisoning. Postgrad. Med. J. 45(527):635-637.

A case report of diquat poisoning after accidental ingestion is presented. An 18-year-old man accidentally ingested diquat and attempted to spit it out. During the next 10 hours, he had diarrhea. Ten hours after the ingestion he was hospitalized and treated by gastric lavage. After 56 hours no symptoms were apparent and forced diuresis was initiated as a prophylactic means. A good diuretic response ensued and treatment continued for 11 days because diquat metabolites were detected qualitatively for 9 days. All clinical test results were normal during and after treatment and no clinical symptoms emerged. The authors concluded that acute poisoning by diquat followed a different course than paraquat poisoning and differences in tissue distribution and elimination by man and by other species may exist.

752. Orians, G. H., and Pfeiffer, E. W. (1970) Ecological effects of the war in Vietnam. Science 168:544-554.

[Review article.]

753. Ott, M. G., Holder, B. B., and Olson, R. D. (1980) A mortality analysis of employees engaged in the manufacture of 2,4,5-trichlorophenoxyacetic acid. J. Occup. Med. 22(1):47-49.

Mortality data is reported for workers that were occupationally exposed to 2,4,5-T during its manufacture at Dow Chemical Co. A group of 204 people were occupationally exposed to 2,4,5-T for at least 1 month between 1950 and 1971. Only 3 of these workers could not be traced for this study. Eleven deaths occurred in the total group. Observed deaths for the group of 11 workers, for the group of 6 workers with less than 1 year of exposure, and for the group of 5 workers with more than 1 year of exposure were lower than expected for all categories, except external causes (accidents and suicides). The categories were malignant neoplasms, diseases of cardiovascular system and all other causes (no deaths fell into this last category). Six deaths, including 5 in the low exposure group, were from external causes, including 2 suicides (that occurred more than 10 years after exposure), 3 car accidents and 1 fire. The expected incidence was 3.7. The authors concluded that no adverse effects detectable by mortality statistics were observed in workers exposed to 2,4,5-T during its manufacture. The small number of deaths that have occurred in the exposure group indicate that is premature to evaluate the effects of 2,4,5-T exposure of these workers by mortality statistics.

754. Ott, M. G., Holder, B. B., and Olson, R. D. (1979) A longevity survey of employees exposed to 2,4,5-trichlorophenoxyacetic acid. Unpublished report of the Medical Department, Dow Chemical Company, Midland, Mich. 15 pp.

[Not available.]

755. Paggiaro, P. L., Martino, E., and Mariotti, S. (1974) A case of 2,4-dichlorophenoxyacetic acid (2,4-D) poisoning. Med. Lavoro 65(3-4):128-135.

A case of accidental poisoning by 2,4-D is reported. The subject, a 63-year-old man, inhaled fumes of a solution comprised of 44% 2,4-D isopropyl ester. Within 2-3 hours the subject lost consciousness and within 2 days cephalgia and fever appeared, followed by myalgia in the neck and lower limbs, anorexia, muscular hypertonia, tachyarrhythmia, urinary incontinence and constipation. Sepsis of the urinary tract was identified by urine culture analysis. No compromise in thyroid function was observed. Therapy with hydroquinidine corrected the intermittent nodal tachycardia in 5 days and muscular hypertonia in 4 days. The authors reviewed the symptoms produced from acute and chronic 2,4-D exposures in humans and observed that cephalgia, constipation and tachyarrhythmia were symptoms that were observed only in the current case. The authors concluded that 2,4-D poisoning caused the nodal tachycardia of the patient and that a large amount of 2,4-D was inhaled (the amount was not estimated) and suggested that 2,4-D interfered with membrane polarization of striated muscle fibers.

756. Palm, C. E. (1968) Original arsenical herbicides. In Classification and chemistry of herbicides, chapter 10, Principles of Plant and Animal Pest Control, Volume 2. No. 1597 [Washington, D.C.: National Academy of Sciences] pp. 167-169.

[Review article.]

757. Palmer, J. S. (1963) Chronic toxicity of 2,4-D alkanolamine salts to cattle. J.A.V.M.A. 143(4):398-399.

The toxic effect of 2,4-D was described in cattle. Yearling steer (1 per dosage level) were administered daily doses of 50-250 mg/kg of 2,4-D alkanolamine salt (comprising 65% of a commercial formulation) for up to 112 days. Body weights and signs of intoxication were recorded during the exposure period and gross pathology was assessed at necropsy. A total of 112 treatments of 50 mg/kg doses caused no intoxication. Doses of 100, 200, and 250 mg/kg produced signs of toxicity after 86, 34, and 15 treatments, respectively. These signs included cracked muzzles, ulcerated mucous membranes, epistaxis upon restraint, nasal discharge, apathy, and depression. The steer that received 20 treatments of 250 mg/kg doses had tympany, severe hemorrhages of the large intestine, and pulmonary congestion and the steer given 44 treatments of 200 mg/kg showed hind leg muscle weakness and a staggering gait. Death (in unspecified animals) was attributed to kidney inflammation and a pneumonic condition. The authors concluded that 2,4-D had a low order of toxicity and probably did not accumulate in bovine tissues.

758. Palmer, J. S., and Radeleff, R. D. (1964) The toxicologic effects of certain fungicides and herbicides on sheep and cattle. Ann. N. Y. Acad. Sci. 111:729-736.

The toxic effects of orally administered 2,4-D and 2,4,5-T to sheep and cattle were described. Sheep, primarily Delaine breed, and cattle, primarily dairy breeds, were orally administered commercial preparations of 2,4-D alkanolamine salt, 2,4-D propylene glycol butyl ether esters, 2,4,5-T propylene glycol butyl ether esters, or 2,4,5-T triethylamine salt and dalapon in gelatin capsules daily or five times per week. One animal was treated with each test dosage of each compound. Clinical signs of toxicity and necropsy findings were reported. Sheep given 481 doses of 100 mg/kg of either form of 2,4-D were unaffected by treatment; 7 doses of 500 mg/kg of 2,4-D salt were lethal to another sheep and 9 doses of 250 mg/kg of the ester was lethal. The tolerated and lethal doses of 2,4-D in cattle were 50 mg/kg for 112 doses and 200 mg/kg for 44 days respectively. Poisoned animals showed anorexia, depression, weight loss, muscle weakness, ataxia, and ulceration of bovine nasal mucous membranes. In sheep, 481 doses of 100 mg/kg of the 2,4,5-T salt was tolerated, and 369 doses of 100 mg/kg of the 2,4,5-T ester or 7 doses of 250 mg/kg were lethal. In the cow, 7 doses of 250 mg/kg of the 2,4,5-T ester was also lethal. Anorexia and weight loss occurred in 2,4,5-T poisoned animals. Necropsy findings for these animals included degeneration of the kidneys and liver, cardiac hemorrhages, congestion of visceral blood vessels, and rumen atony. Dalapon was tolerated at doses of 100 mg/kg for 481 days or 500 mg/kg for 10 days to sheep or 500 mg/kg for 8 days to cattle. The authors concluded that accidental ingestion of any of these compounds is unlikely to present a significant health hazard to sheep or cattle.

759. Park, J., Darrien, I., and Prescott, L. F. (1977) Pharmacokinetic studies in severe intoxication with 2,4-D and Mecoprop. Clin. Toxicol. 18:154-155.

The pharmacokinetics of 2,4-D is described from data from a male subject who ingested 70 ml (estimated) of an herbicide comprised of 10% 2,4-D and 20% 4-chloro-2-methylphenoxypropionic acid (Mecoprop), as the amine salts. Clinical symptoms 3 hours after ingestion included neuropathy, myopathy, hyperventilation, pyrexia, and coma. Alkaline diuresis, which was initiated to force excretion of the herbicides reversed the coma, pyrexia, and hyperventilation. The levels of 2,4-D and Mecoprop in blood and urine were analyzed by gas liquid chromatography and the plasma half-lives and renal clearances were calculated before and during diuresis. Urine samples were acid-hydrolyzed to determine the proportion of each conjugate that was excreted as acid-labile conjugates. The plasma half-lives of 219 hours for 2,4-D and 39 hours for Mecoprop were reduced to 5 and 14 hours, respectively during alkaline diuresis, with concomitant increases in the clearances of both compounds during diuresis. About 10% of total 2,4-D and 40% of Mecoprop were metabolized to acid-labile conjugates. The authors concluded that renal clearance at alkaline urine pH and the higher efficacy of alkaline diuresis for 2,4-D than Mecoprop reflected the

higher pKa value for 2,4-D and more limited metabolism compared with Mecoprop.

760. Pasi, A., and Embree, J. W., Jr. (1976) Further comments on the assessment of the mutagenic properties of diquat and paraquat in the murine dominant lethal test. Mutat. Res. 31:123-125.

[Editorial.]

761. Pasi, A., Embree, J. W. Jr., Eisenlord, G. H., and Hine, C. H. (1974) Assessment of the mutagenic properties of diquat and paraquat in the murine dominant lethal test. Mutat. Res. 26:171-175.

The authors tested the mutagenic effects of diquat in a dominant lethal test in Swiss-Webster mice. Five males 8-10 weeks old were given a single intraperitoneal injection of 6 nmole/kg body weight diquat dibromide monohydrate which corresponded to the LD₅₀ for this compound. A control group of 10 males received a single intraperitoneal injection of physiological saline (10 ml/kg). No positive control was included in the study. Two hours after treatment each male was caged with 3 untreated females, 8-10 weeks old. After one week, males were removed and placed with another group of 3 untreated females. This was repeated for eight consecutive weeks, in order to assess the effects of diquat on the entire spermatogenic cycle of the mouse. Fifteen days after being caged with the male, the females were killed and uteri scored for pregnancy, total number of implants, early fetal deaths and late fetal deaths. The following statistical analyses were performed on the results: analysis of variance, chi-square, and student's t-test. No significant differences in total implants or early fetal deaths were observed indicating no mutagenic effect. However, the pregnancy rate between diquat treated weeks 1 through 5 (p less than 0.05 week 1 and 4, p less than 0.01 than week 2,3,5) and untreated controls decreased significantly. This antifertility effect corresponds to effects on postmeiotic early and late spermatids and epididymal sperm for weeks 1, 2 and 3. For weeks 4 and 5 the antifertility effect corresponds to effects on premeiotic early and late spermatocytes.

762. Paynter, O. E., Tusing, T. W., McCollister, D. D., and Rowe, V. K. (1960) Toxicology of dalapon sodium (2,2-dichloropropionic acid, sodium salt). J. Agric. Food Chem. 8(1):47-51.

The acute, subacute, chronic, and reproductive toxicities, of dalapon were studied in various animal species and tissue levels of dalapon were determined in the rat and dog. Single doses of dalapon (83-85% purity) were administered by intubation to rabbits, guinea pigs, rats, mice, and chickens. The LD₅₀ values were calculated and gross and histopathological examinations were performed. Dermal irritation of 10 daily applications undiluted, 1% and 10% aqueous solutions of dalapon was tested on intact and abraded rabbit skin. Eye irritation produced

by powdered dalapon or 1% or 10% aqueous solutions was tested in the rabbit. A heifer and a calf were administered 1 g/kg dalapon by oral intubation orally for 10 successive days. Two dogs were orally administered dalapon 5 days per week for 80 days. Doses were increased from 50 mg/kg per day initially to 1000 mg/kg per day. Wistar-Dow rats (10 per group) were fed diets with from 0.0115 to 1.15% dalapon for 97 days. Dogs (3 per group) were administered daily doses of 15, 50 or 100 mg/kg dalapon in capsules, 5 days per week for 52 weeks. Carworth Farm rats (44 per group) were fed diets with 100, 300, or 1000 ppm dalapon for 2 years. A 3-generation reproductive study was performed on rats fed diets of 0.03 to 0.3% (3000 ppm) dalapon. The LD₅₀ values ranged from 3.86 g/kg for the rabbit and guinea pig to 9.3 g/kg for the male rat. Death occurred within 1 day and the only autopsy finding was the presence of a large amount of fluid and gas in the gastrointestinal tract. Undiluted dalapon produced hyperemia and dermal necrosis on both intact and abraded skin, 10% solutions were moderately irritating and 1% solutions were ineffective. Powdered dalapon produced severe conjunctivitis and corneal injury, while aqueous solutions were less potent eye irritants. Dalapon produced anorexia, lassitude, and diarrhea in the heifer, but no clinical effects in the calf or gross or histopathological changes except mild renal lesions in the calf. No subacute effects were observed in dogs that received 161-200 grams total of dalapon except vomiting. Biochemical and hematological analyses, urinalyses, and gross and histologic pathology exams revealed no adverse effects in dogs or rats after subacute or chronic treatment. An increase in kidney weights occurred in both species after chronic treatment. No reproductive effects were detected in any generation of the rat study. Levels of dalapon in dog and rat tissues and milk after chronic exposure were low relative to the administered doses, with maximum levels of 28 ppm in the rat kidney and 78 ppm in the dog kidney. The authors concluded that dalapon has a low order of toxicity, but protection against eye exposure should be used when handling the compound and dermally applied dalapon should be washed off with water.

763. Pazderova, J., Lukas, E., Nemcova, M., Spacilova, M., Jirasek, L., Kalensky, J., John, J., Jirasek, A., and Pickova, J. (1974) Chronic intoxication by chlorinated hydrocarbons formed during the production of sodium 2,4,5-trichlorophenoxyacetate. Prac. Lek. 26(9):332-339.

The health of 55 workers from a 2,4,5-T factory in Czechoslovakia is described. All of the information was presented in two previous reports; see Jirasek et al. (1973) and Jirasek et al. (1974).

764. Pazderova-Vejlupkova, J., Lukas, E., Nemcova, M., Pickova, J., and Jirasek, L. (1980) Chronic poisoning by 2,3,7,8-tetrachlordibenzo-p-dioxin. Pracov. Lek. 32:204-209.

The health of 55 workers involved in the manufacture of 2,4,5-T was observed over 5 years. The contaminant, TCDD, was detected on the walls and furnishings of the factory, as well as in the 2,4,5-T. Each

worker had an average of five medical examinations. Chloracne was observed in 95 percent of the workers, and increased elimination of uroporphyrins occurred in 41 percent (half of these workers also showed dermal symptoms of porphyria cutanea tarda). Alterations in lipid metabolism occurred frequently: hyperlipemia was seen in 67 percent of the workers, hypercholesterolemia in 56 percent, and hyperphospholipemia in 42 percent. These lipid-metabolic parameters improved over the 5-year observation period. Polyneuropathy was present in one-third of the workers at the end of the observation period. No renal, cardiovascular, hematologic, or optic problems were encountered in the workers. At the time of publication, the only deaths among the exposed workers were: two in traffic accidents, one from cirrhosis of the liver and infectious hepatitis (age 40), one from arteriosclerosis (age 57), and two from bronchogenic carcinoma (ages 47 and 50). The authors indicated that the number of observed cases of bronchogenic carcinoma was too small to imply a causal relationship with exposure. Two of the workers' wives each reported having one miscarriage, and 18 children were born during the period in which the exposed workers remained ill. The authors indicated that this incidence of 2 abortions in 18 pregnancies was far below the worldwide incidence of 15-20 percent for spontaneous abortions. The authors did not indicate that they used a systematic method to collect information on reproduction or that they considered psychological factors which would increase voluntary birth control. The authors suggested that polyneuropathy was a direct response of nerve tissue to TCDD and potentiated negative effects on fat and carbohydrate metabolism. The authors attributed some of the psychological disturbances they observed to the fear of death, disfigurement, and disability. This TCDD exposure is significant, but the authors do not present the time of the appearance of symptoms or their duration, or thoroughly describe the incidence or extent of each symptom.

765. Pegg, D. G., Hewitt, W. R., McCormack, K. M., and Hook, J. B. (1976) Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on renal function in the rat. J. Toxicol. Environ. Health 2:55-65.

Renal function of rats treated with TCDD was studied in vivo and in vitro. Male Sprague-Dawley rats were administered a single dose of 10-50 ug/kg TCDD intraperitoneally or 1 or 5 ug intragastrically. Some rats were then given drinking water containing ammonium chloride until the kidneys were removed to establish acidosis. After 3 to 7 days, the kidneys were removed and accumulation of organic molecules was measured in kidney slices incubated in medium containing [¹⁴C]-labeled compounds. Glucose and ammonia production during a 1 hour incubation period were analyzed in renal slices. [¹⁴C]-paraaminohippurate (PAH) and [³H]-inulin clearances were measured in vivo under a saline load and during control periods sleeping times were recorded for rats administered 115 mg/kg hexobarbital. Growth rates of rats treated with 10 or 25 ug/kg TCDD were significantly below control rats after 3 and 7 days and hexobarbital sleeping times were increased substantially by 25 ug/kg of TCDD. PAH uptake was decreased 7 days after 1 or 5 ug of TCDD

was given orally or 25 ug/kg TCDD was injected but was unaltered 3 or 7 days after 10 ug/kg TCDD was given. A dose of 25 ug/kg TCDD did not alter 2-deoxyglucose uptake 7 days later. Ammonia and glucose production were unaltered by TCDD in non-acidotic and acidic rats. Glomerular filtration rate, measured as inulin clearance and effective renal plasma flow (PAH clearance) were decreased 7 days after 25 or 50 ug/kg of TCDD was given, although their ratio was not altered by TCDD. Urine flow rates following volume expansion were lower in TCDD-treated rats than in control rats. Fractional sodium excretion was affected minimally by TCDD treatment. The authors concluded that TCDD-induced impairment of renal function reflected the systemic toxicity of TCDD and not a specific effect on a particular aspect of renal function.

766. Peoples, S. A. (1975) Review of arsenical pesticides. In Arsenical Pesticides, ACS Symposium Series Vol. 7. [Washington, D.C.: American Chemical Society] pp. 1-12.

[Review article.]

767. Peoples, S. A., Maddy, K. T., Peifer, W. R., and Edmiston, S. (1979) Occupational exposures to pesticides containing organoarsenicals in California. Vet. Hum. Toxicol. 21(6):417-421.

The authors tabulated occupational accidents involving cacodylic acid, methanearsenic acid, and their salts which occurred in California from 1975 to 1977. At least 22 of the 34 incidents involved herbicides containing cacodylic acid or its sodium salt (or both). Nine of the 34 incidents involved systemic symptoms, including vomiting, diarrhea, and abdominal pain. Of the remaining cases, 13 involved eye irritation and 12 involved skin injuries, including contact dermatitis and allergic rash. All cases responded well to treatment. Most accidents resulted from equipment failure or lack of use of protective gear.

768. "Perspectives on Chlorinated Dibenzodioxins and Dibenzofurans." (1973) Lee, D. H. K.; Falk, H. L.; Dixon, R. L.; Fishbein, L. G. R.; Malling, H.; Moore, J. A.; Hoel, D.; Hart, L., eds. Environmental Health Perspectives Vol. 5. (Research Triangle Park, North Carolina: DHEW.) 313 pp.

[Review article.]

769. Peterson, J. (1978) Seveso: The event. Ambio. 7(5-6):232-233.

[Editorial.]

770. Peterson, R. E., Modhukar, B. V., Yang, K. H., and Matsumura, F. (1979) Depression of adenosine triphosphate activities in isolated liver surface membranes of 2,3,7,8-tetrachlorodibenzo-p-dioxin treated rats: Correlation with effects of ouabain biliary excretion and bile flow. J. Pharmacol. Exp. Ther. 210(2):275-282.

The effects of TCDD on liver surface membrane adenosine triphosphatase (ATPase) levels and on biliary excretion are described. Male Holtzman rats were administered a single oral dose of 10 or 25 ug/kg TCDD in acetone-corn oil (1:19). After 2-40 days, [³H]-ouabain was administered intravenously; bile and blood samples were collected over the next 60 minutes and biliary excretion of ouabain was calculated. In other animals, biliary excretion of [¹⁴C]-erythritol was determined. Liver surface membranes (LSM) were isolated from some TCDD-treated rats, examined by electron microscopy to confirm purity of the preparation and Na⁺, K⁺-ATPase and Mg⁺⁺-ATPase were both assayed. [³H]-TCDD was administered to a group of rats and the radioactivity associated with the isolated LSM fraction was determined. The effect of TCDD added to LSM preparations from control rats on ATPase activity was also reported. Both types of ATPase levels from TCDD-treated rats were depressed over the 40 day period, compared to vehicle controls and to pair-fed controls. Bile flow, bile salt excretion and ouabain and erythritol biliary excretions were all depressed by TCDD, with signs of recovery evident by the last time point. Blood clearance of ouabain was also decreased. Ten days after [³H]-TCDD treatment, the LSM fraction contained 0.0136 picomol TCDD per ug LSM protein. The equivalent of this concentration in vitro was 10⁻¹⁸ to 10⁻⁹ M; even concentrations of 10⁻³ M TCDD in vitro failed to alter ATPase activity. The authors concluded that the LSM was a site of TCDD action and the effect of TCDD on LSM ATPase activity and biliary excretion of ouabain correlated (but were not necessarily causally related).

771. Pfeiffer, E. W., and Orians, G. H. (1972) "Chapter 3: The Military Uses of Herbicides in Vietnam," In Harvest of Death. Neilands, J. B., ed., (Free Press) p. 117-176.

[Background material.]

772. The Phenoxy Herbicides - Second Edition. (1978) Council for Agricultural Science and Technology, Report No. 77. 28 pp.

[Review article.]

773. Pilinskaya, M. A. (1974) Cytogenetic effect of the herbicide 2,4-D on human and animal chromosomes. Tsitologiya i Genetika 8(3):202-206.

The author describes the cytogenetic effects of 2,4-D on human lymphocytes in vitro and in mouse bone marrow cells in vivo. Human lymphocyte cultures were established from two healthy donors with no known exposure to mutagens. 2,4-D was added to cultures at 0.002,

0.02, 0.2, 2.0, 20 or 50 ug/ml. Chromosome spreads were prepared according to the author's laboratory methods, which were unspecified. Metaphases (500 per dose level) were examined and scored for chromatid fragments and exchanges and chromosome fragments, dicentrics, exchanges, and rings. Results were analyzed by the X^2 method. No increase in chromosome damage was observed at the lowest dose of 2,4-D, 0.002 ug/ml. At 0.02 and 0.2 ug/ml chromosomal aberrations increased with increasing dose and were significantly different from the controls. At doses of 2.0 ug/ml, high cytotoxicity was observed. Chromosome damage did not increase above the levels observed at 0.2 ug/ml. The most frequent type of chromatid damage observed was single acentric fragments. Chromosome aberrations were mainly paired acentric fragments; 81.4% of the chromosome breaks occurred in the long arms. The authors concluded that 2,4-D was a potential genetic hazard to humans. The cytogenetic effects of 2,4-D in mice were also examined. Male outbred white mice (18-200g; 6 mice per dose level) received a single oral dose of 10, 50, 100 or 300 mg 2,4-D/kg body weight. Ten control mice were also included in the study. Mice were killed 20 hr after administration of the herbicide. Chromosome spreads were prepared and 200 metaphases per animal were scored. Increased chromosome aberrations were observed only at the toxic doses of 100 and 300 mg/kg. Single acentric fragments were the most common form of chromosome damage; paired fragments occurred much less frequently; and translocations were rare. From these results the author concluded that 2,4-D is a weak mutagen in mice.

774. Piper, W. N., Rose, J. Q., and Gehring, P. J. (1973b) Excretion and tissue distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat. Environ. Health Persp. 5:241-244.

The experiments published in this report were presented in a previous publication, see Piper, W.N., Rose, J.Q., and Gehring, P.J. (1973a).

775. Piper, W. N., Rose, J. Q., and Gehring, P. J. (1973a) Excretion and tissue distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat. Symposium on chlorodioxins, origin and fate, Washington, DC. Advances in Chemistry Series 120:85-91.

The levels of TCDD excreted and distributed in various rat tissues up to 7 days after administration was determined. Male Sprague-Dawley rats were administered 50 ug/kg [^{14}C]-TCDD (specific activity, 2.8 uCi/mg; 93-95% purity) in acetone-corn oil (1:9) by oral intubation. The CO_2 of expired air was trapped and urine and feces were collected; all samples were analyzed for radioactivity as well as tissue samples. About 30% of the dose was excreted in feces in 48 hours, 53% was excreted in feces in 21 days, 13% in urine and 3% in expired air. Beyond the first 2 days (after unabsorbed TCDD had been excreted in the feces), clearance of radioactivity from the body showed first order kinetics, with a half-life of 17 days. After 3, 7, and 21 days the liver contained 3.2, 4.5, and 1.3% of the dose of TCDD per gram and fat contained 2.6, 3.2, and 0.4%, respectively. Tissue levels were lower

for 12 other tissues reported at the same time periods. The carcass was analyzed for radioactivity (result was not reported) and total recovery was calculated as 96.8% of the dose. The authors concluded that some of the dose was not absorbed and the liver and fat are the primary sites for TCDD localization; some metabolism of TCDD occurred because $^{14}\text{CO}_2$ was recovered in expired air. The authors noted that the administered dose was toxic to the animals, and TCDD may have been excreted differently by animals in better physical condition.

776. Piper, W. N., Rose, J.Q., Gehring, P. J. (1971) Paper presented at the meeting of the American Chemical Society, Washington, DC.

[Not available.]

777. Piper, W. N., Rose, J. Q., Leng, M. L., and Gehring, P. J. (1973) The fate of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) following oral administration to rats and dogs. Toxicol. Appl. Pharmacol. 26(3):339-351.

The pharmacokinetics of 2,4,5-T plasma clearance and urinary excretion were studied in the rat and the dog. Single doses of 5 to 200 mg/kg [^{14}C]-2,4,5-T (98+% purity) in acetone-corn oil (1:9) were administered by intubation to Sprague-Dawley rats (6 per dosage group) and a 5 mg/kg dose was administered by the same procedure to 4 beagle dogs. Blood, urine, feces, and expired air were collected and analyzed for radioactivity. Plasma protein binding of [^{14}C]-2,4,5-T was determined in vitro as the amount of isotope that exchanged freely between plasma retained in dialysis tubing and the surrounding 2,4,5-T solution. Urinary metabolites were extracted and separated by thin-layer chromatography. Data for clearance of 2,4,5-T in the rat was simulated by a one-compartment model with apparent first-order rates of absorption and clearance. Rate constants and corresponding half-lives were from the computerized best-fit. The values that provided the closest simulation of the actual data when used in the equations derived from the kinetics model. The half-lives and volumes of distribution both increased with higher doses. Doses of 5 and 200 mg/kg resulted in half-lives of 4.7 and 25.2 hours, respectively, and 14.4 and 43.7 liters/kg, respectively. The rate of excretion decreased with higher doses and was comprised primarily of urinary excretion. The proportion of the dose excreted in feces increased at higher doses and less than 1% was recovered in expired air. Urinary metabolites were separated in urine from the highest doses groups and comprised up to 5% of the urinary radioactivity. Total recovery of radioactivity, (including radioactivity in the carcass) was 91.1%. The same kinetic pattern was seen for 2,4,5-T clearance in the dog although the half-life of 77 hours and volume of distribution were considerably larger than for the rat. After 9 days 42% of the dose was recovered from the urine and 20% from feces. (Cross-contamination of their samples was suspected). Three urinary metabolites accounted for 10% of the total urinary radioactivity, the remainder recovered as 2,4,5-T. No sex-related differences in results were observed. When 2,4,5-T was present at

concentrations of 0.04 to 425 mg/l; 89.7% of 2,4,5-T bound reversibly to plasma protein. The authors concluded that detoxification mechanisms were altered at high doses of 2,4,5-T, decreasing the rate of clearance and that the slower rate of 2,4,5-T excretion of dogs compared to rats could account for the higher toxicity in dogs.

778. Pirie, A., and Rees, J. R. (1970) Diquat cataract in the rat. Exp. Eye Res. 9(2):198-203.

The pathological changes associated with diquat-induced cataracts and the distribution of diquat to the eye are described for the rat. Wistar rats were fed a diet that contained 0.05 or 0.075% diquat dibromide for at least 1 year. The rats were killed and the eyes were removed and prepared for histology or analyzed for ascorbic acid, enzymes, and GSH levels (reducing groups). Four male rats were administered 0.5-1.0 mg of [¹⁴C]-diquat intraperitoneally and 1-3 hours later ocular tissues, intraperitoneal fluid, and blood were analyzed for radioactivity. Gross examination of treated rats within 4-8 months revealed the development of a thin sheet of opacity which formed under the posterior capsule of the lens and took on an irregular pattern. This stage was followed by formation of a clearly defined nuclear cataract and then shrinkage and complete opacity ensued. At the earliest stage a thin plaque of ribonuclease-sensitive, basophilic material was observed histologically. No consistent decrease in mitosis occurred in lens epithelia cells of treated rats. The concentration of [¹⁴C]-diquat remained below plasma concentrations 1-3 hours after treatment. Ascorbic acid levels in the ocular fluids decreased gradually with age and decreased more rapidly in diquat-treated rats. The decrease in ascorbic acid levels were evident in rats with posterior opacity only. In rats with severe cataracts, lens weight was increased, total protein was diminished, and GSH levels remained unaffected. The authors concluded that very little of the administered dose of diquat reached the lung, and, therefore, diquat probably acted catalytically to produce cataracts that had different properties than cataracts caused by other agents.

779. Pirie, A., Rees, J. R., and Holmberg, N. J. (1970) Diquat cataract: formation of the free radical and its reaction with constituents of the eye. Exper. Eye Res. 9(2):204-218.

Reactions of diquat with various components of the eye were studied in vitro and distribution of diquat to the eye was studied in the rabbit. Diquat was added to extracts of bovine lens, aqueous humor and vitreous humor and the effects of added chemicals and sources of light on the formation of diquat radicals was monitored as a decrease in ascorbate concentration in the incubation mixture. At 0.2 mM diquat concentration, catalysis of diquat radical formation in extracts of aqueous humor was accelerated in the presence of sunlight, was independent of metals, and produced hydrogen peroxide at a slower rate than the rate of ascorbate loss. In extracts of vitreous humor, in addition to ascorbate utilization hyaluronic acid depolymerization occurred in the

presence of 0.05 mM diquat. At 0.5 mM, diquat also caused a 33% reduction in ascorbic acid of rat lens in vitro. Dutch rabbits (2) were administered 30 mg/kg diquat dichloride intraperitoneally. Controls were given saline. One hour after treatment, aqueous and vitreous humors were removed, exposed to ultraviolet light, and analyzed for ascorbic acid. Ascorbic acid levels were from 10% to 75% of control levels. Reduction of glutathione by glutathione reductase in the presence of diquat in aqueous humor or bovine lens extracts was demonstrated by an increase in the production of reduced glutathione end product measured after exposure to sunlight. Diquat had no effect on reduced glutathione levels in the absence of ascorbic acid. The authors concluded that diquat radical formation could lead to the formation of cataracts and to an alteration in ascorbic acid levels without a change in reduced glutathione levels.

780. Pirie, A., Rees, J. R., Holmberg, N. J. (1969) Diquat cataract in the rat. Biochem. J. 114(4): 89 pp.

[Abstract, only.]

781. Pocchiari, F. 2,3,7-Tetrachlorodibenzo-para-dioxin decontamination. In Chlorinated Phenoxy Acids and Their Dioxins, C. Ramel, ed. (Ecol. Bull. No. 27, Stockholm: Swedish Natural Science Research Council, 1978a) pp. 67-70.

[Background material.]

782. Pocchiari, F., Silano, V., Zampieri, A. (1979) Human health effects from accidental release of tetrachlorodibenzo-p-dioxin (TCDD) at Seveso, Italy. Ann. N. Y. Acad. Sci. 320:311-320.

The areas southeast of a chemical plant in Meda, Italy, are categorized according to the degree of TCDD contamination in the soil, and a summary of results to date of a study of the health status of residents in this area is presented. An explosion at the Meda chemical plant that was synthesizing trichlorophenol resulted in TCDD contamination of the surrounding area. Zone A, with an area of 110 ha received about 2 kg of TCDD, and the 733 residents were evacuated 2 weeks after the explosion. Zone B, 270 ha, and zone R, 1,430 ha, each received 20 g of TCDD. The 4,800 residents of zone B and 22,000 in zone R were prohibited from cultivating or consuming local crops or raising poultry or other animals. At the time of publication, 50 cases of chloracne were detected in residents of zone A, two-thirds of them in children under 12 years old. In the other two zones, 0.5 percent to 1.2 percent of the residents exhibited skin lesions, compared to 0.3 percent observed in other towns in northern Italy. Neurological screenings in 1977 and 1978 revealed 7 percent and 12 percent respectively of the group from zone A with idiopathic clinical neural damage, compared to 1 percent of those from zones B and R identified in 1977. Subclinical damage, including reduced nerve conduction velocity, was seen in less than 5

percent of any group. Of approximately 200 factory workers from the Meda plant and another factory in zone A, 4 percent showed clinical damage in peripheral nerve fibers and 3 workers showed polyneuropathy of the legs. No decrease in immune response was detected in 45 children from zone A when laboratory tests performed triannually from 1976 were compared with those from a control group of 45 unexposed children of the same age. Of 34 aborted fetuses examined (including 4 spontaneous and 30 therapeutic abortions), none had any alterations that were attributed to TCDD exposures. The frequency of malformations in newborn infants in the contaminated areas matched the expected frequency. The difficulties in obtaining reliable reproductive data were discussed. Of a group of 1,654 subjects from all three zones who were examined, 32 percent had evidence of hepatomegaly. Transient rises were observed in the percentage of abnormal liver function test results in 1976. An increased incidence of aberrations in peripheral lymphocytes was observed in factory workers and zone A inhabitants, compared to control groups. An increased incidence of childhood infectious diseases was attributed to the increased medical surveillance. The authors chose to postpone their conclusions on the effects of TCDD on human health until more data from their ongoing studies could be compiled and reviewed.

783. Pohl, R. J., Philpot, R. M., and Fouts, J. R. (1976) Cytochrome P-450 content and mixed function oxidase activity in microsomes isolated from mouse skin. Drug Metab. Dispos. 4(5):442-450.

Induction of skin microsomal enzyme activities by dermally applied TCDD in the mouse is described. Swiss-Webster mice were pretreated with 0.3 (ug) of TCDD applied in acetone to about 30 cm² of shaved skin. Controls were treated with acetone only. Microsomes prepared from the skin and the liver were assayed for aryl hydrocarbon hydroxylase activity, 7-ethoxy-coumarin deethylase activity and cytochrome P-450 content. Hepatic enzyme levels were induced three-fold 24 hours after TCDD treatment and five-fold after 72 hours. Skin microsomal enzymes were induced four to eight-fold after 24 hours and six to 30-fold after 72 hours. The P-450 content of both tissues was elevated. A spectral shift of the peak wavelength to 447 nm occurred for TCDD-induced cytochrome P-450 from skin. The authors concluded that mixed function oxidase activity in the skin is potentially capable of detoxifying topically applied chemicals or metabolizing them locally to irritants.

784. Poiger, H., and Schlatter, Ch. (1979) Biological degradation of TCDD in rats. Nature 281:706-707.

Isolation of biliary metabolites of TCDD in the rat is described. Female Sprague-Dawley rats were orally administered 20 uCi of [³H]-TCDD (41 Ci per mmol; 98.7% purity of radiochemical), several days after their bile ducts were cannulated. Bile was collected for the subsequent 4 days and then the animals were killed and the livers were removed. Total radioactivity of the bile and liver were determined and the chemical nature of the biliary radioactivity was indentified by

dichloromethane extractability, dialyzability (exclusion limit or type of dialysis tubing was not stated), enzyme susceptibility, and removal by lyophilization and by thin layer and gas liquid chromatographies. About 1% of the dose was excreted daily in bile and 8.5-12% was recovered in liver. Two metabolites were isolated from bile, as water soluble conjugates. The authors concluded that TCDD was excreted in the bile in 2 distinct metabolic forms, which may have resulted from cleavage of the ether group.

785. Poland, A., Greenlee, W. F., and Kende, A. S. (1979) Studies on the mechanism of action of the chlorinated dibenzo-p-dioxins and related compounds. Ann. N. Y. Acad. Sci. 320:214-230.

[Review article.]

786. Poland, A. and Glover, E. 2,3,7,8-Tetrachlorodibenzo-para-dioxin and enzyme induction. In Chlorinated Phenoxy Acids and Their Dioxins, C. Ramel, ed. (Ecol. Bull. No 27, (Stokholm: Swedish Natural Science Research Council, 1978) p 145-148.

[Review article.]

787. Poland, A., and Glover, E. (1975) Genetic expression of aryl hydrocarbon hydroxylase by 2,3,7,8-tetrachlorodibenzo-p-dioxin: Evidence for a receptor mutation in genetically non-responsive mice. Mol. Pharmacol. 11:389-398.

The potency of TCDD in inducing hepatic aryl hydrocarbon hydroxylase (AHH) activity is described for 15 strains of mice. Female mice, 4-9 weeks of age, were administered TCDD in para-dioxane, intraperitoneally or methylcholanthrene in corn oil. 10-16 hours later, they were killed and hepatic microsomal AHH toxicity was determined. The dose of TCDD that produced half of the maximal level of induction was found to be about 1 nmole/kg for 3 strains of mice that also show an AHH-inductive response to MC and about 10 nmoles/kg for 3 strains that are non-responsive to MC. A single dose of 3 or 30 nmoles/kg TCDD or 0.3 m moles/kg MC were administered to 3 treatment groups for each of 14 strains of mice. For 7 strains, only the higher dose of TCDD induced AHH activity and for the remainder all 3 treatment groups showed induced AHH activities. Heterozygous offspring of a cross between a non-responsive strain and a responsive strain had sensitivity to TCDD that was intermediate between the 2 parental strains. The authors concluded that nonresponsive strains of mice were the consequence of a genetic mutation that produced an induction receptor with diminished affinity for the enzyme inducers, including TCDD and MC.

788. Poland, A., and Glover, E. (1974) Comparison of 2,3,7,8-tetrachlorodibenzo-p-dioxin, a potent inducer of aryl hydrocarbon hydroxylase, with 3-methyl-cholanthrene. Mol. Pharmacol. 10:349-359.

The potency, time-course and species specificity of TCDD induction of aryl hydrocarbon hydroxylase (AHH) was compared to these parameters for 3-methylchloranthrene induction of AHH. Male Sprague-Dawley rats were administered TCDD in paradioxane or methylcholanthrene (MC) in corn oil or sodium pentobarbital in saline, intraperitoneally. One day later, livers were removed and microsomes were isolated and assayed for AHH activity. At 0.22 ug/kg TCDD produced half-maximal induction and was estimated to be 28,640 times as potent as MC on a molar basis. Both inducers produced the same level of maximum stimulation and this level was not exceeded when the 2 compounds were tested in combination. Some rats were maintained for up to 35 days after an inducer had been administered and then AHH activity was assayed. MC-induced AHH activity and carbon monoxide-binding cytochrome peaked 4 days after treatment, then dropped sharply, whereas TCDD-induced AHH activity and CO-binding cytochrome remained elevated for 21 days, and showed a slight decrease by 35 days. Shifts in spectral maximas and changes in the ethyl isocyanide difference spectra induced by each agent followed the same time course as shown for AHH activity. Both agents induced AHH activity in rat kidney, intestine and lung, but not in the testicle. TCDD and MC produced 30-50% increases in aminopyrine N-demethylase and NADPH-cytochrome C reductase activities, compared to 4-5 fold increases induced by phenobarbital. Data on species differences in TCDD potency from other reports were discussed. The authors concluded that TCDD induction resembled MC induction in all respects studied except for a higher potency of TCDD; higher apparent affinity for an "induction receptor" and resistance to biotransformation were presented as possible explanation for TCDD's higher potency.

789. Poland, A., and Glover, E. (1973a) 2,3,7,8-Tetrachlorodibenzo-p-dioxin: A potent inducer of delta-aminolevulinic acid synthetase. Science 179:476-477.

TCDD was shown to inhibit 8-aminolevulinic acid (ALA) synthetase activity in chick embryos. At 4.7×10^{-2} moles per egg, TCDD injected into the air sac of eggs on day 17 of gestation, caused a 2-fold increase in ALA synthetase activity and at 1.6×10^{-2} mole/egg caused a 35-fold increase in activity 2 days after treatment. The TCDD effect was blocked by actinomycin D and by cycloheximide after 5 days, treated eggs maintained 70% of the maximally induced enzyme activity. Other dibenzo-para-dioxins which were reported (elsewhere) to be lethal and acnegenic at low doses also inhibited ALA synthetase. The ability of TCDD to inhibit ALA synthetase was considered relevant to its presumed ability to cause porphyria cutanea tarda in exposed workers. (This data is published in more detail in Poland and Glover, 1973b).

790. Poland, A., and Glover, E. (1973b) Studies on the mechanism of toxicity of the chlorinated dibenzo-para-dioxins. Environ. Health Perspec. 5:245-251.

The effect of TCDD on chick embryo and rat-aminolevulinic acid (ALA) synthetase and aryl hydrocarbon hydroxylase activities was studied. TCDD, dissolved in para-dioxane, was injected into chick embryos and after 8 hours, hepatic enzyme activities were assayed. At 4.7×10^{-12} mole per egg, TCDD caused a 2-fold increase in ALA synthetase activity and at 1.6×10^{-9} mole per egg, TCDD caused a 35-fold increase compared to activity of control eggs that were injected with para-dioxane only. Similarly at 1.6×10^{-12} moles per egg, TCDD doubled AHH activity, and maximal induction was produced by 1.6×10^{-10} mole per egg. Induction of AHH by TCDD was maximum 18 hours after treatment was initiated, and persisted for 5 days before declining. The inductive effects in both enzymes were produced by other dibenzo-para-dioxins with carbons at positions 2, 3, 7, and 8 substituted with halogens in 3 positions with the 4th position free. TCDD produced a 5 fold increase in rat liver AHH activity at 3.1×10^{-8} mole/kg and was 3×10^4 times more potent than 3-methylcholanthrene (3-MC) in its capacity to induce AHH activity. Although both TCDD and 3-MC showed the same level of maximum response, induction by TCDD persisted for over 1 month, while AHH activity of 3-MC treated rats returned to control values in 8 days. Spectral characteristics of the cytochrome P-450 enzyme system indicated that both TCDD and 3-MC induced formation of the same enzyme system. The authors discussed their results in terms of proposed mechanisms of toxicity of TCDD.

791. Poland, A., and Glover, E. (1980) 2,3,7,8-tetrachlorodibenzo-p-dioxin: Segregation of toxicity with the Ah locus. Molec. Pharmacol. 17:86-94.

The potency of TCDD in eliciting thymic atrophy and, in fetuses, cleft palate were determined in responsive, non-responsive and hybrid strains of mice. C57BL/6J, DBA/2J and hybrid mice of these 2 strains were administered a single intraperitoneal dose of TCDD. (Prior to TCDD treatment, hybrid mice were phenotyped by their zoxazolamine paralysis times after beta-naphthoflavone induction.) Five to six days after TCDD treatment, the animals were killed and the thymuses were removed and weighed. Aryl hydrocarbon hydroxylase (AHH) and 7-ethoxycoumarin-O-deethylase activities were assayed in the liver and thymus. [³H]-TCDD binding was determined in hepatic and thymic cytosol. Cleft palates and fetal deaths were determined on day 18 of gestation, in litters of mice that were administered a single subcutaneous dose of TCDD on day 10 of gestation. C57BL/6J mice showed 10 fold higher sensitivity to thymic involution, high incidence of cleft palate, high level of enzyme induction and a high level of specific cytosol binding TCDD, compared to the responses by DBA/2J mice. Hybrid mice showed intermediate responses and were correlated with their phenotype. The potencies of other compounds as enzyme inducers correlated with their potencies in producing thymic atrophy. Four of 5 strains of responsive mice with high affinity cytosol receptors showed high incidences of cleft palate,

after TCDD treatment while 5 non-responsive strains showed low incidences at the same dose. The authors concluded that TCDD binding to the cytosol receptor was a necessary step for toxic responses to be elicited by TCDD, because toxic responses segregated with the Ah locus, which determines the receptor.

792. Poland, A.; Glover, E., and Kende, A. S. (1976) Stereospecific, high affinity binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin by hepatic cytosol. J. Biol. Chem. 251(16):4936-4946.

The characteristics of a cytosol "receptor" species from mouse liver that binds TCDD is described for methyl cholanthrene (MC) responsive and non-responsive strains of mice. C57BL/6J mice and DBA/2J mice were administered a single intraperitoneal dose of [¹⁴C]-TCDD and killed from 3 hours to 14 days later. Livers were removed and fractionated into cytosol, nuclear, mitochondrial and microsomal fractions. Radioactivity associated with each fraction was determined. The extent of high-affinity binding was determined in the cytosol fraction by subtracting the total cytosol-bound radioactivity and the radioactivity bound nonspecifically to low affinity sites (i.e. the amount of radioactivity that was not displaced from the binding site when a 200-fold excess of 2,3,7,8-tetrachlorodibenzofuran was added to the sample). Hepatic uptake of [¹⁴C]-TCDD, expressed as a percentage of the total dose, was about 2-3 times higher in MC-responsive (C57BL/6J) mice than in nonresponsive mice; hepatic uptake of radioactivity remained elevated for 14 days in both species, and uptake increased in DBA mice at higher doses of TCDD and decreased in C57BL mice at higher TCDD doses. Hepatic uptake of radioactivity for 2 hybrid strains of mice from crosses of DBA mice with 2 responsive strains) were intermediate between the parental values. Each of the 4 subcellular fractions retained 20-40% of the total hepatic radioactivity and only the level in the supernatant fraction was reduced in mice that were pretreated with a large excess of TCDD. A small pool of high-affinity binding sites were identified in C57BL mouse cytosol; an equilibrium dissociation constant of 0.27 nM and a total of 8.4 pmol of binding sites per g liver were calculated for this pool. The high-affinity binding pool was absent in DBA cytosol. The binding affinities of various structural analogues of TCDD and other arylhydrocarbon hydroxylase inducers for the high-affinity receptor (in C57BL mice) correlated highly with their potencies in inducing hydroxylase activity in vivo. About 60% of the high specific binding of TCDD to cytosol was lost in the presence of trypsin while no loss occurred when RNase and DNase were tested. The authors concluded that a single locus codes for the induction reception protein that has a high affinity for TCDD and activates AHH induction as a consequence of TCDD binding; nonresponsive mice have an altered receptor with lowered affinity for inducers.

793. Poland, A. P., Glover, E., Robinson, J. R., and Nebert, D. W. (1974) Genetic expression of aryl hydrocarbon hydroxylase activity. J. Biol. Chem. 249(17):5599-5606.

Microsomal enzyme induction by TCDD was studied in several inbred strains of mice, including strains that are responsive to 3-methylcholanthrene (3MC) enzyme induction and strains that are non-responsive to 3MC. Responsive strains included C57BL/6N, C57BL/6J, C3H/HeN, BALB/cAnN and CBA/HN strains and the nonresponsive strains studied were DBA/ZN, DBA/2J, AKR/N, NZW/BLN, and NZB/BLN strains. Mice were administered 40 ug/kg TCDD in dioxane intraperitoneally or 4 ug (in 4 applications over 2 days) dermally. Enzyme activities were assayed in microsomal preparations 48 hours after TCDD administration. Benzo(a)pyrene hydroxylase, 7-ethoxycoumarin Ortho-deethylase, para-nitroanisole O-demethylase, and 3 methyl-4-methyl aminoazobenzene N-demethylase activities were assayed. The total cytochrome P-450 content of microsomes was determined spectrophotometrically from the carbon monoxide and ethyl isocyanide difference spectra. TCDD (ip) induced hydroxylase activity to the same extent in 3MC-responsive and 3MC-non-responsive mice. Microsomal enzymes from the liver, bowel, lung, and kidney responded to TCDD treatment. Dermal application of TCDD induced hydroxylase activity in the skin and liver of both responsive and non-responsive strains. Formation of new hepatic cytochrome P-450 was demonstrated in non-responsive mice after TCDD administration spectrophotometrically and by changes in the EPR spectra at low temperatures. TCDD-induced enzymes were sensitive to alpha-naphthoflavone inhibition but not to metyrapone inhibition. Spectral changes characteristic of induction of new cytochrome P-450 also occurred in lung, bowel, kidney, and skin microsomal preparation from 3MC-non-responsive mice after TCDD treatment. The authors concluded that non-responsive mice probably do not recognize most polycyclic hydrocarbon inducers (i.e. 3MC) while they possess the regulatory and structure genes required for new cytochrome P-450 formation. They proposed that defect in inducer recognition involved a mutation yielding a defective receptor with diminished ability to bind inducers. TCDD was proposed to interact with the same receptor that other inducers bind. These conclusions are well founded, based on the experiments reported here, which represent a thorough consideration of induction of various enzymes in various tissues and strains of mouse in which appropriate and specific technical assays were applied.

794. Poland, A., and Kende, A. (1976) 2,3,7,8-Tetrachlorodibenzo-p-dioxin: Environmental contaminant and molecular probe. Federation Proceedings. 35(12):2404-2411.

[Review article.]

795. Poland, A. P., Smith, D., Metter, G., and Possick, P. (1971) A health survey of workers in a 2,4-D and 2,4,5-T plant. With special attention to chloracne, porphyria cutanea tarda, and psychologic parameters. Arch. Environ. Health 22(3):316-327.

Results are reported of physical examinations of 73 male employees in a factory that manufactures 2,4-D and 2,4,5-T. Some of the workers in the factory had been included in a study conducted 6 years previously (Bleiberg et al. 1964). Medical histories were obtained and physical examinations were performed, with special emphasis on neurologic and dermatologic conditions. Each worker also responded to the Minnesota Multiphasic Personality Inventory (MMPI). [Routine automated blood analyses were performed on serum samples from workers, and urine was analyzed for porphyrins and porphyrin precursors.] Acne was present in 66 percent of the workers and was considered severe in 18 percent. The presence of scarring, hyperpigmentation, hirsutism, and complaints of eye irritation each correlated with the severity of acne, while the length of employment or the employee's location within the plant was not related to severity. Only three workers had conjunctivitis, although hyperemia of the buccal and nasal mucosa was present in 11 percent and 32 percent, respectively, of the employees. No cases of porphyria cutanea tarda were identified. Maintenance men had statistically higher coproporphyrin excretion levels than workers in administrative positions, but both sets of values were within the normal range. No hyperferremia was observed. Blood analyses revealed minimal liver dysfunction. About 30 percent of the employees had gastrointestinal complaints. The employees had the expected frequencies of abnormal conditions related to the cardiovascular, pulmonary, metabolic, and hematologic systems. The MMPI correlated only hypomania with severity of chloracne. The authors concluded that uroporphyrinuria identified in workers in a previous study and chloracne can occur independently and have different clinical courses, even though they may potentially have a common etiology. The etiology of both conditions was not known, but the authors suggested dichlorophenol, a precursor of 2,4-D as the etiologic agent of porphyria cutanea tarda, and TCDD as a potentially acnegenic compound.

796. Possible Long-Range Adverse Health Effects on Individuals that were Exposed to The Herbicide Agent Orange. Letter to Ralph H. Metcalf, U.S. House of Representatives. (1978) U.S. General Accounting Office, Washington, DC, 20 pp.

[Editorial.]

797. Pratt, I. S., Keeling, P. L., and Smith, L. L. (1980) The effect of high concentrations of oxygen on paraquat and diquat toxicity in rats. Arch. Toxicol. [Suppl] 4:415-418.

The effects of diquat on mortality and lung pathology were evaluated in the rat in an atmosphere of 85% oxygen. Wistar rats were administered 10 or 20 mg/kg diquat subcutaneously and then placed in air or in an

exposure chamber with 85% oxygen. Deaths were recorded daily and some rats were killed between 2 and 24 hours after dosing and lung tissue was examined by electron microscopy. After the higher dose was administered, 50% of the rats exposed to air died, with a mean time to death of 48 hours. Slight damage of type I, II, and epithelial cells was observed. No rats given the lower dose and exposed to air died and no lung damage was observed. All of the rats given the higher dose and exposed to high oxygen died, with a mean time to death of 17 hours. Severe lung edema and moderate damage to type II cells occurred. Half of the rats given the lower dose and high oxygen died (in an average of 41 hours), but no tissue damage occurred. Rats exposed to 85% oxygen alone showed no tissue damage or mortality. Paraquat toxicity was potentiated 10 fold by high oxygen, compared to the 2 fold increase in diquat toxicity. The authors proposed the hypothesis that bipyridyls exert their toxicity by generating superoxide anions and suggested that this anion generation is likely to be facilitated by high oxygen concentrations. They did not indicate whether their results supported this hypothesis.

798. A preliminary assessment of herbicides and defoliation. (1968)
Environ. Sci. Technol. 2(3):176-181.

[Review article.]

799. Preliminary dioxin implementation plan. U.S. Environmental Protection Agency - Office of Pesticide Programs, Criteria and Evaluation Division, Washington, DC, 18 pp.

[Background material.]

800. Prescott, L. F., Park, J, and Darrien, I. (1979) Treatment of severe 2,4-D and mecoprop intoxication with alkaline diuresis. Br. J. Clin. Pharmac. 7:111-116.

A case involving intentional ingestion of a weedkiller comprised of 10% 2,4-D and 20% 4-chloro-2-methylphenoxypropionic acid (mecoprop) is described. The ingestion induced vomiting. The patient became aggressive, then confused, and finally lost consciousness for 4 days. The patient remained confused for 4 days after he regained consciousness. Pyrexia and hyperventilation both responded to alkaline diuresis, which was initiated 42 hours after the ingestion. Myotonia persisted for several days after the patient regained consciousness. Plasma enzyme levels indicated severe muscle injury and electromyography, which was performed 6 days after the ingestion, indicated mild hypopathy. Weakness of the legs was noted 2 months after the incident. The plasma concentration was 400 ug/ml for 2,4-D and remained constant until the pH of the urine rose above pH7 after initiating alkaline diuretic therapy. The clearance half-life then fell to 3.7 h. Consciousness was regained when the blood levels of 2,4-D and mecoprop each were 100 ug/ml.

801. Pritchard, J. B. (1980) Accumulation of anionic pesticides by rabbit choroid plexus in vitro. J. Pharmacol. Exper. Therap. 212(2):354-359.

Uptake of 2,4-D by rabbit choroid plexus and the effects of 2,4-D on uptake of other organic ions, including neurotransmitter metabolites were studied in the rabbit. Lateral plexi from male New Zealand rabbits were excised and incubated in medium that contained the ¹⁴C-labeled anion to be tested. After incubation, radioactivity in the tissue and medium were counted. The tissue-to-medium ratio of radioactivity was 40-60 at 2,4-D concentrations up to 1μM. Above this 2,4-D concentration, the transport mechanism was saturated. Uptake was inhibited by 90% when oxygen was replaced by nitrogen or by 1mM cyanide. DDA (a polar DDT metabolite) and chlorophenol red each produced dose-related inhibition of 2,4-D uptake which could be partially overcome with higher doses of 2,4-D. Likewise, 2,4-D produced a dose-dependent inhibition in DDA uptake and in uptake of the serotonin metabolite, 5-hydroxy-3-indoleacetic acid. The authors concluded that 2,4-D are actively transported into the choroid plexus by the organic anion system and then may also be bound within the plexus; 2,4-D accumulation in the plexus was thought to be similar to transport by the kidney and by the liver, described by other investigators and a potential for neurotoxicity of 2,4-D was suggested.

802. Pushkar, M. S. (1969) Morphological changes in the organism under the effect of the herbicide reglone (diquat). Vrach. Delo. 9:92-96.

[Foreign language.]

803. Radosevich, S. R., and Winterlin, W. L. (1977) Persistence of 2,4-D and 2,4,5-T in chaparral vegetation and soil. Weed Sci. 25(5):423-425.

The authors studied the distribution, persistence, and vertical movement of 2,4-D and 2,4,5-T in soil after herbicide application to chaparral. Four replicate plots (186 m²) were sprayed with 4.5 kg/ha 2,4-D (butoxy propyl ester) or 2,4,5-T (propylene glycol butyl ester). Samples of soil surface litter and of the terminal 10-15 cm of chamise foliage, understory grass, and forbs were collected before and immediately after spraying and 30, 60, 90, 180, and 360 days after herbicide application. Soil samples were collected at the same times after herbicide application at 0-5, 10-15, 25-30, and 55-60 cm depths. Determination of 2,4-D and 2,4,5-T in the samples collected was by gas-liquid chromatography. After application, soil surface litter contained over 50% of the 2,4-D or 2,4,5-T applied, followed by understory grass and forbs (25.2% 2,4-D; 31.2% 2,4,5-T), and chamise (20.7% 2,4-D; 18% 2,4,5-T). Residues of both herbicides decreased up to 93% on foliage and litter and within 30 days. In the soil, residues of only 0.1% 2,4-D and 0.07% 2,4,5-T were detected immediately after application. No detectable herbicide was found below 5 cm. Soil residues of both herbicides remained constant for 90 days after application. However, by day 180 for 2,4-D and 360 for 2,4,5-T, only 0.01% of the original application could be detected in the soil. This experiment took place during the dry season, therefore, the effects of surface runoff and percolation through the soil could not be measured. The authors also studied the loss of herbicide from paper disks in a greenhouse bioassay under conditions similar to the chaparral. In seven days, nearly all the 2,4-D and 2,4,5-T had disappeared. The authors felt that either volatilization or decomposition was responsible for herbicide disappearance. From the work of other scientists, they eliminated photodecomposition as a possibility and concluded that volatilization was responsible for rapid herbicide disappearance. In addition, the authors demonstrated that 2,4-D and 2,4,5-T does not accumulate in soils and vegetation and is an unlikely contaminant of water supplies.

804. Raju, K. S., and Rangaswami, G. (1972) Studies on microbial degradation of herbicides in soil. Biochem. J. 128(1):40.

[Abstract, only.]

805. Ramel, G. Genetic effects of phenoxyacetic acids in animals. In Chlorinated Phenoxy Acids and Their Dioxins, C. Ramel, ed. (Ecol. Bull. No. 27, Stockholm: Swedish Natural Science Research Council, 1978a) p. 182-185.

[Review article.]

806. Ramel, C. Editor's preface, Conclusions and recommendations, Chemistry and Summary. In Chlorinated Phenoxy Acid and Their Dioxins, C. Ramel, ed. (Ecol. Bull No. 27, Stockholm: Swedish Natural Science Research Council, 1978b) p. 9-27.

[Review article.]

807. Ramsey, J. C., Hefner, J. G., Karbowski, R. J., et al. (1979) The in vivo biotransformation of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the rat. Toxicol. Appl. Pharmacol. 48(1)Pt. 2:A162.

808. Ramsey, J. C., Lavy, T. L., and Braun, W. H. Exposure of forest workers to 2,4,5-T: calculated dose levels. Report from the toxicology laboratory, Dow Chemical Company, Midland, Mich.

Pharmacokinetic analysis of data for 2,4,5-T urine levels of workers is described. Exposure conditions and urinary 2,4,5-T data were described in another report (see Lavy, T.L., 1978 report to the National Forest Products Association). From previous animal and human studies, the excretion of 2,4,5-T was established as a first order process that is independent of the route of administration; a 1 compartment pharmacokinetic model which incorporated these characteristics was used to estimate absorption levels by 3 methods. For 12 of the 41 exposures, the absorbed doses and rate constants were estimated as the values providing the best fit of the urinary excretion data by the kinetic model using the human excretion rate from a previous study. The second method involved mathematically estimating the proportion of the initial dose that was excreted on 7 successive days from the established rate constants; cumulative urine levels were then divided by this proportion to estimate absorbed doses for 39 of the 41 exposures. The third method, applied to all 41 exposures, used an equation that estimated exposures from data for urinary excretion over a limited interval, independent of the total quantity of urine collected. All 3 methods yielded similar results, with maximum exposure of 0.132 mg/kg and 0.156 mg/kg for a backsprayer and mixer, respectively, and lowest exposure of 0.001 mg/kg for helicopter flagmen. The average dose for all workers was 0.063 mg/kg. The authors estimated body burdens from their model. The authors concluded that the close fit of observed and calculated urinary 2,4,5-T values attested to the validity of the proposed kinetic model and that method 3 was the method of choice. Worker exposure levels were concluded to be far below the no-effect level of 20 mg/kg (for teratogenic and fetotoxic effects) put forth by the EPA, indicating that 2,4,5-T exposure presents a negligible toxic hazard to workers.

809. Ranish, N. A., Dettbarn, W. D., and Iyer, V. (1977) The influence of nerve stump length on (2,4-dichlorophenoxy)acetic acid-induced myotonia. Exp. Neurol. 54:393-396.

The influence of nerve stump length on 2,4-D induced myotonia in the rat was investigated. Adult rats were subjected to unilateral sciatic

nerve transections. Denervation was performed to either the level of the spinal cord or at the distal end of the nerves. This resulted in nerve stumps of two lengths in the different rats. 2,4-D (200 mg/kg) was administered intraperitoneally at 6 to 8 hour intervals and the myotonia evaluated 2 hours after each administration. After the first 48 hours, the 2,4-D was given daily for 10 to 12 days. Myotonia was measured by electromyographic examination of the gastrocnemius muscle. After 12 days of examination the animals were sacrificed and the nerve stump lengths measured. The times of appearance of recorded changes in myotonia, the loss of myotonic activity and the onset of fibrillation were measured and compared to the length of the nerve stump. Longer time intervals between denervation and these myotonic parameters were associated with longer nerve stump lengths. The muscle retained its capacity to become myotonic, and the length of this period was found to be related to the nerve stump length. By one week after denervation, however, the muscle was no longer able to become myotonic in response to 2,4-D. The authors compared their results to results of other experiments that induced myotonia with 20, 25-diazacholesterol. The authors made no attempt to relate their study with studies of others that have induced myotonia with 2,4-D. The experiment lacked control animals and the purity and preparation of the 2,4-D compound was not described.

810. Rao, K. S., and Dad, N. K. (1979) Studies of herbicide toxicity in some freshwater fishes and ectoprocta. J. Fish Biol. 14:517-522.

The authors studied the acute toxicity of 2,4-D to ectoprocta and dalapon to fish. According to the authors, dalapon toxicity was measured in Heteropneutes fossilis, Saratherodan mossambicus, and Puntius ticto caught locally in India. Concentrations of dalapon tested and results of the acute toxicity test were not presented. The authors reported that fish survived for 8 days in concentrations ranging from 1-2500 mg/l. 2,4-D butyl ester was tested in three species of ectoprocta, Plumatella casmiana, Lophopodella carteri, and Hyalinella punctata, at 3 and 4 mg/l. No toxicity was observed in L. carteri or H. punctata 3 mg/l for up to 84 hours. P. casmiana was more sensitive to 3 mg/l 2,4-D; 50 percent of the organisms were dead at 72 hours. Sluggishness was observed as early as 24 hours. In all three species, 4.0 mg/l 2,4-D produced toxicity; all P. casmiana and H. punctata were dead at 48 hours while all L. carteri were dead at 36 hours. In addition germination of P. casmiana leptoblasts was not affected by 0.01 - 1.5 mg/l 2,4-D.

811. Rappe, C. (1979) Dioxiner och dibensofuraner tva substansgrupper i blickpunkten. Lakartidningen. 76:(1-2):21-24.

[Not available.]

812. Rasmuson, B., and Svahlin, H. Mutagenicity tests of 2,4-dichlorophenoxyacetic acid and 2,4,5,-trichlorophenoxyacetic acid in genetically stable and unstable strains of Drosophila melanogaster. In Chlorinated Phenoxy Acids and Their Dioxins, C. Ramel, ed. (Ecol. Bull. No. 27, Stockholm: Swedish Natural Science Research Council, 1978) p. 190-192.

The author tested 2,4-D and 2,4,5-T in a mutation assay in Drosophila melanogaster. The test involves use of a sex-linked genetically unstable system in Drosophila. The instability is caused by the insertion of foreign DNA into the regulatory part of the structural gene of the white locus. Mutations induced within the regulatory part of the gene influence control of the white locus somatically which results in different pigment synthesis. Mutagenic frequency is measured by the number of hatched males with pigmented eye sectors after larval exposure to a mutagen. In addition the authors also used a genetically stable strain to compare strain differences in mutation frequencies. Larvae of each strain were kept until eclosion on food containing 25 ppm 2,4-D or 100 ppm 2,4,5-T. Ethylmethane sulfonate (500 ppm) was used as a positive control. 2,4-D induced an increased mutation frequency in the unstable strain, but not in the stable strain. 2,4,5-T did not cause increases in mutation frequencies in either strain. These results differ from recessive lethal studies in which both 2,4-D and 2,4,5-T were mutagenic.

813. Rawls, R. L. (1980) Reproductive hazards in the workplace. Part I. Chem. Eng. News 58(6):28-31.

[Editorial.]

814. Rawls, R. L. (1979) DOW finds support, doubt for dioxin ideas. Chem. Eng. News 57(7):23-29.

[Not available.]

815. Rawls, R. L., and O'Sullivan, D. A. (1976) Italy seeks answers following toxic release. Chem. Eng. News Aug 23:27-28,33-35.

[Review article.]

816. Recommended Classification of Pesticides by Hazard. (1975) WHO Chronicle 29:397-401.

[Review article.]

817. Reggiani, G. (1980) Acute human exposure to TCDD in Seveso, Italy. J. Toxicol. Environ. Health 6:27-43.

The health status of 17 people who were exposed to TCDD from a factory explosion in Seveso, Italy, in 1976 were reported. The subjects who were examined had been referred to the judge who held the inquest on the accident and included 13 children below 15 years of age. The environmental levels of TCDD in the vicinity of the factory were reported, and the tissue levels in one woman from the contaminated area who died 6 months after the accident were reported. Clinical and neurological examinations, which included electroencephalography, electromyography, and nerve conduction velocity measurements, were performed. Circulating complement, serum immunoglobulin levels, and lymphocytic response to nonspecific mitogens were determined. Chromosomal aberrations were analyzed, and laboratory tests were performed to assess hepatic function, metabolism of protein, lipids, and carbohydrates, porphyrin excretion, hematology, and urine content. Burnlike lesions of the skin developed in almost all cases. Within 3-7 days after the accident, exposed areas of the skin showed signs of acute inflammation, which subsided in 2-3 months. Hyperkeratosis, vermicular atrophy, and vesicles and pustules occurred in some cases. Chloracne developed in 12 cases, usually 2 weeks to 2 months after exposure, and involved the formation of blackheads and cysts on the face and in some cases also on the neck, shoulders, chest, lower trunk, and limbs. The duration of chloracne was 8 to 26 months, and residual scars remained on the faces of two of the most severely affected and youngest patients. None of the cases was classified as severe by the grading system recommended by an expert panel for evaluating the severity of chloracne. The only other changes identified in the patients were gastrointestinal disturbances within a few days of the onset of skin lesions and transient increases in serum enzyme levels, which were not considered severe enough to indicate hepatic injury. The authors concluded that visceral lesions had not occurred in the subjects of this study and attributed acute dermatitis to chemicals other than TCDD, which were released during the accident. Varied exposure to TCDD and varied individual susceptibility were presented as possible reasons why some people developed dermatitis only, and others developed chloracne, as well.

818. Reggiani, G. (1979) Estimation of the TCDD toxic potential in the light of the Seveso accident. Arch. Toxicol. 2:291-302.

The results of a health survey of residents near an Italian factory that released TCDD are described. Chloracne observed in periodic screening of thousands of residents during a two-year period after the accident showed a trend to healing with few new cases reported. The expected incidence of adolescent acne is 0.1-0.4 percent; the observed incidence of chloracne was 0.6-1.2 percent. Clinical chemistry test results revealed no increase in hematologic or renal problems. Some abnormal results of hepatic function tests were reported briefly, as well as abnormal porphyrin patterns in an undisclosed proportion of examined people. Hepatomegaly was found in 8 percent of the factory

workers. No adverse health effects were reported in a group of Swiss workers involved in the decontamination of the most contaminated area (zone A). The only neurologic effects attributed to TCDD contamination were subclinical, involving a reduction in sensory and motor conduction velocity of l-2 nerves. The incidences of abortions and of congenital malformations were considered to be below the expected values. No fetal malformations in 34 aborted fetuses (including 4 spontaneous abortions) examined were attributed to the action of exogenous agents. A "representative group" of children born after the accident was examined, and no developmental abnormalities have been observed. Cytologic tests revealed no increase in chromosomal aberrations in blood cells from exposed individuals nor a decrease in immune capability in children. Tissue samples of a woman from Zone A who died of carcinoma of the pancreas 7 months after the accident were analyzed for TCDD. A total body burden of 40 (u)g was estimated, with tissue levels from 0.04 ppb TCDD in the kidney to 1.84 ppb in the fat. The author concluded that humans have a higher tolerance to TCDD than would be expected from animal data. The significance of these findings cannot be evaluated, because the data do not include details on the methods used to evaluate each health parameter, or the specific groups studied, or the controls.

819. Reggiani, G. (1979) Abstracts: Eighteenth annual meeting; 361. TCDD contamination in Itlay: The risk assessment of low level exposure, cumulative effect and long term consequences. Toxicology and Applied Pharmacology 48(1):A180.

[Abstract, only.]

820. Reggiani, G. (1978) Medical problems raised by the TCDD contamination. Arch. Toxicol. 40:161-188.

[Review article.]

821. Rehder, H., Sanchioni, F., Cefes, F., and Gropp, A. (1978) Pathological-embryological investigations in cases of abortion related to the Seveso accident. J. Swiss Med. 108(42):1617-1625.

Results are presented of examinations of 34 aborted fetuses from mothers who were exposed to TCDD from the industrial accident in Seveso, Italy in July, 1976. An estimated 150 women in the affected area were in their first trimester of pregnancy when the accident occurred. A total of 125 women in the affected and bordering areas had decided by October, 1976, to terminate their pregnancies and 30 cases were approved. Fetuses from these elected abortions and from 4 spontaneous abortions that occurred concurrently, in this region were examined. Of the group of mothers who elected abortions, 3 were from zone A of highest contamination, 5 from zone B of moderate contamination and 13 from zone R of low contamination; 7 of the remaining mothers resided outside of these zones, but worked, travelled, visited

or consumed food from the contaminated zones and 2 abortions were elected for psychological reasons. Fetuses ranged from a few millimeters (5 weeks, estimated gestational age) to 17.5 cm from crown to heel (20 weeks of age). Most fetuses were 10-12 weeks of age. Twenty-five fetuses, between 5 and 12 weeks of age, were removed by instrument curettage and many were mutilated, some with only a few organs remaining for examination. Five fetuses, 13-17.5 cm in length, were removed after prostaglandins were administered, sometimes by extraction which also damaged the fetal skin and sometimes internal organs, as well. The 4 spontaneous abortions occurred in zone A and R in week 11-24 of pregnancy; 3 fetuses were 11-19 cm in length, the fourth was resorbed. Fetal organs were weighed, fixed in Bouin's fluid, and examined by light microscopy. In 23 of the fetuses, no abnormalities in organ size, structures or developmental stage (compared to fetal age) were observed. One of these mothers showed mild symptoms of chloracne. Six fetuses showed changes from normal development and included a secondary obliterated duodenum (a potentially temporary condition), a fetus with a subepidermal cyst and a defective ventricle septum (considered an artifact from manipulation), 2 cases of prolapse of the retina and 1 of displaced liver and intestine (all considered to be artifacts), and 1 case of slightly retarded renal and tracheal development. The remaining 5 fetuses, including 4 from spontaneous abortions and 1 which showed signs of intrauterine death prior to the elected abortion, were described in detail and were attributed to heterogeneous mixture of alleged reasons for fetal death or abortion. The authors concluded that no evidence of a fetotoxic or teratogenic effect of human exposure to TCDD was documented by their results, although the study was limited by incomplete fetal material and limited examination techniques.

822. Rehwoldt, R. E., Kelley, E., and Mahoney, M. (1977) Investigations into the acute toxicity and some chronic effects of selected herbicides and pesticides on several fresh water fish species. Bull. Environ. Contam. Toxicol. 18(3):361-365.

The authors briefly describe the acute and chronic toxicity of 2,4-D and 2,4,5-T in seven species of fish. In the acute toxicity experiments, striped bass, banded killyfish, pumpkinseeds, white perch, American eels, carp, and guppies were exposed to unspecified concentrations of the two herbicides for 24, 48, and 96 hours. Mean threshold limit values (TL) were then calculated. At 24 hours, TL values for 2,4,5-T varied from 27.3-73.2 mg/l depending on species. 2,4-D was less toxic than 2,4,5-T. TL values for 2,4-D at 24 hours varied from 55.5 - 427.2 mg/l depending on species. Ages and sizes of fish used in the experiment were not reported. In a long term 10 month experiment fish were exposed to aquaria concentrations of 0.1 ppm 2,4-D or 2,4,5-T. Numbers and kinds of fish used were not specified by the authors. No toxic effects were seen in exposed fish after 10 months. However, no histological examinations were performed. In a breeding experiment using 4 female and an unspecified number of male guppies, an unspecified concentration of 2,4-D or 2,4,5-T had no effect on offspring compared to controls. However, the lack of details presented in

this paper prevents any form of complete evaluation. From the data, it appears that 2,4-D is less toxic than 2,4,5-T and species sensitivity to the herbicides seems to vary.

823. Renner, H. W. (1979) Monitoring of genetic environmental risk with new mutagenicity tests. Ecol. Environ. Safety 3:122-125.

[Not available.]

824. Report on 2,4,5-T - A Report of the Panel on Herbicides of the President's Science Advisory Committee. By Dr. Colin M. MacLeod, Chairman. Washington, DC: Government Printing Office. (1971) 68 pp.

[Background material.]

825. Riimaki, V., Asp., S., Seppalainen, A. M., and Heinberg, S. (1978) Symptomology, morbidity, and mortality experience of chlorinated phenoxy herbicide (2,4-D and 2,4,5-T) sprayers in Finland. A clinical and epidemiological study. Working paper for an IARC working group meeting on "Coordination of Epidemiological Studies on the Long Term Hazards of Chlorinated Dibenzodioxins and Chlorinated Dibenzofurans." Lyon, France, 10-11 January.

[Not available.]

826. Rip, J. W., and Cherry, J. H. (1976) Liver enlargement induced by the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). J. Agric. Food Chem. 24(2):245-250.

The effects of 2,4,5-T on rat liver weight, hepatic RNA and DNA syntheses, and enzyme induction were examined. One month old male Long-Evans rats were administered 10 mg of 2,4,5-T (less than 0.05 ppm TCDD contaminant) in 6 g of feed, daily for 1-11 days and were killed 18 hours after the final feeding or were fed the 2,4,5-T diet for 6 days and then a normal diet for 8 days before being killed. Liver, kidney, spleen, and body weights were recorded. Liver homogenates were fractionated into subcellular components and assayed for nucleic acids and protein. Hepatic glucose-6-phosphatase and acid phosphatase activities were assayed and the 2,4,5-T content of the liver was determined. Rats fed 2,4,5-T for 7 days showed a 28% increase in both wet and dry liver weights, but no changes in other organ wet or dry weights or body weight, compared to controls. The increase in absolute and in relative liver weight was 20% after 2 days and 150% after 6-7 days in another experiment. Total DNA content of the liver remained constant for 11 days of treatment and DNA per gram of liver decreased. RNA content per liver increased up to 20% in 2 days and by 26% at 6 days. Protein content per liver increased by 50% with 8 days of treatment and protein per gram of tissue remained constant. The increases in protein and RNA were comparable for each subcellular liver

fraction and the crude homogenate. Liver weights were normal in rats fed a normal diet after treatment. Moderate inhibitions of both enzymes occurred. The ultraviolet absorption spectrum of 2,4,5-T extracted from treated rat livers showed the same profile as the 2,4,5-T standard and only 1.5% of the administered dose of 2,4,5-T remained in the liver after 24 hours. The authors concluded that the 2,4,5-T induced liver enlargement was caused by cellular hypertrophy, with minimal enlargement from herbicide hepatotoxicity and the increase in protein synthesis did not involve induction of 2,4,5-T metabolizing enzymes.

827. Roan, C. C., and Morgan, D. P. (1972) Alleged effects on human health of the use of herbicides in the area around Globe, Arizona. Arizona Community Pesticide Studies Project, 6 March.

The authors reported on the analysis of human body fluids and tissues and water samples for 2,4-D, 2,4,5-T, and TCDD in 1969 and 1970. Four urine samples, three serum samples, and three adipose tissue samples were negative for all three compounds (detection limit 2 ppb, method of analysis not specified). From these few samples, the authors concluded that the general population in the Globe, Ariz., area did not have any chronic exposure to 2,4-D, 2,4,5-T, and TCDD. Twenty-two water samples over a one-year period were also collected and analyzed by gas chromatography (detection limit 0.01 ppb). All samples were negative for 2,4-D, 2,4,5-T, and TCDD. The authors concluded that there was no widespread environmental contamination of the area by 2,4-D, 2,4,5-T, and TCDD.

828. Robbins, A. (1979) Dioxin studies. [letter to the editor] Science 205(4413):1332.

[Editorial.]

829. Robens, J. F. (1978) Tests for possible carcinogenicity of 20 pesticides in Osborne-Mendel rats and B6C3F1 mice. Toxicol. Appl. Pharmacol. 45(1):236.

[Abstract, only.]

830. Robson, J. M. (1970) Testing drugs for teratogenicity and their effects on fertility. Brit. Med. Bull. 26(3):212-216.

[Review article.]

831. Rocchi, P., Perocco, P., Alberghini, W., Fini, A., and Prodi, G. (1980) Effect of pesticides on scheduled and unscheduled DNA synthesis of rat thymocytes and human lymphocytes. Arch. Toxicol. 45:101-108.

The authors studied the action of diquat and 16 other pesticides on scheduled and unscheduled DNA synthesis in rat thymocytes and human lymphocytes. Diquat (95% purity) in DMSO was added to rat thymocyte cell cultures to determine the concentration of diquat which gave 50-70% DNA synthesis inhibition. This dose was then used to determine the effect of diquat on DNA synthesis in human lymphocytes. Diquat (500 ug/ml) was added to cultures of human lymphocytes with and without hydroxyurea for 4 hours. During this culture period, the cells were labelled with tritiated thymidine to measure DNA synthesis. Cells treated with ultraviolet radiation were used to compare scheduled and unscheduled DNA synthesis. Diquat at 500 ug/ml produced approximately the same amount of inhibition of scheduled DNA synthesis in rat thymocytes as well as in human lymphocytes. In this system inhibition of scheduled DNA synthesis was comparable to inhibition of unscheduled DNA synthesis. The authors did not discuss the relevancy of these data. However, it appears from the data presented that diquat at the dose tested in human lymphocytes did not induce unscheduled DNA synthesis compared to controls.

832. Rodgers, C. A., and Stalling D. L. (1972) Dynamics of an ester of 2,4-D in organs of three fish species. Weed Sci. 20(1):101-105.

[Background material.]

833. Roll, R. (1971) Studies of the teratogenic effect of 2,4,5-T in mice. Fd. Cosmet. Toxicol. 9:671.

Incidences of fetal malformations and deaths in mice administered 2,4,5-T are described. NMRI mice were administered 20-130 mg/kg 2,4,5-T 99.6% purity; 0.05 ppm TCDD contaminant) in peanut oil, by oral intubation on days 6-15 of gestation. On day 18 of pregnancy (1 day prior to expected delivery) fetuses were removed, weighed, and examined for visceral malformations by Dawson's method) or for skeletal abnormalities in Alizarin red-stained specimens. The numbers of implantation sites, resorption sites and dead fetuses were noted. The LD₅₀ doses were calculated to be 674 mg/kg for adult NMRI male mice and 778 mg/kg for females. Maternal weight gains were reduced in rats administered 90 or 130 mg/kg doses. A small decreases in the numbers of full-term fetuses and small decreases in early and late resorption and dead fetuses occurred from treatment with 90 mg/kg doses and these changes were all substantial in the 130 mg/kg dosage group. A dose-related decrease in fetal weight and increase in cleft palate incidence occurred; these changes ranged from 1.23 g. of fetal weight for controls to 0.73 g. for the highest dosage group and 1.9% of controls with cleft palate to 48.8% for the highest dosage group. Dose-dependent breast bone anomalies were considered to represent slow development, rather than lack of development that would produce

permanent abnormal structures. The authors concluded that 2,4,5-T (and not the dioxin contaminant was responsible for the high incidence of cleft palate they observed and suggested that the mouse was more susceptible than the rat (studied by other investigators) to these teratogenic effects.

834. Rose, H. A., and Rose, S. P. (1972) Chemical spraying as reported by refugees from South Vietnam. Science 177(4050):710-712.

The authors describe the findings they obtained from questionnaires they issued to 98 South Vietnamese refugees who had migrated to Hanoi. Eighty of the group were men, mostly between 25 and 45 years of age. Most of the 98 respondents (95%) were sprayed with herbicide at least 2 times, with 2 people reporting that they were sprayed 21-30 times. The remainder of the questions referred only to the last spraying mission they experienced. Chemicals were sprayed by air in 91% of the cases and the authors indicated that descriptions were compatible with spraying of Agents Orange, White, and Blue and of (ortho-chloro-benzylmalononitrile (CS). When asked about environmental effects, 95% indicated that trees were damaged or killed, 89% that crops were destroyed or became inedible, 48% that fish died, 39% that domestic animals died and 5% that cattle died. Abortions or monstrous births of animals were voluntarily mentioned by 5% of the group. Health effects reported were ocular problems, 79% of the cases; transient nausea and vomiting, 58%; skin burns and reddening, 56%, persistent skin effects including pustules, scabs and eczema, 16%; fatigue or dizziness after spraying, 92%; prolonged fatigue, 17%. Respondents also reported health effects of others in their village, including long illnesses in the old and in children (observed by 11% of the respondents), deaths due to chemicals (8%), and human abortions (4%). The authors suggested that the reported eye defects could be attributed to exposure to CS and that fatigue was a common symptom of people exposed to 2,4-D or to organophosphorus insecticides. The authors concluded that the toxicology of individual agents could not be assessed because complex combinations of agents were sprayed in each episode and frequent episodes of spraying occurred.

835. Rose, J. Q., Ramsey, J. C., Wentzler, T. H., Hummel, R. A., and Gehring, P. J. (1976) The fate of 2,3,7,8-tetrachlorodibenzo-p-dioxin following single and repeated oral doses to the rat. Toxicol. Appl. Pharmacol. 36:209-226.

The pharmacokinetics of TCDD distribution and excretion in the rat is described. Sprague-Dawley rats (6 per group) were administered a single oral dose of 1 ug/kg [¹⁴C]-TCDD (99+% radiochemical purity) in acetone-corn oil (1:25) by oral gavage. Other rats (18 per group) were administered 0.01, 0.1 or 1.0 ug/kg/day of [¹⁴C]-TCDD, 5 days per week for 7 weeks. Urine, feces and expired air samples and tissue sample of rats killed after 1, 3, or 7 weeks of exposure were analyzed for radioactivity. Liver homogenates were extracted with organic solvents to determine extractable radioactivity and were analyzed by gas

chromatography-mass spectrometry to determine the proportion of radioactivity present as non-metabolized TCDD. Toxic effects of TCDD in the rat were not mentioned. The body burden for 24 days following a single oral dose of TCDD was calculated by subtracting fecal excretion levels from the total dose administered. No radioactivity was detected in urine or expired air. Body burdens followed apparent first-order kinetics, with a half-life of 31 days for both sexes. Twenty-two days after a single dose, fat and liver each contained 1.25% of the dose, and kidney, thymus and spleen each had less than 0.1%. When repeated doses were administered, radioactivity was detected in the urine of only the highest dosage group, accounting for 3% and 12.5% of the cumulative doses for males and females, respectively. Body burdens calculated from excretion data were close to the levels of radioactivity detected in carcasses at the end of exposure of 37% and 48% for the 1 and 0.1 ug/kg/day dosage groups, respectively. Body and burden data were fitted to a one compartment pharmacokinetic model and half-times and rate constants were calculated. About 75% of the 1 ug/kg doses were absorbed by male rats and about 90% by females; 90% of the 0.1 ug/kg doses were absorbed by both sexes. The half-life (from data for all groups) was 23.7 days. The steady state body burden was estimated to be 29 times the daily dose and 90% of the steady state value would be reached after 78.5 days of exposure. Radioactivity accumulated in the liver and fat and whole body at the same ratio; the concentration in the liver was about 5 times that of fat, and both levels plateaued with continuous administration. Other tissue levels of radioactivity were less than one-tenth of the hepatic levels. Recovery of TCDD by chemical methods confirmed the radiochemical data, indicating that TCDD biotransformation was not detectable. The authors concluded that low, continuous exposure to TCDD is unlikely to result in tissue accumulation of TCDD; TCDD approached a constant concentration in the fat, liver, and whole body within 13 weeks of repeated administrations and the rate constant was independent of dose.

836. Rose, M. S., Crabtree, H. C., Fletcher, K., and Wyatt, I. (1974) Biochemical effects of diquat and paraquat. Disturbance of the control of corticosteroid synthesis in rat adrenal and subsequent effects on the control of liver glycogen utilization. Biochem. J. 138(3):437-443.

The effects of diquat administration on glycogen depletion, and on adrenocorticotrophic hormone (ACTH) levels, plasma corticosteroid levels and cyclic AMP content of the adrenal glands are described in normal, hypophysectomized and adrenalectomized rats. An intraperitoneal dose of 20 mg (cation/kg) of diquat dichloride was administered to male Alderley Park rats, including adrenalectomized and hypophysectomized rats. Vehicle controls received an injection of saline. Liver glycogen, blood glucose, plasma corticosteroids and ACTH, and adrenal cyclic AMP and catecholamine levels were determined at various times after administration. Rats administered diquat and starved for 24 hr. had a mean hepatic glycogen level of 27.7 mg/g liver (wet weight) compared to 0.8 mg/g in starved vehicle controls and 67.2 mg/g in fed vehicle controls. Rats administered diquat after a 28 hr. starvation period had levels of 11.5 mg/g of hepatic glycogen, compared to 4.6-4.8

mg/g for the vehicle and untreated controls. In adrenalectomized rats, the liver glycogen level fell more rapidly in diquat-treated rats than in vehicle controls. The increase in blood glucose levels was maximum 1 hr. after diquat treatment at 110 mg of glucose per 100 ml blood and was less in hypophysectomized rats and minimal in adrenalectomized rats. Diquat administration did not cause a change in adrenal catecholamine levels or in clearance of corticosteroids from ACTH-treated, hypophysectomized rats. Diquat caused an increase in cyclic AMP and a sustained increase in plasma corticosteroids in starved rats, compared to a transient increase observed in vehicle controls and was ineffective in changing corticosteroid or cyclic AHP levels in hypophysectomized rats. Diquat caused an increase in ACTH levels (in intact rats) which disappeared by 24 hr. after diquat administration. Paraquat was also studied in these experiments. The authors concluded that diquat stimulated synthesis of adrenal corticosteroid synthesis possibly by increasing adrenal sensitivity to ACTH, and suggested that some toxic effects observed histologically after diquat and paraquat poisoning were related to increased corticosteroid synthesis.

837. Rose, M. S., Lock, E. A., Smith, L. L., and Wyatt, I. (1976) Paraquat accumulation: tissue and species specificity. Biochem. Pharmacol. 25(4):419-423.

The uptake of diquat by rat tissues in vivo and in vitro is described. Male Alderley Park rats (3-4 per group) were administered 680 umoles/kg [¹⁴C]-diquat orally and tissues were removed after 2-30 hr. and radioactivity was determined. Tissues were removed from non-treated rats and were sliced and incubated in the presence of 10⁻⁶M diquat. Radioactivity taken up by the tissues was determined at the end of the incubation period. The concentrations of diquat in lung tissue 2-30 hr. after in vivo treatment remained close to the plasma levels, whereas the kidney levels were 5-10 times the plasma levels, the levels for the liver and adrenals were 1-2 times plasma levels, and levels for brain and muscle were below plasma levels. Slices of renal cortex concentrated diquat to levels about double those in the medium and the effect was established within 1 hr. Tissues studied in vitro included those analyzed after in vivo exposure, as well as skin, heart, small intestine and spleen. No other tissue accumulated diquat after 1-2 hr. in culture. Uptake of paraquat was also studied. The authors concluded that only the kidney and possibly the adrenals and liver were capable of accumulating or retaining diquat.

838. Rose, M. S., and Smith, L. L. (1977) Minireview. Tissue uptake of paraquat and diquat. Gen. Pharmacol. 8:173-176.

[Review article.]

839. Rose, M. S., Smith, L. L., and Wyatt, I. (1976) The relevance of pentose phosphate pathway stimulation in rat lung to the mechanism of paraquat toxicity. Biochem. Pharmacol. 25(15):1763-1767.

The effect of diquat on glucose metabolism by rat lung slices and diquat accumulation in the lung were studied. Rat lung slices were incubated in the presence of [^{14}C]-glucose and metabolism was determined by trapping and counting $^{14}\text{CO}_2$ generated. Diquat was introduced to the incubation medium at concentrations of 10^{-4} to 10^{-6}M or was administered intravenously (65 $\mu\text{moles/kg}$ in saline) 0.5-18 hr. before the lungs were removed or orally (680 $\mu\text{moles/kg}$) 4-30 hr. before the lungs were removed, sliced, and incubated. Vehicle controls were used for the u.v. study. At 10^{-4}M and 10^{-5}M , diquat caused twofold and fourfold stimulation, respectively, of [$1\text{-}^{14}\text{C}$] glucose metabolism. At all times after u.v. treatment and 30 hr. after ingestion, [$1\text{-}^{14}\text{C}$]-metabolism was elevated about twofold. The effect of diquat at 10^{-5}M was not inhibited by imipranine (10^{-4}M). The half-time for [^{14}C]-diquat to equilibrate with the volume of tissue available to [^3H]-water was 80 min. The metabolism of [$6\text{-}^{14}\text{C}$]-glucose was not altered by diquat administered by any route. The effects of paraquat were also examined. The authors concluded that diquat and paraquat entered lung cells and stimulated the pentose phosphate pathway, generating free radicals; accumulation of paraquat was suggested as the reason why paraquat was toxic to the lung and diquat was not toxic.

840. Rose, M. S., Smith, L. L., and Wyatt, I. (1974) Evidence for energy-dependent accumulation of paraquat into rat lung. Nature 252(5481):314-315.

Uptake of diquat by rat lung slices is described. Lung slices were prepared from male Alderley Park rats and were incubated in the presence of 10^{-3}M to 10^{-5}M [^{14}C]-diquat for up to 2 hr. Radioactivity of the tissues was analyzed after the incubation period ended. The amount of diquat taken up by lung slices plateaued by 30 min. of incubation and the plateau level was dependent upon the concentration of diquat in the medium. The pattern of uptake of paraquat by lung slices was also described. The authors concluded that paraquat, but not diquat, was actively accumulated by liver slices by an energy-dependent process.

841. Rowe, V. K. (1980) Direct testimony before the U.S. Environmental Protection Agency, FIFRA Docket No. 415 et al., Nov 13.

[Testimony.]

842. Rowe, V. K., and Hymas, T. A. (1954) Summary of toxicological information of 2,4-D and 2,4,5-T type herbicides and an evaluation of the hazards to livestock associated with their use. Amer. J. Vet. Res. 15:622-629.

Acute and subacute toxicity of 2,4-D and 2,4,5-T is described for the dog, rabbit, guinea pig, rat and chicken. Various salts and esters of 2,4-D and 2,4,5-T were administered orally in water, olive oil, corn oil, or undiluted. For acute studies, mortality rates were determined 14 days after a single dose was administered. Brief descriptions of the toxic effects observed in treated animals were given. The LD₅₀ values ranged from 300-1000 mg/kg for both compounds for all species except the dog, with an LD₅₀ of 100 mg/kg. The chicken was the least sensitive species. LD₅₀ values for the various forms of the phenoxy acids were comparable and commercial formulations had LD₅₀ values that were proportional to the levels of active ingredients they contained. Neither compounds had cumulative effects, since repeated oral doses that were ineffective were only slightly lower than an ineffective single oral dose. For both compounds, symptoms in affected animals included muscular tenseness and weight loss. Irritation of the stomach and minor liver and kidney damage was also observed. The authors concluded that 2,4-D and 2,4,5-T used by recommended agricultural methods presented a negligible hazard to livestock and wildlife.

843. RPAR Assessment Team. (1979) Biologic and economic assessment of 2,4,5-T. A report of the USDA-STATES-EPA 2,4,5-T RPAR Assessment Team, Washington, DC, Feb 15.

Exposure of 2,4,5-T and TCDD are estimated after assumptions regarding conditions of exposure are stated. Dermal absorption of 2,4,5-T, measured for four human volunteers in a laboratory experiment, and deposition and absorption determined for field workers were reported elsewhere (Newton, 1978; Lavy 1978a; and Lavy 1978b, respectively) were used to calculate exposure. Assumptions, including the concentration of 2,4,5-T used for spraying by workers with specific tasks, and the types of protective clothing worn, are stated for 10 different exposure situations. Maximum possible exposure levels were estimated from the rates of absorption, given in the previous laboratory reports, and were applied to the specific, assumed exposure conditions. Dosages were presented for 2,4,5-T and TCDD, based on a level of contamination of 3×10^{-8} ppm TCDD in the 2,4,5-T mixture and an absorption rate for TCDD twice the rate of 2,4,5-T absorption. Maximum absorption was estimated for a backpack spray operator without protective clothing administering 40 lb of acid equivalent per 100 gallons, with a predicted maximum absorption rate of 0.85 mg/kg/hr of 2,4,5-T and 2.1×10^{-5} ug/kg/hr of TCDD. The authors concluded that the levels of exposure observed after actual operational applications were substantially lower than those calculated from laboratory data with maximum exposure of 0.095 mg/kg/hr of 2,4,5-T calculated from data on workers under specific conditions of exposure.

844. Rubenchik, B. L., Botsman, N. E., and Groban, G. P. (1970) The carcinogenic effect of the herbicide monuron. Vopr. Onkol. 16(10):51-53.

[Foreign language.]

845. Rubenchik, B. L., Botsman, N. E., and Shipko, G. P. (1969a) Study of carcinogenic properties of the herbicide monuron. Vopr. Ratsion Pitanii 5:85-86.

[Not available.]

846. Rubenchik, B. L., Petrun, A. S., Pliss, M. B., and Shipko, G. P. (1969b) Monuron action on the liver when this herbicide is administered with food. Vopr. Pitan. 28(6):13-18.

[Foreign language.]

847. Safety of the herbicide 2,4,5-T. [Letters to the editor] 1977. New Zea. Med. J. July 13, 1977:35-36.

[Editorial.]

848. Saint-Ruf, G. (1972) Formation of dioxin in the pyrolysis of sodium a-(2,3,7,8-trichlorophenoxy)-propionate. Naturwissen Schäften 59(12):648

[Background material.]

849. Saint-Ruf, G., and Do-Phuoc Hien. (1975) Similarity of the biochemical effects of 2,3,7,8-tetrachloridibenzo-p-dioxin and 2,3,7,8-tetrabromodibenzo-p-dioxin in the rat. C. R. Hebd. Seances Acad. Sci. Ser. D. 280(23):2709-2711.

[Foreign language.]

850. Samman, P. D., and Johnston, E. N. M. (1969) Nail damage associated with handling of paraquat and diquat. Br. Med. J. 1:818-819.

Two cases of nail damage that were suspected to be caused by contact with Preeglone Extra are described. A 75-year-old man who diluted Preeglone Extra (which contains equal amounts of diquat and paraquat) concentrate with water frequently over a number of years, presented with damage to most of his fingernails. Damage included subacute paronychia, partial nail loss, discoloration, and hemorrhages below the nail. Two months later all nails were regrowing satisfactorily. A 50-year-old man, who also diluted Preeglone Extra, frequently had nail damage on most fingers, which involved nail loss, discoloration, and infection. The toe nails were not involved in either case. A case of nail discoloration after handling paraquat was also reported. The authors concluded that local contact of the nails with the herbicide in concentrated form resulted in discoloration, nail softening and loss, accompanied by susceptibility to infection.

851. Sanders, H. O. (1970) Toxicities of some herbicides to six species of freshwater crustaceans. J. Water Poll. Cont. Fed. 42:1544-.

[Background material.]

852. Sanderson, C. A., and Rogers, L. J. (1981) 2,4,5-Trichlorophenoxy-acetic acid causes behavioral effects in chickens at environmentally relevant doses. Science 211(4482):593-595.

Alterations in behavioral development of chickens treated with 2,4,5-T in ovo are described. A single dose of 2,4,5-T (0.03 ppm TCDD contaminant) suspended in 0.5% gum tragacanth was injected into chicken

eggs on day 8 or day 15 of incubation or was administered intraperitoneally to 2-day-old chicks. Doses of 7-53 mg/kg and 75-225 mg/kg were administered to eggs and chicks, respectively. Median lethal doses (LD₅₀), gross abnormalities and body weights were established. Behavioral activities measured included general activity (scored by an activity meter), ambulation (the frequency each chicken crossed into a new quadrant; the floor of the test box was divided into 4 quadrants), visual learning (the number of incorrect pecks at background pebbles by chickens seeking food) and jumping behavior (frequency of jumping). The LD₅₀ doses were 53 mg/kg administered on day 15 of incubation and 200 mg/kg to 2-day-old chicks. In chicks given 27 mg/kg on day 15, 70% hatched. No dose-related gross malformations or weight loss from doses below 150 mg/kg on day 2 were observed. Jumping behavior increased significantly in chickens given 13 or 27 mg/kg on day 15 or 150 mg/kg after hatching. General activity increased significantly in chicks treated in ovo on day 15 with a 27 mg/kg dose and visual learning decreased in chicks treated in ovo on day 15 with 7 or 27 (but not 13) mg/kg TCDD. The authors concluded that doses of 22 mg/kg could reach the egg from recommended agriculture rates of 2,4,5-T spraying and these levels caused adverse behavioral effects in chickens. The relationship of the behavioral parameters measured to mammalian behavioral development are not obvious; the biological significance of the observed changes in measured frequencies and the possibility all of a 22 mg/kg dose of 2,4,5-T deposited on an eggshell of reaching the embryo remain unknown.

853. Sare, W. M. (1979) 2,4,5-T and the problems of toxicity. [letter to the editor] Med. J. Austr. 1(11):526.

[Editorial.]

854. Sare, W. M. (1972) The weedicide 2,4-D as a cause of headaches and diplopia. [letter to the editor] New Zea. Med. J. 75(478):173-174.

[Editorial.]

855. Sare, W. M., and Forbes, P. I. (1977) The herbicide 2,4,5-T and its possible dysmorphogenic effects. New Zea. Med. J. 85(588):439.

[Editorial.]

856. Sare, W. M., and Forbes, P. I. (1972) Possible dysmorphogenic effects of an agricultural chemical: 2,4,5-T. New Zea. Med. J. 75(476):37-38.

Two lethal cases of congenital deformities were reported. In the previous few years 2,4,5-T, which can be contaminated with dioxin, had been sprayed in the rural area of New Zealand where the births occurred. Both infants had gross myelo-meningocele and autopsy of one infant revealed other malformations (which were not described), in

support of the potential causal relationship between TCDD exposure and birth defects. The authors indicated that the farms of both mothers had been sprayed during the first trimester of each pregnancy and rain water collected from the roof of each house was used as the domestic water supply. A neighbor in the sprayed area who did not collect roof water gave birth to a normal child. The farm of one of the affected families reported the lowest calving rate for pregnancy-tested cattle. The authors also calculated the potential exposure levels of TCDD from aerial spraying and an estimated fetotoxic dose of TCDD in humans extrapolated from animal data.

857. Sauerhoff, M. W., Braun, W. H., Blau, G. E., and Gehring, P. J. (1977) The fate of 2,4-dichlorophenoxyacetic acid (2,4-D) following oral administration to man. Toxicology 8:3-11.

The pharmacokinetics of 2,4-D clearance from plasma was determined in five men. The men, aged 29-40 (and in good health, as evidenced by results in the normal range for all tests including physical exams and clinical blood and urine analyses) were administered 5 mg/kg analytical grade 2,4-D in milk (2) or as a powder followed by water. Urine and plasma samples were analyzed over the subsequent 144 hours by gas chromatography-mass spectrometry. Acid-labile urinary conjugates of 2,4-D were determined by acid-hydrolysis of urine samples prior to analysis. No signs of toxicity were detected by any subject. A two-compartment model was used to analyze data from the one subject, a one-compartment model was used for two additional subjects, and the data for the last two subjects were not analyzed kinetically. Neither model adequately described the data at the 95% confidence level. The half-lives for absorption were between 1.7 and 4.2 hours and for elimination from blood were 7-11 hours for the one-compartment model and 4 and 15 hours for the fast and slow components of the two-compartment model. The volumes of distribution were 283-294 ml/kg for the one-compartment model and 84 and 218 ml/kg for the central and slow exchange compartments of the two-compartment model. Urinary elimination was analyzed pharmacokinetically for all five subjects. From 48 to 97% of the dose was excreted in the urine, with a half-life of 10.2-28.4 hours. From 0 to 27% (mean 12.8%) of the dose of 2,4-D was excreted as urinary conjugates. The authors concluded that an oral dose of 2,4-D was well absorbed in man and was excreted from the body and cleared from plasma by apparent first-order rate processes.

858. Sauerhoff, M. W., Braun, W. H., Blau, G. E., and Gehring, P. J. (1976) The dose-dependent pharmacokinetic profile of 2,4,5-trichlorophenoxyacetic acid following intravenous administration to rats. Toxicol. Appl. Pharmacol. 36(3):491-501.

The effect of the dosage of 2,4,5-T administered to rats on the pharmacokinetics of excretion were examined. Sprague-Dawley rats were administered 5 or 100 mg/kg of 2,4,5-T (99% purity) by intravenous infusion. Blood, urine, feces, and tissue samples were analyzed for radioactivity and urinary metabolites were extracted and separated by

thin layer chromatography and gas chromatography-mass spectrometry. Plasma clearance of 2,4,5-T followed first order kinetics after the lower dose only. For the higher dose, the rate constant for the linear phase of plasma clearance (the faster component) was close to the value for the lower dose (0.16 per hour). The half-lives were 4.3 hours and 5.3 hours for the low and high doses respectively. The excretion of 2,4,5-T showed the same dose-dependent kinetics with the constants for the faster components from the high dose corresponding to the constants for excretion of the low dose. Less than 5% of either dose remained in the body after 48 hours. Tissue-to-plasma ratios of radioactivity were the same after the low and high doses, except for a higher kidney-to-plasma ratio after the lower dose. Over 94% of the urinary radioactivity was associated with unchanged 2,4,5-T. The authors concluded that the higher dose of 2,4,5-T saturated the active transport process of renal excretion and that projections of toxicity of low doses of 2,4,5-T based on data from high doses are not justified because mechanisms of detoxification have been compromised at high doses.

859. Sauerhoff, M. W., Braun, W. H., Blau, J. E., and LeBeau, J. E. (no date) The fate of 2,4-dichlorophenoxyacetic acid (2,4-D) following oral administration to man. No reference pp. 136-137.

[Abstract, only.]

860. Sauerhoff, M. W., Chenoweth, M. B., Karbowski, R. J., Braun, W. H., Ramsey, J. C., et al. (1977) Fate of silvex following oral administration to humans. J. Toxicol. Environ. Health 3:941-952.

The pharmacokinetics of [2-(2,4,5-trichlorophenoxy) proprionic acid](silvex) was studied in human volunteers. Six men and one woman were each administered orally 1 mg/kg silvex which contained less than 0.01 ppm TCDD. Blood, urine, and fecal samples collected in the subsequent 168 hours were analyzed for silvex, by gas chromatography-mass spectrometry, and urine and feces were analyzed for metabolites by hydrolysis, extraction, or derivitization of samples prior to the silvex analysis. No adverse effects were detected in the subjects after ingesting silvex and all clinical chemistry analyses on blood and urine were within normal ranges. Blood levels of silvex peaked 1-2 hours after ingestion. Blood clearance was biphasic, with half-lives of 4 and 16.5 hours for the two components. The best fit of the data was provided from a two-compartment model and the volume of distribution for each compartment was 115 ml/kg and 107 ml/kg. A mean of 80% of the dose of silvex was excreted in the urine in 144 hours, with 15-44% of the dose recovered as conjugates in the urine. 2,4,5-trichlorophenol was not detected in the urine. Urinary excretion was biphasic, with half-lives for each component of 5 and 26 hours. Fecal excretion of silvex accounted for 3.2% of the dose in one subject and below 1% for four other subjects. The authors noted that the rate for the slow phase of plasma clearance resembled the rate of clearance of 2,4,5-T described elsewhere and noted that the biliary route of excretion which predominates in the rat is not important in silvex excretion in man.

861. Savidge, J. A. (1978) Wildlife in a herbicide-treated Jeffrey pine plantation in eastern California. J. For. 76(8):476-478.

The author compares the populations of vegetation and wildlife on a 2,4,5-T sprayed area and an unsprayed area of a Jeffrey pine plantation. The study was conducted 6 years after an aerial spraying of 2,4,5-T (1.8 kg/acre acid equivalent). Each study area contained approximately 11 acres. The biggest difference between the two areas was the elimination of most of the Ceanothus velutinus (snowbrush) in the sprayed area. Live Ceanothus covered 30% of the unsprayed plot, but only 2% of the sprayed plot. According to the author, this difference was responsible for the changes seen in wildlife populations. Elimination of Ceanothus allowed a currant species (Ribes cereum) that was dormant at the time of spraying to thrive in the sprayed area. Other vegetation, such as the Jeffrey pine, were similar in dominance in both areas. The total number and number of species of birds on the unsprayed plot were approximately twice those of the sprayed plot. Most of the species eliminated on the sprayed plot used Ceanothus as a source of food or shelter. Nearly twice as many small mammals (mice, chipmunks, squirrels) were found in the sprayed plot. The author speculated that food and/or habitat changes might have been responsible for the increase. Deer usage also varied between the two plots. On the unsprayed areas, deer and deer trails were observed, while on the sprayed area neither were observed. The author did not make any conclusions on the significance of these changes.

862. Sawinsky, A., and Pasztor, G. (1977) Exposure tests in reglone spraying by aircraft. Z. Gesamte Hyg. 23(11):845-846.

[Not available.]

863. Scarpelli, D. G., Rao, M. S., Subbarao, V., et al. (1980) Activation of nitrosamines to mutagens by postmitochondrial fraction of hamster pancreas. Cancer Research 40:67-74.

864. Schantz, S. L., Barsott, D. A., and Allen, J. R. (1979) Toxicological effects produced in nonhuman primates chronically exposed to fifty parts per trillion 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Toxicol. Appl. Pharmacol. 48(1):A180.

[Abstract, only.]

865. Schardt, C., and Heathfield, C. (1979) "Agent Orange" - A Selected Bibliography. Prepared by the Veterans Administration. 13 pp.

[Bibliography.]

866. Schiller, C. M. (1979) Chemical exposure and intestinal function. Environ. Health Perspec. 33:91-100.

[Review article.]

867. Schonborn, H., Schuster, H. P., and Kossling, F. K. (1971) Clinical and morphological findings in an acute oral intoxication with diquat (reglone). Arch. Toxicol. 27(3):204-216.

[Foreign language.]

868. Schreiweis, D. O., and Murray, G. J. (1976) Cardiovascular malformations in Oryzias latipes embryos treated with 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). Teratology 14(3):287-290.

The teratogenic effects of 2,4,5-T were examined in developing fish eggs. Oryzias latipes eggs were grown in the presence of 10-50 ppm 2,4,5-T (2,4,5-T had less than 0.05 ppm dioxin contamination), which was introduced in ethanol (final volume of ethanol, 0.4%) at stage 10 (blastula stage) of development. Ethanol was added to control dishes of eggs. The rate of hatching and developmental features, including axis formation, initiation of heartbeat, and normal body pigmentation were followed. The rates of hatching were 100% in control and 10 ppm-treatment groups, 96% for the 14 ppm group, 47% for the 20 ppm group, 4% at 25 ppm and none at higher doses. Interruption of each developmental feature monitored showed a dose-related pattern. Cardiovascular anomalies also occurred at a frequency related to 2,4,5-T dose. These anomalies included enlarged veins, enlarged cardinals, and enlarged heart chambers, tube heart and hemorrhages. No other anomalies in other organ systems were discussed. The authors suggested that 2,4,5-T is selectively taken up by differentiating cardiovascular tissue and recommended rates of spraying for 2,4,5-T that would not interfere with reproduction in this fish species.

869. Schulz, K. H. (1968) Clinical picture and etiology of chloracne. Arbeitsmedizin-Sozialmedizin-Arbeitshygiene 3(2):25-29.

[Review article.]

870. Schulz, K. H. (1957) Arch. Klin. Exp. Derm. 206:589-596.

The clinical symptoms of workers in Hamburg, Germany involved in 2,4,5-T synthesis are described briefly and experimental results of efforts to elucidate the etiology of the observed pathology are presented. Thirty-one workers developed dermatological symptoms between the middle of 1954 and the spring of 1955. Comedones, pustules, furuncles and retention cysts appeared on the face, neck, back, chest, genitals, arms and legs. (The frequencies of these symptoms or temporal or clinical patterns were not described). Other

symptoms reported were chronic conjunctivitis, blepharitis and complaints of nausea, debility and loss of appetite. Clinical tests did not reveal liver, kidney, central nervous or hemopoietic disorders (the tests used and the frequency of testing were not indicated). A chemical laboratory worker outside of Hamburg developed chloracne 10-14 days after working with a tetrachlorodibenzodioxin; no other details of this incident were provided. Various compounds used in the synthesis of 2,4,5-T or proposed as contaminants during the manufacturing process were tested for acnegenic activity in the rabbit ear; hepatotoxicity was tested with a bromosulfthalein method which was not described further. Technical and chemical grades of 2,4,5-trichlorophenol and its distillation residue, 1,2,4,5-tetrachlorobenzene, diphenyl ether, mono- to tetra-chlorinated diphenyl ethers, dibenzofuran, mono- to tetra-chlorinated dibenzofurans and a tetra-chlorinated dibenzo dioxin (chlorine positions were unknown) were each painted on rabbit ears and the development of chloracne at the site was monitored. Technical grade 2,4,5-trichlorophenol, its distillation residue, tri- and tetra-chlorinated dibenzofurans and tetrachlorodibenzo-dioxin each elicited an acnegenic response of inflammation in 5-7 days and keratotic-containing follicles several days later. These compounds all produced slight hepatotoxicity, except for the furans, which elicited a strong response and the dioxin, which was not tested for hepatotoxicity. Chlorinated diphenyl ethers also produced slight hepatotoxicity. The authors concluded that the tetrachlorodibenzo dioxin was likely to have been a contaminant in the distillation residue and was likely to be the etiologic agent in causing the observed dermatitis.

871. Schulze, J. A., Manigold, D. B., and Andrews, F. L. (1973) Pesticides in selected western streams 1968-71. Pest. Monit. J. 7(1):73-84.

The authors describe the occurrence of 2,4-D and 2,4,5-T in 20 western streams October 1968 through September 1971. Samples were analyzed by gas chromatography (detection limit 0.05 ug/l 2,4,5-T; 0.02 ug/l 2,4-D). The highest concentration of 2,4-D detected was 0.99 ug/l while the highest concentration of 2,4,5-T was 0.40 ug/l. Of the total 109 2,4,5-T occurrence, 47% were detected at 2 stations. 2,4-D was detected 103 times. Amounts of herbicide applied to the watersheds under study was not presented.

872. Schultz, D. P., and Harmon, P. D. (1974) Residues of 2,4-D in pond waters, mud, and fish, 1971. Pestic. Monit. J. 8(3):173-179.

The authors 1) measured residue levels and the rate of dissipation of 2,4-D in water, bottom mud and fish, 2) determined the effect of physical and chemical characteristics on uptake and dissipation and 3) assessed the efficacy of 2,4-D on waterhyacinth in Florida and Georgia ponds. Nine ponds of varying ecological and geographical types, located in Florida, Georgia and Missouri, were treated with DMA-2,4-D at 2, 4 or 8 lb/acre in 1971. The formulation contained 4 lb 2,4-b acid equivalent per gallon. Fish, mud and water samples were taken at intervals up to 147 days after application. Approximately 98% of the

waterhyacinth was killed within 7 days. The highest residue level in Florida pond water was 0.345 mg/l 3 days after application of 8 lb/acre; it then dissipated to less than or equal to 0.005 mg/l 14 days after treatment. The highest level in Georgia pond water was 0.692 mg/l 3 days after treatment with 8 lb/acre 2,4-D. Levels decreased to less than 0.005 mg/l 28 days after treatment. In Missouri pond water, the highest level was 0.630 mg/l 14 days after application of 8 lb/acre decreasing to less than detectable 56 days after application. After treatment, residues in mud were 0.046 mg/kg in Florida, 0.042 mg/kg in Georgia, and 0.170 mg/kg in Missouri, all treated at levels of 8 lb/acre 2,4-D. Trace amounts of 2,4-D in mud were detected 112 days after application in the Florida and Georgia ponds and no residues were detected in the Missouri ponds 56 days after sampling. Residues in fish from Florida and Georgia ranged from 0.005 mg/kg to 1.075 mg/kg. In the Florida ponds, only one sample contained detectable levels after 14 days. In the Georgia ponds, only one sample showed detectable levels after 28 days. All fish samples from Missouri ponds showed no detectable amount of 2,4-D except 6 samples which showed trace amounts. The authors conclude that no difference in waterhyacinth kill was evident among the three treatment levels. Since the Missouri ponds had no waterhyacinth before treatment, the persistence of the herbicide in Missouri ponds is due to the lack of sufficient surface biomass to degrade 2,4-D. It is also mentioned that initial concentrations were twice as much in the Missouri pond due to a wrong assumption before spraying.

873. Schuth, C. K., Isensee, A. R., Woolson, E. A., and Kearney, P. C. (1974) Distribution of ^{14}C and arsenic derived from [^{14}C] cacodylic acid in an aquatic ecosystem. J. Agr. Fd. Chem. 22(6):999-1003.

The authors studied the accumulation and distribution of cacodyli acid in model aquatic ecosystem using three soil types, Willacy sandy loam, Hidalgo clay loam, and Laredo silt loam. Duplicate soil samples were treated with 21 ppm ^{14}C -cacodylic acid and layered on the bottom of glass aquarium tanks. Water was added to each of the tanks and allowed to equilibrate for weeks. Catfish, crayfish, daphnids, snails, filamentous algae, and duckweed were then added to each tank. Water samples were removed from each tank at 2-day intervals for 59 days for scintillation counting. In addition, water samples were removed for arsenite, arsenate, and total arsenic analysis by colorimetric and atomic absorption spectrometry at 8, 22, 38, 50, and 59 days. Soil and organisms were also analyzed by the same methods. In the soil (average of all 3 types), after 59 days 13.5% of the ^{14}C radioactivity and 40% of the As from the original cacodylic acid remained. According to the authors, the differences in ^{14}C and as loss indicate that the C-As bond of cacodylic acid in the soil is being split. In water, the cacodylic acid increased nearly linearly for 30-50 days, leveled off, then decreased. Water analysis after 59 days did not show any cacodylic acid present. The authors concluded that ^{14}C in the water was associated with volatile chemical forms such as $^{14}\text{CO}_2$ or organoarsenicals. Analysis of aquatic organisms for ^{14}C and total arsenic revealed little bioaccumulation. Bioaccumulation ratios decreased as food chain

position of the organism increased. According to the authors, the ^{14}C data indicate degradation of cacodylic acid and uptake of ^{14}C by the plants.

874. Schwetz, B. A., Norris, J. M., Sparschu, G. L., Rowe V. K., and Gehring, P. J. (1973) Toxicology of chlorinated dibenzo-p-dioxins. Environ. Health Perspect. 5:87-99.

The acute oral, parenteral, and dermal toxicities of TCDD, as well as the production of eye irritation, acne, and chick edema disease are summarized. TCDD was administered in corn oil: acetone (9:1) by gavage to Sherman rats, Swiss Webster mice, New Zealand albino rabbits, Hartley guinea pigs, and Beagle dogs, and the animals were observed for 2-8 weeks. Female rats were more sensitive than male rats, and rabbits were more sensitive than dogs to TCDD. The oral LD_{50} s of the same TCDD preparation were 0.6 (u)g/kg in the guinea pig, 22-45 (u)g/kg in the rat, and 115 (u)g/kg in the rabbit. Death was usually delayed, occurring several weeks after TCDD was administered. Weight loss was seen in all species after TCDD treatment, while other symptoms were species-specific. Histological lesions observed in most species included hepatic necrosis, fat atrophy, and periarteritis. Single doses of 32-500 (u)g/kg TCDD in acetone were applied to the shorn abdominal skin of rabbits, and the site of administration was wrapped in cotton after the acetone evaporated. Rabbits were administered single doses of 32-500 (u)g/kg TCDD in corn oil suspension intraperitoneally and observed for 4 weeks. Lethality of TCDD in the rabbit was the same by all routes of administration. Eye irritation was evaluated in rabbits to which 2 mg (article does state 2 milligrams) of TCDD was instilled into the conjunctival sac of one eye. Delayed conjunctival chemosis was observed 13-22 days after TCDD was applied ocularly. Acnegenic activity was evaluated in rabbits that received 0.1 ml of the supernatant of a TCDD suspension in benzene applied to the inner surface of the ear 5 days per week for 4 weeks. Acne was produced by TCDD concentrations above 0.04 (u)g/ml, with the severity of the response dependent upon the concentration applied to the ear. White Leghorn chickens were administered 1 or 10 (u)g/kg TCDD daily in their diets and were observed for symptoms of chick edema syndrome. Daily doses of 1 or 10 (u)g/kg of TCDD produced chick edema syndrome, characterized by increased pericardial fluid volume, dyspnea, subcutaneous and pulmonary edema, and enlarged, mottled liver, and histologically identified atrophy of the germinal centers of the spleen, few bursal lymphocytes, and fatty degeneration. The toxicities of other chlorodibenzo-dioxins were also examined. The authors concluded that TCDD had a very high order of toxicity relative to the other dioxins tested, and the toxicological properties of the various chlorodibenzodioxins were different. The authors suggested that repeated contact with small amounts of TCDD would probably produce chloracne in man, but TCDD contact with the eyes would probably not impair vision seriously.

875. Schwetz, B. A., Sparschu, G. L., and Gehring, P. J. (1971) The effect of 2,4-dichlorophenoxyacetic acid (2,4-D) and esters of 2,4-D on rat embryonal, foetal and neonatal growth and development. Fd. Cosmet. Toxicol. 9:801-817.

The teratologic and reproductive effects of 2,4-D and two derivatives of 2,4-D were evaluated in rats. Pregnant rats were orally administered 12.5-87.5 mg/kg of 2,4-D daily from day 6-15 of gestation. Other rats received the molar equivalents of 2,4-D propylene glycol butyl ether (PGBE) or 2,4-D isooctyl ester (IO) on days 6-15 or on days 5-8 or IO on days 8-11 or days 12-15. All compounds were administered in corn oil and the control rats received only corn oil. On day 20 of gestation weights, and skeletal malformations at 21 days of age were recorded. No maternal toxicity, measured as body-weight gain, was observed with the doses administered. All 3 compounds caused a decrease in fetal body weight at doses at 75 and 87.5 mg/kg. Only PGBE produced a reduction in percent resorptions, but this effect was small and was not dose-related. The incidence of subcutaneous edema was increased by all 3 compounds administered at doses of 50 mg/kg or greater. Other skeletal defects included delayed ossification of sternbrae, wavy ribs, missing sternbrae (all significantly increased by high doses of 2,4-D or PGBE), sternbrae with split centers of ossification (significantly increased by high doses of 2,4-D or IO), lumbar ribs (caused by all 3 compounds) and delayed ossification of skull bones (significantly increased by IO). Administration of 87.5 mg/kg molar equivalents of PGBE or IO on days 5-8 did not alter the numbers of implantations or of corpora lutea per litter. When administered on days 8-11, this treatment produced a significant increase in resorption but did not produce a change in fetal size. The only alterations in post natal indices were decreases in viability and lactation indices in the 75 and 87.5 mg/kg (equivalent) groups treated with PGBE and IO. The delayed bone ossification was a temporary condition that was not observed in 3-week-old neonates. The authors concluded that 25 mg/kg or its molar equivalent was the no-effect dose for each of the 3 compounds and the toxicity increased in the order: PGBE, 2,4-D, IO. The authors also concluded that all 3 compounds were embryotoxic and fetotoxic, but were not teratogenic or detrimental to survival of offspring to 21 days of age.

876. Scientific Dispute Resolution Conference on 2,4,5-T. (1979) Sponsored by The American Farm Bureau Federation, 101 p.

[Review article.]

877. Scifres, C. J., McCall, H. G., Maxey, R., and Tai, H. (1977) Residual properties of 2,4,5-T and picloram in sandy rangeland soils. J. Environ. Qual. 6(1):36-42.

The authors studied the movement and persistence of 2,4,5-T and picloram in a sandy rangeland ecosystem. Three study sites were chosen: 1) Bastrop, consisting of 8.1ha of Coastal Bermuda grass in

Edge-Tabor sandy clay loam soil; 2) Caldwell, 3.8ha of Coastal Bermuda grass in Laverne-Tabor-Lakeland loamy fine sand, and 3) San Perlita which had coastal prairie climax vegetation. The Bastrop and Caldwell sites were sprayed with 0.56 Kg/ha 2,4,5-T and 0.56 kg/ha picloram using a ground sprayer in 1973. Bastrop was sprayed again in 1974. San Perlita received only one spraying in 1974. Twenty five vegetation and soil, samples water samples were collected from each site and analyzed by gas-liquid chromatography (detection limit 10 ppb soil and 5 ppb water). Immediately after the first spraying at Bastrop, grass tissue contained 58,840 ppb 2,4,5-T and 109,351 ppb picloram. Levels of both herbicides decreased by about 90% during the 1st 7 days after application. No 2,4,5-T was detected at 112 days. Picloram persisted for 224, and detectable levels were measured at 365 days. Twice as much 2,4,5-T was detected on Bermuda grass after the 2nd spraying at Bastrop, the primary source of variation being a 50% decrease in the quantity of vegetation at application in 1974. Persistence of 2,4,5-T in 1974 was similar to 1973. By 112 days, no 2,4,5-T was detected. Picloram persisted for 112 days in 1974. At Caldwell, both herbicides persisted for longer periods. 112 days for 2,4,5-T and 224 days for picloram. In Bastrop soil samples, levels of 2,4,5-T increased from 0 to 7 days probably as a result of runoff. Only trace amounts (10ppb) of 2,4,5-T were detected on days 28 and 56; no 2,4,5-T was detected at 112 days. For the most part, 2,4,5-T residues were restricted to the top 2.5 cm of soil. A similar pattern of 2,4,5-T residues was observed at Bastrop in 1974 and at Caldwell in 1973. At Bastrop in 1973, picloram residues were similar to the pattern five to 2,4,5-T. In 1974, picloram residues in soil did not peak until 28 days after spraying occurred in the top 18 cm of soil. Trace amounts of picloram were detected up the top 15 cm of soil up to 112 days after spraying. Except on day 56, no picloram residues were detected below 15 cm. At Caldwell, 2,5,5-T residue were present immediately after spraying. Only trace residues were detected at 7, 28, and 56 days and no residues were detected at 112 days. Except for trace amounts of picloram at 2,5-15 cm on days 7, 28, and 56, no picloram residues were detected below the top 2.5 cm of soil at Caldwell. At San Perlita, neither 2,4,5-T nor picloram residues were detected below the top 2.5 cm of soil. 2,4,5-T persisted for 34 days, while picloram was still being detected at 68 days. At Bastrop, residues of both herbicides were present in water runoff 27 days after application. At Caldwell, 2,4,5-T residues were detected in runoff at 27 days and picloram residues were present at 56 days. The authors made no conclusions regarding the effects of 2,4,5-T and picloram on the rangeland ecosystem. However, it appears that both 2,4,5-T are not persistent compounds in a rangeland environment, although persists slightly longer than 2,4,5-T. Neither compound appears to accumulate in vegetation or soil.

878. Seabury, J. H. (1963) Toxicity of 2,4-dichlorophenoxyacetic acid for man and dog. Arch. Environ. Health 7:202-209.

The therapeutic efficacy of 2,4-D for fungal infections was evaluated in dogs and in man. Three dogs were infected with Histoplasma

capsulatum and were treated with intravenous doses of 1.2-3.2 mg/kg of the sodium salt of 2,4-D for approximately one month (total doses for the three dogs were 277 mg, 389 mg and 464 mg). The dogs were autopsied from 85 to 202 days after the last injection. No gross or microscopic lesions were observed in the tissues examined except those attributed to the infection. No neurological tissues were examined and no comparison was made of the extent of infection of the 3 treated dogs compared to 3 control dogs that received only the fungus. Two patients with disseminated coccidioidomycosis (and poor prognoses) were treated with 2,4-D. The first patient was given intramuscular injections over 4 days of 40 mg (total) of the sodium salt of 2,4-D and 3.3 mg each of indole-3-propionic acid and indole-3-butyric acid. The patient died on the fifth day; no symptoms or autopsy findings were attributed to 2,4-D treatment. The second patient received a total of 12.7 g of 2,4-D over a period of 34 days (along with 369 mg indole-3-butyric acid and 38 mg of naphthaleneacetic acid) with no toxicity observed. An intravenous dose of 3600 mg of sodium 2,4-D 2 days later caused the patient to lapse into a deep stupor, accompanied by hyporeflexia and urinary incontinence. These symptoms were alleviated within 24 hours, although muscle weakness and lethargy persisted for an additional 24 hours. The patient died 2 weeks later and all lesions observed grossly and microscopically at autopsy were attributed to the fungal infection, including hepatic and renal congestion. Other tissues, including the treated muscle, heart, and pancreas appeared normal except for leptomeninges over the brain stem. Death was attributed to disseminated coccidioidomycosis.

879. Seefeld, M. D., Albrecht, R. M., and Peterson, R. E. (1979) Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on indocyanine green blood clearance in Rhesus monkeys. Toxicology 14(3):263-272.

Hepatic function was assessed in monkeys treated with a single dose of TCDD. Adult male Rhesus monkeys were given TCDD in acetone-corn oil (0.5:9.5) by gastric intubation. One animal each received 5 (u)g/kg and 75 (u)g/kg bitol dehydrogenase (SDH), and gamma glutamyl transpeptidase (GTP) were also measured. [Histopathological examination of the liver was performed.] Monkeys died or became moribund and were killed 4 weeks after the high dose, and 6-7 weeks after the middle dose. The monkey that received the low dose survived, and the only changes observed were elevated SDH and SGPT levels 2-5 weeks after treatment. The 2 monkeys given 25 (u)g/kg TCDD showed: elevated ICG clearance for 4 weeks after treatment, followed by a decrease until death occurred; weight losses of 27 percent and 42 percent of pretreatment weights; and elevated SDH and SGPT levels. The same changes occurred after the high dose, but the changes occurred sooner and were generally greater. Weight loss was 45 percent of pretreatment weight. Monkeys at the highest two doses had symptoms which included blepharitis, facial edema, changes in the skin and nails, and loss of hair. Only the last symptom was observed from the lowest dose. Lipid accumulation in the liver and occasional necrotic hepatocytes were observed. The authors concluded that serum SDH and SGPT levels were the most sensitive parameters of toxicity of those studied and suggested that the

transient increase in ICG clearance observed after TCDD treatment reflected an adaptive response in increased hepatic blood flow. The authors suggested that the fall in ICG clearance may reflect hepatotoxicity or could be caused by the starvation state of the monkeys.

880. Seiler, J. P. (1979b) Phenoxyacids as inhibitors of testicular DNA synthesis in male mice. Bull. Environ. Contam. Toxicol. 21:89-92.

The author tested 2,4-D and 2,4,5-T in a mouse testicular DNA synthesis inhibition test. According to the author this assay correlates well with the carcinogenicity of chemicals. One hour before treatment male mice (number unspecified) received 1 μ C¹⁴-thymidine intraperitoneally. Herbicides were administered in a single oral dose: 200 mg/kg 2,4-D, 200 mg/kg 2,4,5-T acid, or 50, 100, 200, or 400 mg/kg 2,4,5-T isooctyl ester. The animals were given intraperitoneal injections of 10 μ Ci ³H-thymidine 3-96 hours later. The ratios of ³H counts per μ g DNA and of ³H counts per ¹⁴C counts were then compared to controls. Criteria for a positive result was that thymidine uptake was depressed significantly (statistical test not specified). All three compounds significantly inhibited testicular DNA synthesis. The author concluded that more work needs to be done on the carcinogenicity of these compounds.

881. Seiler, J. P. (1978a) The genetic toxicology of phenoxy acids other than 2,4,5-T. Mutat. Res. 55:197-226.

[Review article.]

882. Seiler, J. P. (1978b) Herbicidal phenylalkylureas as possible mutagens. I. Mutagenicity test with some urea herbicides. Mutat. Res. 58:353-359.

The authors evaluated the mutagenicity of diuron, monuron and several other phenylalkylurea herbicides in the mouse testicular DNA synthesis inhibition (DSI) test, the Ames test, and the micronucleus test in mice. The herbicides used in the tests were either pure substances or commercial formulations. No specific designation was made for each compound. In the DSI test, each herbicide was administered in a single oral aqueous dose given by stomach tube to 4 male mice (age and strain unspecified) per dose level. Three hours later each mouse received an intraperitoneal injection of 10 μ Ci ³[H]-thymidine. Thirty minutes later the mice were killed, testes removed and homogenized [³H]-TdR incorporation measured. Both monuron and diuron significantly inhibited (p less than 0.05) testicular DNA synthesis. Each compound was also screened for mutagenicity in the Ames test. Data for only one tester strain (Salmonella typhimurium TA1535) were reported. This strain reverts from histidine requiring to prototrophy by base pair substitution. Rat liver S9 was used as the metabolic activation system. Both compounds induced dose dependent increases in numbers of revertants in the plate assay and also in a number of tubes with

microbial growth in the fluctuation assay. No positive or negative controls or numbers of replicate plates were reported. The authors also tested monuron and diuron in a micronucleus test in mice. Very few details were reported. Six mice per dose level (age, strain, sex unspecified) received doses of 1000 or 2000 mg/kg monuron or 1000 or 2000 mg/kg diuron. Six mice served as controls. Percentage of micronucleated polychromatic erythrocytes in bone marrow cells were scored. No differences between control and treated mice were observed. The testing presented in this paper cannot be viewed as reliable evidence of mutagenic effects primarily because the author fails to present methodology, description of animals used and controls, and criteria used in evaluating the results of his study.

883. Seiler, J. P. (1973) A survey on the mutagenicity of various pesticides. Experientia 15(5):622-623.

The author tested 2,3,7,8-TCDD and 25 other pesticides in Salmonella typhimurium strains his G46, TA1530, TA1531, TA1532 and TA1534. In this system, the tester strain reverts from histidine dependence to histidine independence when treated with a mutagen. According to criteria set by the author, a strong mutagenic response was defined as a relative mutagenicity (number of revertants from treated plates per 10^8 bacteria/spontaneous reversions per 10^8 bacteria) of greater than 10. A medium mutagenic response was defined as a relative mutagenicity of 5-10, a weak response was a relative mutagenicity value of 3-5, a doubtful response was a relative mutagenicity of 1-2, and a negative response was defined as a relative mutagenicity of 1. 2,3,7,8-TCDD was a strong mutagen in strain TA1532, a doubtful mutagen in strains TA1531 and TA1534, strains which are reverted by frameshift mutagens. The compound was a direct acting mutagen, that is, it did not require exogenous metabolic activation for a mutagenic effect 2,3,7,8-TCDD was negative in G46 and TA1530, which are reverted through base pair substitutions. No numerical data for chemically induced-revertants or spontaneous revertants were presented by the authors. This prevents a proper evaluation of the test system in that laboratory. The author also did not include the numbers of replicate plates used in the experiment, the dose of TCDD used, or the number of times the experiment was repeated. Failure to include this information severely limits the value of the data since no quality of experimental design can be evaluated.

884. Shadoff, L. A., Hummel, R. A., Lamparski, L., and Davidson, J. H. (1977) A search for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in an environment exposed annually to 2,4,5-trichlorophenoxyacetic acid ester (2,4,5-T) herbicides. Bull. Environ. Contam. Toxicol. 18(4):478-485.

The authors measured TCDD residues in environmental samples taken from two areas where 2,4,5-T was extensively used as a herbicide. The first sample area was a Texas drainage impoundment that is part of a watershed in which large areas have been sprayed with 2,4,5-T for control of mesquite (0.5 lb/acre, 2,4,5-T acid equivalent) and brush

(3-4 lbs/acre, 2,4,5-T). Samples of water, mud, catfish, and walleyed pike were collected from the impoundment. Human milk samples from mothers residing in the area were also collected. The second sampling area was a pond in Arkansas used to flood to rice fields that had been sprayed with 1.25 lb/acre/2,4,5-T 4-8 weeks previously. Application of 2,4,5-T had occurred for 18 years up to the sampling time. Water, mud, catfish, and walleyed pike samples were collected. Analysis of the samples for TCDD was performed by GC-MS; limits of detection for the system used in the study averaged less than 10 ppt. Samples sizes were 10 of fish and mud, 20 of human milk, and 500 ml water. No TCDD was detected under the conditions of the study.

885. Shafik, M. T., Sullivan, H. C., and Enos, H. F. (1971) A method for determination of low levels of exposure to 2,4-D and 2,4,5-T. Intern. J. Environ. Anal. Chem. 1:23-33.

Levels of 2,4-D and 2,4,5-T in the urine of occupationally exposed workers and of rats after oral administration are presented. Twenty-two workers occupationally exposed to 2,4-D, 2,4,5-T or both were categorized by occupation and urine samples were analyzed for these herbicides by gas chromatography. No other details about the characteristics of the exposed group or the timing of urine collection were given. 2,4-D was detected in urine of farmers, spray operators (method of spraying was not given), and aircraft spray operators. Each group consisted of 2 people and urine levels in each of these workers ranged from 0.2 to 1.0 ppm. 2,4,5-T was detected in all samples from a group of 6 spray operators, 2 spray crew foremen and 2 aircraft spray operators; levels ranged from 0.05 to 3.6 ppm. No 2,4,-D was detected in urine from 2 herdsmen, 4 farm laborers and 2 project officers exposed to 2,4-D. 2,4,5-T was not detected in 2 farmers exposed to 2,4,5-T. Male Sprague-Dawley rats (two per group) were administered daily doses of 3.75 ug/kg to 37.5 mg/kg 2,4-D or 5 ug/kg to 50 mg/kg 2,4,5-T for 3 days. Urine samples were collected for 7 days after the start of exposure and were analyzed for 2,4-D and 2,4,5-T by gas chromatography. After the 7 days, from 28% of the highest dose to 75% of the lowest dose of 2,4-D had been recovered in urine and from 49% of the lowest dose of 2,4,5-T and all of the highest doses were excreted in urine. The authors described methods for detecting derivatives of these herbicides in biological samples and concluded that their methods were suitable for detecting low levels of exposure from analysis of urine collected within 24 hours of exposure.

886. Shapley, D. (1973) Herbicides: Agent Orange stockpile may go to the South Americans. No reference.

[Editorial.]

887. Sharma, R. P., and Gehring, P. J. (1979) Effects of 2,3,7,8-tetra-chlorodibenzo-p-dioxin (TCDD) on splenic lymphocyte transformation in mice after single and repeated exposures. Ann. N. Y. Acad. Sci. 320:487-497.

Cellular immunity is described from in vitro studies of splenic lymphocytes of mice treated with TCDD acutely or subacutely. Male CD-1 mice were orally administered 0.01-10 ug/kg TCDD in acetone-corn oil (1:20), weekly for 8 weeks or 10 ug/kg, once. Animals were killed 2, 4 and 8 weeks after treatment started and splenic lymphocytes were isolated and cultured with phytohemagglutinin or pokeweed mitogen. Incorporation of [³H]-thymidine by lymphocytes from TCDD-treated mice and lymphocytes treated with TCDD in vitro was assessed. Total serum immunoglobulins were assayed by quantitative immunoelectrophoresis. Increased liver weights and hepatic necrosis were observed in mice administered 10 ug/kg/week of TCDD; spleen, thymus and kidney weights were not significantly altered by TCDD treatment. Lymphocyte transformation in the absence of mitogens was stimulated 2 and 4 weeks after TCDD treatments started; in the presence of mitogens, TCDD-treated lymphocytes showed less stimulation than non-TCDD-treated controls, after 2 and 4 weeks. The acute dose produced the same effects as the subacute dose although stimulation by the acute dose was more transient than the subacute effect. In vitro doses of 10^{-9} to 10^{-4} M TCDD produced a dose-related decrease in [³H]-thymidine incorporation of lymphocytes and no change in the responses of these cells to mitogens. Immunoglobulin levels were increased in mice administered 0.01-0.1 ug/kg TCDD doses and decreased at higher doses. The authors concluded that TCDD was immunosuppressive in mice and the toxic effect on thymic tissue may be transient.

888. Sharp, C. W., Ottolenghi, A., and Posner, H. S. (1972) Correlation of paraquat toxicity with tissue concentrations and weight loss of the rat. Toxicol. Appl. Pharmacol. 22(2):241-251.

The tissue distribution of diquat is described for the rat. Male Sprague-Dawley rats were administered 20 mg/kg of [¹⁴C]-diquat dichloride (containing diquat-dibromide carrier) intravenously. Other rats were administered 20 mg/kg [¹⁴C]-paraquat dichloride intravenously (both radioactive compounds 99+% pure). Animals were killed 1-10 days later and tissues were removed. Paraquat was analyzed colorimetrically in acid-extracts of lung, muscle, liver, and kidney homogenates. (Determination of diquat concentrations was not described.) The ratio of the concentrations of paraquat to diquat was reported. The ratios for the lung and muscle were about 10, ranging from 2-16 in muscle and 8-33 in lung. The ratios were below one for kidney and liver and were between 2 and 8 for heart, adrenal, spleen, stomach, ileum, testes, and thymus. The distribution and toxicity of paraquat were described further. The authors concluded that diquat, in contrast to paraquat, did not concentrate in the lung or cause pulmonary toxicity.

889. Shavgulidze, M. M., Nanobashvili, V. I., and Mirianashvili, M. N. (1976) On the toxicity of the herbicide 2,4-D. Veterinaria (4):103-104.

The acute and chronic toxicities of 2,4-D were studied in sheep. Single doses of 200-900 mg/kg 2,4-D sodium salt were administered perorally to sheep. Other sheep were administered 120 daily doses of 18 mg/kg 2,4-D amine salt orally. Mortality and clinical signs from the acute doses recorded and after subacute exposure, clinical signs were recorded and hematological and biochemical blood tests were performed. A dose of 900 mg/kg killed all sheep. Clinical signs of intoxication occurred after doses of 500 mg/kg or greater and included general weakness, ataxia, immobility, muscular paralysis of the hind legs, difficulty in breathing, sensitivity to light, and lack of appetite. Death occurred 2-4 days after exposure. Fluctuations in both directions occurred during the first month for the erythrocyte count, leukocyte counts, hemoglobin levels, blood nitrogen levels, and "alkaline reserve" (no further explanation given for this parameter). No traces of 2,4-D, administered as a single dose of 300-400 mg/kg of the amine or sodium salt, were detected 12 days after exposure (no other methodology was reported for this experiment). The authors concluded that meat from sheep was safe for human consumption 12 days after sheep had ingested 2,4-D and that 2,4-D was a compound of moderate toxicity.

890. Shaw, B., and Hopke, P. K. (1975) The dynamics of diquat in a model eco-system. Environ. Letters 8(4):325-335.

The authors report on the distribution and accumulation of diquat in a model aquatic ecosystem. An aquarium tank (20 gal.) containing Lake Chautauqua water and sediments and plants, Norlunge fingerlings, snails, and Hyalella was treated with 64.5 mg diquat of which 13.5 mg was labeled with ^{14}C . A control tank was also included in the experiment. Water samples were removed daily for 9 days and analyzed for ^{14}C by scintillation counting. When radioactivity in the water reached background levels, the experiment was terminated. Plants, animals, and sediments were analyzed for ^{14}C uptake. C-diquat and not a metabolite or $^{14}\text{CO}_2$ was being taken up by the components of this model. The authors also performed a 96-hour acute toxicity study in muskelunge using diquat. However, more than 10% of the fish in the control tank died, which invalidates the experiment.

891. Shepard, B. (1980) Presentation at the 2d Continuing Education Conference on Agent Orange, Washington, DC, May 28-30.

[Background material.]

892. Sherman, H., and Kaplan, A. M. (1975) Toxicity studies with 5-bromo-3-sec-butyl-6-methyluracil. Toxicol. Appl. Pharmacol. 34(2):189-196.

A series of reproductive, acute, and chronic toxicity studies of 5-bromo-3-sec-butyl-6-methyluracil (bromacil) were performed in mammals, fish, and wildlife. An outline of the methods used for the studies and a brief summary of the results were presented. Acute oral toxicity was evaluated in male Charles River animals (presumably rats) after a 14 day evaluation period and in male mongrel dogs. Subchronic toxicity was studied in male Charles River rats given 10 doses of bromacil by gastric intubation over 2 weeks. Acute dermal toxicity was tested by applying a 70% aqueous paste of bromacil to the clipped skin of 3 rabbits and fitting each animal with a plastic collar to prevent ingestion. After 24 hours, the compound was removed and the animals were observed for toxic effects for 2 weeks. Skin irritation and sensitization of 50% bromacil pastes were tested in guinea pigs given a single or 3 doses of bromacil. The dry powder and a 10% solution of bromacil in mineral oil were tested for ocular irritation in rabbits. Acute inhalation toxicity was tested in rats exposed to 2.1 mg/liter or 4.8 mg/liter bromacil in air for 4 hours. Acute toxicity of bromacil to fish and wildlife was tested by previously published methods that were not described. Pregnant rabbits were administered dietary levels of 50 or 250 ppm bromacil during days 8-16 of gestation. Fetuses were removed on day 28 or 29 and examined for gross malformations or stained with alizarin red to observe skeletal malformations. Resorptions were also counted. Other litters were delivered. A ninety day feeding study was performed on rats. The doses initially were 50, 500, and 250 ppm for 3 treatment groups. The highest dose was increased several times to a final dose of 7,500 ppm. Urine, hematology, and renal function tests were performed monthly and histopathology was conducted at the end of the feeding period. A 2-year feeding study was also performed in rats with groups administered 50, 250, and 1,250 ppm bromacil diet. In addition to parameters used for the 90-day study, food consumption, body weights, and tibia growth were monitored. A 2-year feeding study in dogs was performed using the same doses as for the rat study (except the highest dose was administered after a week of lower doses being administered). The same parameters were monitored as in the rat study, and respiration, pulse rate, and body temperatures were monitored twice weekly, as well. A 3-year reproduction study was performed in rats given 250 ppm dietary levels of bromacil. Offspring were maintained on the experimental (or control) diet for 110 days after weaning and then were mated with rats from the same group. The oral LD₅₀ was 5,200 mg/kg in the male rat. Doses of 100 mg/kg or greater produced emesis in the dog, precluding establishing an LD₅₀ for this species. No dermal toxicity, mild dermal and eye irritations, and no skin sensitization were observed. The LC₅₀ for inhalation exposure was above 4.8 mg/liter and for fish was 70-165 ppm. For wildlife the dietary LC₅₀ over 8 days was greater than 10,000 ppm. Subchronic toxicity was elicited as gastrointestinal and central nervous system disturbances from 10 daily doses of 1,035 mg/kg. Mortality of 1,500 mg/kg/day was 67% (4/6) and at 1,035 mg/kg was 17% (1/6). The highest dosage group in the 90-day study showed histological changes of the

thyroid and liver and lowered body weight gain. No other groups showed any adverse effects. No malformations were identified in the teratology study. A decreased weight gain by the highest treatment group was the only deviation noted in both the chronic dog and chronic rat studies. No reproductive effects were observed. No carcinogenicity was observed in the studies with chronically treated animal groups. The authors concluded that bromacil has a very low order of toxicity.

893. Shirasu, Y., Moriya, M., Kato, K., Furuhashi, A., and Kada, T. (1976) Mutagenicity screening of pesticides in the microbial system. Mutat. Res. 40:19-30.

The authors studied the DNA damaging and mutagenic capabilities of bromacil, 2,4-D, diuron, monuron, 2,4,5-T, and 161 other pesticides using three bacterial assays, a rec assay in Bacillus subtilis and reverse mutation assays in Escherichia coli and Salmonella typhimurium. In the rec assay B. subtilis strains H17 Rec+ and M45 Rec- were utilized. Each chemical dissolved in DMSO was added in a 0.02 ml aliquot of a 1 mg/ml solution to a paper disc covering a streak culture of each of the two bacterial strains. After 24 h incubation, the length of inhibition of bacterial growth for each streak (rec+ and rec-) was measured. DNA damaging capability is measured by preferential inhibition of the rec-strain. However, criteria for a positive result was not reported by the authors. However, according to their results none of the above mentioned herbicides was positive in the assay. No metabolic activation was used and no positive controls were mentioned. In the reversion assay, E. coli strains B/R try WP2 and WP2 try hcr were utilized. Both strains revert from tryptophan requiring to prototrophy when exposed to a mutagen that acts by base pair substitutions. Four strains of S. typhimurium were also employed in the reversion assay, TA1535, TA1536, TA1537, and TA1538. These strains revert from histidine dependence to histidine independence by mutagens that induce base-pair substitutions (TA1535) or frameshift mutations (TA1536, TA1537, and TA1538). Each of the herbicides was dissolved in DMSO and added to a filter disc (0.02 ml of a 1 mg/ml solution) which was placed on an agar plate containing bacteria. Criteria for a positive result was not discussed by the authors. No positive controls were reported. In addition, metabolic activation was not included in the testing; therefore, only direct acting mutagens would show a positive result. All of the herbicides mentioned above were negative in both reversion assays.

894. Shirasu, Y. (1975) Mutagens and teratogens. Cong. Anom. 15(4):187-189.

[Review article.]

895. Shu, H., Talcott, R. E., Rice, S. A., and Wei, E. T. (1979) Lipid peroxidation and paraquat toxicity. Biochem. Pharmacol. 28(2):327-331.

Distribution of diquat to the lung and production of lung edema was studied in the rat. A dose of 45 mg/kg [¹⁴C] diquat dibromide in saline was administered subcutaneously to rats and lung tissue was removed after 3 hr. from four rats or 6 hours from seven rats, perfused briefly to remove blood, digested, and counted for radioactivity. Ether anesthetized rats (6-11 per gr.) were administered [¹²⁵I]-bovine serum albumin intravenously, immediately followed by a subcutaneously dose of 32 or 45 mg/kg diquat. Twenty-four hours after the higher dose and 48 hr. after the lower dose, the trachea was cannulated and saline was flushed into the lungs to remove recoverable radioactivity (recovery of radioactivity was estimated at 80% by this method). Blood was also collected and counted for radioactivity. Between 70 and 95% of the radioactivity from the lung lavage and plasma were precipitable with 10% trichloroacetic acid. Diquat was present in the lung at higher concentrations after 3 hr. than after 6 hrs. and the levels at both times were less than half of paraquat levels after equimolar doses had been administered. After 24 and 48 hours, diquat produced a 1.6 fold and 2.1 fold increase, respectively, in alveolar albumin content and minimal effects in plasma levels of [¹²⁵I]-radioactivity. Other effects of paraquat were described. The authors concluded that diquat produced less pulmonary edema and reached the lung in lower amounts than paraquat and indicated that enhanced in vitro stimulation of lipid peroxidation of diquat over paraquat was not consistent with in vivo mechanisms of toxicity.

896. Siebert, D., and Lemperle, E. (1974) Genetic effects of herbicides: Induction of mitotic gene conversion in Saccharomyces cerevisiae. Mutat. Res. 22:111-120.

The authors tested commercial preparation of dalapon, bromacil, diuron, diquat, 2,4,5-T amyl ester, and 2,4-D and 24 other herbicides in a mitotic gene conversion assay in Saccharomyces cerevisiae. In the assay S. cerevisiae strain D4 was employed. This strain is a diploid heteroallelic at two loci, ade 2 and trp 5 which makes the yeast adenine and tryptophan requiring. The induction of gene conversion by a genetically active compound results in cells that no longer require either adenine or tryptophan. Convertants are measured by use of selective medium. Yeast cells in the logarithmic growth phase were treated with 1,000 ppm of each of the herbicides in a liquid suspension culture at pH 4.5 or 7.0 for 16 hours after which cells reported by the authors. Purity of the commercial preparations tested was not reported. In addition criteria for a positive result were not presented by the authors. However, according to the authors, diquat and 2,4-D induced a significant increase in mitotic gene conversion compared to controls. Both compounds were tested at pH 4.5. 2,4-D increased convertants 5 fold at the ade 2 locus and 6 fold at the trp 5 locus, while diquat induced a 7 fold increase at the ade 2 locus and 4-5 fold at the trp 5 locus. Bromacil (pH 4.5) and 2,4,5-T (pH 7.0) amyl ester increased

convertants slightly but not significantly over controls while convertant frequencies induced by dalapon (pH 4.5) or diuron (pH 4.5) did not differ from controls. The rationale behind using 2 different pH values in the testing was not presented by the authors.

897. Sigmon, C. F. (1979a) Influence of 2,4-D and 2,4,5-T on life history characteristics of Chironomus (Diptera: Chironomidae). Bull. Environ. Contam. Toxicol. 21:596-599.

The author studies the effects of 2,4-D and 2,4,5-T butoxyethanol esters on pupation, emergence, and mortality in Chironomus. Midges were collected from sewage oxidation ponds and transferred to culture dishes (10 animals/dish) of lake water in the laboratory. After overnight acclimation to the water, midges were exposed to 1 or 3 ppm 2,4-D or 2,4,5-T acid equivalent at 20, 25, and 30°C. Commercial preparations of herbicide were used; no purity data were available. Fresh herbicide solutions were added daily, and the mortality, pupation, and emerging of animals was recorded. Only 2,4-D at 30°C had any effect on Chironomus. At both 1 and 3 ppm, 2,4-D caused increased larval mortality and decreased pupation and emergence. The author stated that concentrations of herbicides in the experiment represented the highest values observed in waterways and runoff. Although effects from 2,4-D were observed, the author concluded that these effects would not be important in nature.

898. Sigmon, C. (1979b) Oxygen consumption in Lepomis macrochirus exposed to 2,4-D or 2,4,5-T. Bull. Environ. Contam. Toxicol.

The author determined the short term uptake of 2,4-D and 2,4,5-T bluegill sunfish and examined the metabolic response of the fish to the herbicides. Fish used in the experiment ranged from 0.28-23.30g and were randomly assigned testing tanks that housed either one fish greater than 2g or four fish less than 2g. Respiration was measured by the azide modification of the Winkler technique. Fish were exposed to water containing 3 ppm 2,4-D or 2,4,5-T, butoxyethanol esters. Respiration was determined at 20, 25, and 30C. The herbicides had no effect on respiration. Fish were also maintained for 8 days in water containing 3 ppm 2,4-D or 2,4,5-T at 20, 25, and 30C. Uptake of herbicides was measured by gas liquid chromatography. Both controls and 2,4-D exposed fish had uptake levels of less than 0.05 ppm while 2,4,5-T exposed fish had 0.06-0.12 ppm uptake. Uptake of 2,4,5-T was not related to temperature. The significance of this study was not discussed by the author.

899. Sigmon, C. F. (1979c) Physiological effects of 2,4-D and 2,4,5-T on selected aquatic organisms. A Report to the Office of Water Research and Technology, Washington, DC. USNTIS Publication No. PB294539/25T. 30 pp.

[Not available.]

900. Simmon, V. F., Mitchell, A. D., and Jorgenson, T. A. (1977) Evaluation of selected pesticides as chemical mutagens: in vitro and in vivo studies. U.S. Environmental Protection Agency, Research Triangle Park, N.C. Report No. EPA-600/1-77-028. 237 p.

Twenty compounds, including bromacil, cacodylic acid, and monuron, were evaluated for mutagenic activity. Only bromacil was among ten compounds selected for assay in the mouse dominant lethal test. There was no time or dose-response effect in this assay associated with bromacil at 1250, 2500, and 5000 mg/kg of diet. Bromacil, cacodylic acid, and monuron were evaluated in the following test systems: unscheduled DNA synthesis in human fibroblast (WI-38 cells, reverse mutation in Salmonella Typhimurinum strain TA1535, TA1537, TA1538, and TA100 and in Escherichia coli WP2; mitotic recombination in Saccharomyces cerevisiae D3, and preferential toxicity assays in DNA repair-proficient and deficient strains of Escherichia coli (strain W3110 and M45, respectively) and Bacillus subtilis (strains H17 and M45, respectively). These studies were reported in sufficient detail to support the author's conclusions and demonstrate that the study was well controlled and apparently well conducted. All of the results were negative with the exception that monuron (assayed at 10^{-5} , 10^{-4} , and 10^{-3} M) induced unscheduled DNA synthesis in the presence of metabolic activation enzymes. The authors concluded that this result may indicate that monuron is a procarcinogen and that this possibility should be evaluated using in vivo studies.

901. Simmon, S. F., Poole, D. C., Riccio, E. S., Robinson, D. E., Mitchell, A. D., and Waters, M. D. (1979) In vitro mutagenicity and genotoxicity assays of 38 pesticides. Environ. Mutagen. 1:142-143.

[Abstract, only.]

902. Simpson, G. R., Higgins, V., and Chapman, J. (1978) Exposure of council and forestry workers to 2,4,5-T. Med. J. Austr. 2(11):536-537.

[Not available.]

903. Simsiman, G. V., Daniel, T. C., and Chesters, G. (1976) Diquat and endothall: Their fates in the environment. Residue Rev. 62:131-174.

[Review article.]

904. Sirons, G. J., Frank, R., and Dell, R. M. (1977) Picloram residues in sprayed Macdonald-Cartier Freeway right-of-way. Bull. Environ. Contam. Toxicol. 18(5):526-533.

The authors describe the persistence and movement of picloram applied to the shoulders and median strip of a major highway. A commercial

formulation Tordon^R 101 containing 62.4 g (acid equivalent) picloram and 250.8 g (acid equivalent) 2,4-D present as triisopropanol amine salts, was applied at 350 g/ha picloram in alternate years beginning May 1968. Soil samples to 45 cm depth were collected at 7 sites along the Macdonald-Cartier Freeway beginning in May 1969 and continuing for 3 years. In 1971, grass samples were also collected at each site. Samples (50 g soil, 109 grass) were analyzed for picloram only by gas liquid chromatography (detection limit 0.01 ppm). No 2,4-D residue data were reported. The authors reported data for the study period 1968-1973. Samples were pooled in data presentation and no distinction was made between samples collected from median strips or shoulders. Picloram residues in the top 15 cm of soil were greatest one week after an accumulation in soils was presented.

905. Sjoden, P. O., Archer, T., and Soderberg, U. (1977) Effects of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) on radioiodine distribution in rats. Bull. Environ. Contam. Toxicol. 17(6):670-678.

Alterations in tissue distribution of radioactive iodine were determined in rat after 2,4,5-T administration. Rats were orally administered 100 mg/kg of 2,4,5-T (with less than 1 ppm TCDD contaminant) in corn oil 1-15 days prior to sacrifice. A 10 ug dose of ¹³¹I was administered intramuscularly 1 day prior to sacrifice. The blood and several tissues were analyzed for radioactivity. Controls were administered corn oil without 2,4,5-T. No differences were observed among the results from male Sprague-Dawley rats or male or female Wistar rats and these results are discussed together. Data from control groups were not presented but all experimental data were presented as percentages of control group data. Serum radioactivity was depressed especially 1 day after 2,4,5-T treatment. Increased brain, liver, and female thyroid levels occurred 1-3 days after 2,4,5-T treatment. The changes in other tissues were moderate or low and the increases in tissue radioactivity were not adequate to account for the loss from the serum. The authors concluded that increased renal clearance of iodine accounted for the serum loss. Measurement of radioactivity in the urine would be a more appropriate way to evaluate iodine clearance after 2,4,5-T administration than the indirect method used which failed to recover the administered dose of isotope in tissues.

906. Sjoden, P. O., and Soderberg, U. Phenoxyacetic acids: Sublethal effects. In Chlorinated Phenoxy Acids and Their Dioxins, C. Ramel, ed. (Ecol. Bull. No. 27, Stockholm: Swedish Natural Science Research Council, 1978) p. 149-164.

The sublethal effects of 2,4,5-T in rats were investigated in several different experiments. Various behavioral parameters were measured in adult and neonatal rats, including open-field activity, maze learning abilities and test-aversion discrimination. In addition, the effect of 2,4,5-T on food and water intake, thyroid regulation, electrolytic maintenance and amino acid metabolism in the basin were examined. For

all experiments, the 2,4,5-T was mixed with corn oil and administered orally at a dose of 100 mg. Adult rats were given a single dose while pregnant rats received the dose on day 7, 8, or 9 of gestation. Neonatal rats that had been exposed to 2,4,5-T in this manner were behaviorally tested at 35, 60 and 90, 95 and 125 days of age. Results of these behavioral tests indicated that prenatal exposure to 2,4,5-T tended to increase the exploratory activity of 90-day old rats in the open-field test. Increased activity was also evident in 35 and 60-day old rats that had been cross-fostered with control dams. Older cross-fostered rats, aged 95 and 125 days, did not exhibit increased exploration in this test. Rats exposed prenatally to 2,4,5-T also exhibited disabilities in maze learning and electric shock-avoidance conditioning. Adult rats fed a combination of saccharin and 2,4,5-T associated saccharin with 2,4,5-T and developed a resistant long-lasting taste aversion to the sweetener. Adult rats that received 100 mg of 2,4,5-T also displayed a reduction in food and water intake. Radioiodine distribution experiments revealed abnormal thyroid activity in adult rats that were given 2,4,5-T. These rats also exhibited electrolytic disturbances in the brain fluids. Reduction of amino acid synthesis, particularly tryptophan, in the brain of adult rats treated with 2,4,5-T was noted. Serotonin levels were also reduced in the brains of neonatal rats that had been exposed to 2,4,5-T prenatally. The authors concluded that acute exposure to 2,4,5-T, either prenatally or as an adult, produced significant effects on motor behavior, taste and learning abilities, food and water ingestion, thyroid activity and amino acid synthesis in the brain. They attempted to relate results of their taste-aversion experiments to animals in the wild that may be exposed to 2,4,5-T. A number of problems are associated with their investigation. The purity of the 2,4,5-T was not specified or even how the 2,4,5-T compound was prepared for administration was not described for any of the experiments. The ages, sex and number of control rats used for some of the experiments were not reported. It is unclear whether some of the radioiodine and amino acid studies were conducted using a separate group of rats or if rats in the other experimental group were examined. The authors also did not discuss other factors that may have influenced their results, particularly for the behavioral effects.

907. Sjoden, P. O., and Soderberg, U. (1975) Long lasting effects of prenatal 2,4,5-trichlorophenoxyacetic acid on open field behavior in rats: pre- and postnatal mediation. Physiol. Psychol. 3(2):175-178.

The behavioral development of rats treated with 2,4,5-T in utero or fostered by a 2,4,5-T treated mother were studied. Pregnant Wistar rats (12) were treated with one dose of 100 mg/kg of 2,4,5-T (less than 1 ppm TCDD contaminant) in vegetable oil by oral intubation on day 8, 9, or 10 of gestation. Ten pregnant control rats received vehicle only. Litters were reduced to 4 males and 4 females. Half of the litters were cross-fostered by dams of the other group and the remainder were fostered by their biological mothers. All offspring were weaned at 24 days of age and were tested on behavioral parameters at 35, 60, 95, and 125 days of age. Each rat was observed for 4

minutes on 4 consecutive days for ambulation activity (number of grids rat traverses), rearing (frequency rat rises on hind limbs), grooming (frequency rat washes face or body), and defecation. The colors used for the floor grid lines and fields were reversed on the fourth day. Offspring exposed prenatally or postnatally showed significantly more ambulation and rearing activities than controls on days 35 and 60. No changes were seen at 90 days of age; at 125 days of age, increased rearing and ambulation of prenatally exposed rats occurred only on day 1 and decreased activity of postnatally exposed rats also occurred on day 1 only. No sex-related differences were seen in any group. The authors concluded that 2,4,5-T exposure prenatally affects offspring during both prenatal and postnatal development, producing behavioral changes at doses that do not affect anatomical development. The authors speculated that altered thymus function may be responsible for observed behavioral modifications. The relationship of the parameters measured in this paper with any other behavioral parameters, particularly those related to human development, has not been established by these authors.

908. Sjoden, P. O., and Soderberg, U. (1972) Sex-dependent effects of prenatal, 2,4,5-trichlorophenoxy-acetic acid on rats' open field behavior. Physiol. Behav. 9:357-360.

Behavioral development was studied in rats exposed to 2,4,5-T in utero. Pregnant Wistar rats (25) were administered 100 mg/kg of 2,4,5-T (less than 1 ppm TCDD contaminant) orally in maize oil on day 7, 8, or 9 of gestation. Control (pregnant) rats were administered oil only. All offspring were observed for anatomical abnormalities grossly at birth and by autopsy at the end of the experiment. At about 90 days of age offspring were selected from the experimental and from the control groups and subjected to an open field behavior test. For this test, each rat was placed in the center of a grid and observed for 2 minutes. Behavior was recorded which included ambulation (number of squares traversed), rearing (frequency rat rose on hind legs), defecation, latency (time to leave starting square), and grooming. The test was repeated on 2 separate days and data were compared for all treated males and control males, and for treated and control females. No anatomical abnormalities were observed in any group and no behavioral differences were observed between the 2 female groups. Litter size and perinatal mortality were adversely affected by treatment. The experimental males showed significantly increased behavior for all parameters except defecation. The authors concluded that the experimental males were more explorative than male controls. The authors concluded that a behavioral effect could be elicited in male rat development at a dose of 2,4,5-T that did not elicit anatomical effects. The significance of the effect presented here is difficult to extrapolate to other situations without more information regarding the relationship of explorative behavior to other aspects of behavioral development. The effect described does not appear to be an adverse effect or the result of a change in the rate of behavioral development, although the authors did not address these issues.

909. Smith, A. E. (1978) Relative persistence of di- and tri-chlorophenoxy alkanolic acid herbicide in Saskatchewan soils. Weed Res. 18(5):275-279.

[Background material.]

910. Smith, A. E. (1976) The hydrolysis of herbicidal phenoxyalkanoic esters to phenoxyalkanoic acids in Saskatchewan soils. Weed Res. 16:19-22.

[Background material.]

911. Smith, F. A., Schwetz, B. A., and Nitschke, K. D. (1976) Teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in CF-1 mice. Toxicol. Appl. Pharmacol. 38:517-523.

The teratologic and embryotoxic effects of TCDD were evaluated in CF-1 mice. Doses of 0.001 to 3.0 ug/kg/day of TCDD in corn oil-acetone (98.2) were administered by oral gavage to pregnant mice on days 6 to 15 of gestation. On day 18 live, dead, and resorbed fetuses were counted and the maternal liver was weighed. Fetuses were examined for gross malformations and for visceral malformations after fixation with Bouin's fluid or skeletal malformations after staining with alizarin red. The maternal liver weight to body weight ratio was significantly increased only in the highest dose group, compared with vehicle controls. Resorptions per implantations increased significantly only in the 1.0 ug/kg/day group, while no significant changes were observed in the percentage of litters with resorptions, maternal or fetal weights, fetal length, or number of implantation sites. Increased incidences of cleft palate in the highest two groups and of dilated renal pelvis in the highest group were observed. The non-teratogenic dose of TCDD was 0.1 ug/kg/day.

912. Smith, L. L., and Rose, M. S. (1977) A comparison of the effects of paraquat and diquat on the water content of rat lung and the incorporation of thymidine into lung DNA. Toxicology 8 (2):223-230.

The effects of diquat administration to rats on lung edema, pulmonary cell proliferation associated with fibrosis, and mortality are described. A group of 50 Alderley Park rats were administered 105 u mole/kg diquat dichloride intraperitoneally and daily incidence of mortality was recorded. Controls were administered, saline [³H]-thymidine was administered intraperitoneally, and DNA synthesis was determined by the amount of radioactivity incorporated into the DNA fraction of lung tissue in 1 hr. in vivo. Pulmonary edema was determined by the loss in weight of the lung tissue when it was dried to a constant weight. About 70% of the rats died within 14 days of diquat treatment and 20% died within 2 days. On days 1, 2, 4, and 8 after diquat treatment, ³H-thymidine incorporation was measured and found to be reduced to about 1/2 of control levels. Small decreases in

water content of the lung were detected on the first 2 days, only. The effects of paraquat were also studied. The authors concluded that diquat did not produce acute damage to the lung (with edema) or fibrosis which were produced by paraquat.

913. Smith, R. J. (1978) Dioxins have been present since the advent of fire, says DOW. Science 202:1166-1167.

[Editorial.]

914. Smith, P., and Heath, D. (1976) Paraquat. CRC Crit. Rev. Toxicol. 4(3):411-445.

[Review article.]

915. Sadykov, R. E., Rabochev, V. K., and Stokov, Y. N. (1972) The effect of butyl 2,4-D treatment of pastures on the reproductive functions of sheep. Zhivotnovodstvo 34(1):73-74.

The effect of 2,4-D butyl ester on reproduction was studied in sheep. A pasture was sprayed by helicopter at the rate of 3 kg per hectare with 2,4-D butyl ester. Groups of 25 mature ewes with 2-3 month old lambs were grazed on the field 72 hr., 144 hr. and 288 hr. After spraying, and a control group grazed on unsprayed pastureland nearby. Animals grazed for 1 month. Clinical hematology and body weights were assessed. The only effects observed in exposed animals were changes in the lambs in the first 4 days of exposure and included an increase in body temperature of 0.5° and a 50% increase in leukocyte counts. All ewes were mated artificially and then spontaneously and reproductive parameters were assessed. No statistical differences were observed in the rate of conception, numbers of newborns or stillborns and birth weights between treated and control groups and no malformations occurred. The authors reported that other farm experiments had indicated that sheep exposed to pastures sprayed with 2,4-D butyl ester showed decreased fertility as high incidences of sterility, abortions, and stillborns, fetal malformations, decreased male mating behavior, and abnormal sperm resulted. No other details or references were provided for these results. The authors concluded that animals should not graze on sprayed pastureland for 20 days for adult and for 45 days for newborns before weaning.

916. Somers, J. D., Moran, E. T., and Reinhart, B. S. (1978a) Influence of hen dietary calcium and phosphorous on the integrity of the egg shell as it would influence hatching success and the consequences of preincubation 2,4,5-T spraying with and without a high TCDD level. Bull. Environ. Contam. Toxicol. 19(6):648-654.

The teratogenicity of 2,4,5-T alone and with 2.0 ppm TCDD sprayed on normal chicken eggs and eggs with deficient shells was studied.

Chickens were fed diets deficient in calcium (with 50% of the normal dietary calcium) or deficient in phosphorous (with 74% of the normal phosphorus) or deficient in both minerals. Females were maintained on the experimental diet for 40 weeks and during the last 3 weeks they were artificially inseminated. Resultant eggs were sprayed at the rate of 746 l/h with 2,4,5-T (with less than 0.1 ppm TCDD) or 2,4,5-T with 2 ppm TCDD. The age(s) of the eggs when they were sprayed was not reported. Hatchability and frequency of malformations were recorded. Egg shells, non-viable embryos, and chicks were analyzed for 2,4,5-T contents by gas-liquid chromatography. Eggs from hens fed low calcium diets had fewer eggs and the egg shells reduced porosity and reduced strength. The low phosphorus diet did not cause these effects. No reductions in hatchability or increases in malformations were observed after the deficient eggs were sprayed with 2,4,5-T with or without TCDD. No increase in 2,4,5-T levels were observed in shells, eggs, embryos, or chicks from the low-calcium diet group compared to levels from the control diet group treated with 2,4,5-T. Mean 2,4,5-T levels were 66 ppm in shells before incubation, 0.35 ppm inside these eggs, and 0.24 ppm in hatched chicks. The authors concluded that 2,4,5-T was unable to penetrate the egg shells from the experimental groups. The crude methods of treating eggs and measuring effects combined with the lack of reported pertinent details limits the usefulness of this report.

917. Somers, J. D., Moran, E. T., and Reinhart, B. S. (1978b) Reproductive success of hens and cockerels originating from eggs sprayed with 2,4-D, 2,4,5-T and picloram followed by early performance of their progeny after a comparable in ovo exposure. Bull. Environ. Contam. Toxicol. 20(1):111-119.

The reproductive effects of spraying 2,4-D, 2,4,5-T or picloram on White Leghorn chicken eggs was evaluated. Eggs (fo generation) were sprayed with one of the three herbicides on day 0, 4, or 18 of gestation. Commercial preparations of herbicides were used and were sprayed at the rate of 746 l/ha. The dioxin content of 2,4,5-T was not reported. Vehicle controls were treated with the inert ingredients of the formulations and a genetic control and a control sprayed with water were also included in the experiments. After fo generation eggs hatched, the chicks were allowed to develop and egg-laying was monitored for the fo hens mated with males of the same group. Egg weight and shell porosity (measured as weight loss after 10 days of storage) and strength (measured as the extent of deformation produced by a 500 g weight) were also determined. Sperm counts were determined by comparing the optical density of sperm samples from males with the optical density of standard boar sperm. At 55 weeks of age the gross appearance and weight of the testes were determined. Eggs (f1 generation) were collected from the fo hens artificially inseminated with sperm from males of the same treatment group. Half of the f1 eggs were treated on day 0 with the same treatment their parental generation received and the other half remained untreated. The viability, incidence of malformations, and weight gain of the f1 generation through 4 weeks of age were monitored. No adverse effects were

produced by any of the herbicides on any of the parameters presented in this study. The design of the experiments were obscured in the poorly written report and the crude techniques used to dose the eggs and evaluate reproductive parameters severely limit the value of this publication. A previous report by these authors documented that only a minor portion of 2,4,5-T sprayed under the conditions of the present study enters the egg.

918. Somers, J. D., Moran, E. T., and Reinhart, B. S. (1978c) Hatching success and early performance of chicks from eggs sprayed with 2,4-D, 2,4,5-T and picloram at various stages of embryonic development. Bull. Environ. Contam. Toxicol. 20(3):289-293.

The effects of 2,4-D, 2,4,5-T, and picloram sprayed on chicken eggs on the hatchability, teratogenicity, chick growth, and mortality was studied. Commercial preparations of each herbicide were sprayed at a rate of 746 l/ha (10 times the recommended rate) on day 0, 4, or 18 of incubation. Eggs stored for up to 24 days prior to incubation were also sprayed. The percentage of hatched eggs, of gross malformations in chicks, of chick mortality, and chick weight gain at 4 weeks of age were monitored. None of the herbicides produced any adverse effects on any parameters studied, regardless of the embryonic age when spraying occurred. A subsequent report by this group of authors (Somers et al, 1978a) demonstrated that 2,4,5-T does not penetrate the egg under the conditions used in the current experiment, which provides one explanation for the negative results presented here.

919. Source assessment: Pesticide manufacturing air emissions-overview and prioritization. (1978) U. S. Environmental Protection Agency. Environmental Protection Technology Series No. EPA-600 (2-78-004d). 135 pp.

[Not available.]

920. Sparschu, G. L., Dunn, F. L., Lisowe, R. W., and Rowe, V. K. (1971) Study of the effects of high levels of 2,4,5-trichlorophenoxyacetic acid on fetal development in the rat. Fd. Cosmet. Toxicol. 9:527-530.

The effects of high doses of 2,4,5-T on fetal rat survival and development were studied. Groups of 25 pregnant rats were administered either 50 mg/kg 2,4,5-T (0.5 ppm TCDD contaminant) in Methocel by oral intubation on days 6-15 or 100 mg/kg on days 6-10 of gestation. On day 20 fetuses were removed and observed for skeletal and visceral malformations. Corpora lutea, implantation sites, and resorptions were counted. Maternal toxicity, including 80% mortality was observed in groups treated with 100 mg/kg 2,4,5-T. One of the four pregnant rats from this group had viable fetuses (13) on day 20. A higher incidence of poor or delayed ossification was observed in these fetuses, but was considered a reversible manifestation of no teratologic significance because 3-week-old rats from other experiments from this lab failed to

show skeletal abnormalities even though delayed cranial ossification was seen in 20-day fetuses from the same group. No statistically significant decrease in any parameters, except delayed cranial ossification, was observed for the 50 mg/kg treatment group. The authors concluded that 2,4,5-T was not teratogenic at doses below those producing maternal toxicity.

921. Sparschu, G. L., Dunn, F. L., and Rowe, V. K. (1971) Study of the teratogenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat. Fd. Cosmet. Toxicol. 9:405-412.

The teratogenicity of TCDD was evaluated in the rat. TCDD in corn oil-acetone (9:1) at doses from 0.03 to 8.0 ug/kg was administered by oral gavage on day 6 to day 15 of gestation to pregnant Sprague-Dawley rats. Viable and dead fetuses, early and late resorptions, corpora lutea, and implantation sites were counted on day 20 of gestation. Fetuses were weighed, examined for gross malformations, hemorrhages in the gastrointestinal tract, and for either skeletal malformations after alizarin red staining or visceral malformations after fixing in Bouin's fluid and histological changes after staining with hematoxylin and eosin. Maternal vaginal hemorrhages were often observed in rats given doses of 2-8 ug/kg/day of TCDD and the maternal weights and numbers of resorptions per litter of the groups that received 0.5-8 ug/kg/day were adversely altered compared with vehicle controls. In the highest dose group all implants had resorbed in early pregnancy. The numbers of implantation sites and corpora lutea were not altered by any dose of TCDD. Fetal weights of the 0.125 and 0.5 ug/kg/day groups were significantly lower than controls. The frequencies of intestinal hemorrhage and subcutaneous edema showed dose-related frequencies. No renal malformations were observed.

922. Sparschu, G. L., Dunn, F. L., and Rowe, V. K. (1970) Teratogenic study of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the rat. Toxicol. Appl. Pharmacol. 17:317.

[Abstract, only.]

923. Spencer, E. Y. (1973) Guide to Chemicals Used in Crop Protection Research Institute, U. of Western Ontario, London, Ontario. p. 414.

[Not available.]

924. Sponsors of Science Inc. (1974) Sponsors of Science Inc. on the safety of 2,4,5-T and dioxin. Clin. Toxicol. 7(4):413-421.

[Editorial.]

925. SRI International. (1981) A case-control study of the relationship between exposure to 2,4-D and spontaneous abortion in humans. National Forest Products Association, Washington, DC: 116 p.

The incidence of spontaneous abortions among women whose husbands were occupationally exposed to 2,4-D was investigated. A study population, which was composed of farmers, forest workers and herbicide applicators in Oregon and Washington, were issued questionnaires by mail which requested information regarding the occurrence of miscarriages and exposure to 2,4-D. Telephone interviews were then conducted with all respondents that indicated having had at least 1 miscarriage and with a select group of those that reported live births. The telephone interviews addressed the time and extent of 2,4-D exposure of both parents, relative to the time of conception, and other confounding factors, including smoking habits, drug consumption and illnesses. 8,287 of the 14,747 questionnaires were returned. 3,787 eligible cases were identified, of which 1,098 were selected for telephone interviews, 604 interviews were completed; 134 cases of miscarriages and 311 controls (cases of live births were validated and studied. No positive association was obtained between 2,4-D exposure of the father and subsequent spontaneous abortions in the wife, for the total group or for groups of only farmers or of forest and commercial workers. A correlation of these 2 variables was observed in a group of 21 young forest and commercial workers, with 54 corresponding controls, but not for the corresponding group of farmers of the same age group. The authors concluded that there was no evident relationship between 2,4-D use and spontaneous abortions, the result obtained in young forest and commercial workers warrants further study, but does not justify imposing restrictions on 2,4-D use until the study is completed.

926. St. John, L. E., Wagner, D. G., and Lisk, D. J. (1964) Fate of atrazine, kuron, silvex, and 2,4,5-T in the dairy cow. J. Dairy Science 47:1267-1270.

The rate of excretion of 2,4,5-T was determined in the cow. One Holstein cow was fed a diet that contained 5 ppm 2,4,5-T for 4 days. Milk and urine samples were collected for 6 days after the start of the experimental diet and these samples were analyzed for 2,4,5-T by gas chromatography. The level of sensitivity for 2,4,5-T was 0.5 ppm. The daily amounts of 2,4,5-T excreted in urine were 93, 108, 112, 104, 9, and 4 mgs for the 6 days of collection, respectively. The total urinary excretion of 2,4,5-T accounted for 430.7 mg, which approximated the total dosage of 454 mg administered. Urinary 2,4,5-T was in the form of soluble salts. No results were reported for levels of 2,4,5-T detected in milk. Atrazine, kuron, and silvex excretion were also studied. The authors concluded that all of the administered 2,4,5-T was excreted intact in urine.

927. Stalling, D. L., and Hackins, J. N. (1978) Metabolism of 2,4-dichlorophenoxyacetic acid (2,4-D) in bluegills and water. J. Agr. Fd. Chem. 26(2):447-452.

The metabolism of 2,4-D by fish in a plastic pool of water outdoors was evaluated over a 12-week period following a single application. Thirty bluegill fish (Lepomis macrochirus) were placed in a pool with 2 mg/L [¹⁴C]-2,4-D dimethylamine salt. Another pool without fish served as a control. Both pools were partially (30%) shaded by dark screens and life forms were allowed to develop in the water for 2 weeks. Water samples were removed weekly and fish removed after 1, 5, 8 and 12 weeks and separated into the fillet and the head-viscera. All analyzed for radioactivity. ¹⁴CO₂ in the water samples was trapped and counted. Tissues were extracted to separate the amino acid plus glycogen fraction, neutral lipids, free fatty acids, and acid soluble fraction. Each fraction was analyzed for radioactivity. After 12 weeks, 49% and 16% of the radioactivity remained in the experimental and control pools, respectively. ¹⁴CO₂ made up none and 89% of the radioactivity in the experimental and control pools, respectively. The experimental pool contained a heavy algae bloom by the end of the test period. About one-third of the radioactivity in the fish was in the fillet and radioactivity increased in the fish over the period. None of the radioactivity was presented as 2,4-D. After 84 days, the distribution of radioactivity in fillet was 59%, 22%, 10%, and 3% for the amino acids, neutral lipid, acid-soluble, and free fatty acid fractions, respectively, and for the head-viscera were 45%, 18%, 10% and 28%, respectively. The authors concluded that the 2,4-D aromatic portion had been completely degraded by microorganisms and other factors within the pools and were then incorporated into biochemicals synthesized by the fish. Rapid degradation of 2,4-D was implied by the observation that no labeled 2,4-D metabolites were detected in any sample.

928. Stehl, R. H., and Lamparski, L. L. (1977) Combustion of several 2,4,5-trichlorophenoxy compounds: Formation of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Science 197(4307):1008-1009.

[Background material.]

929. Stellman, S., and Stellman, J. (1980) Health problems among 535 Vietnam veterans potentially exposed to toxic herbicides. Society for Epidemiological Research: Abstracts. 444.

[Abstract, only.]

930. Sterling, T. D. (1975) Toxic and teratogenic effect of 2,4,5-trichlorophenoxyacetic acid and 2,3,7,8-tetrachlorodibenzo-p-dioxin. Summary of Hearings of the Assembly Committee on Natural Resources of the State of Wisconsin, March 19, 1975, 24 p.

[Background material.]

931. Sterling, T. D. (1971) Difficulty of evaluating the toxicity and teratogenicity of 2,4,5-T from existing animal experiments. Science 174:1358-1359.

[Editorial.]

932. Stevens, J. T., DiPasquale, L. C., and Farmer, J. D. (1976) The acute inhalation toxicology of cacodylic acid. Toxicol. Appl. Pharmacol. 37:0412.

[Abstract, only.]

933. Stevens, J. T., DiPasquale, L. C., and Farmer, J. D. (1979) The acute inhalation toxicology of the technical grade organoarsenical herbicides, cacodylic acid and disodium methanearsonic acid; a route comparison. Bull. Environ. Contam. Toxicol. 21(3):304-311.

The toxicity of cacodylic acid, administered by various routes of exposure, is described. Swiss-Webster mice and Sherman rats were exposed to a commercial preparation of cacodylic acid (Phytar 138; 65.6% cacodylic acid), and to Fisher- or Ansul-purified cacodylic acid (95.5% and 99.5% purity, respectively). Groups of 10 animals of each sex were exposed to an atmosphere of up to 10 mg/liter of cacodylic acid (Phytar 138), produced in an inhalation chamber by a dust generator for 2 hours and then were observed for clinical signs for the next 14 days. At death, or at 14 days for the survivors, animals were necropsied. Of groups of 10 male rats, two died within 14 days of exposure to 4.1 mg/liter cacodylic acid, none died after exposure to 6.9 mg/liter, and one died after exposure to 10.8 mg/l. The LC_{50} for female rats was calculated at 3.9 mg/liter. Mice were exposed to one concentration, 6.4 mg/liter and one death occurred in 14 days. Respiratory distress, rhinorrhea, and porphyrin-like encrustation occurred during exposure and diarrhea, erythematous lesions, and decreased weight gain occurred after exposure. Gross pathology observed in fatal cases included dark spots and redness of the lungs, impacted caecum, and blood and mucous in the intestine. Respiratory irritation was measured as a decrease in respiratory rate in mice (three per group) exposed to cacodylic acid aerosol for 5 minutes. Results were expressed as the concentration of cacodylic acid that produced a 50% decrease in respiratory rate (RD_{50}). Control mice were exposed to diatomaceous dust. The RD_{50} for cacodylic acid was 3.15 mg/liter and equal amounts of cacodylic acid from either the commercial or a purified preparation (99.5% purity) were equally effective. Exposure to 3.5 mg/liter of diatomaceous earth produced a minimal response on respiratory rate. Acute doses of 400-625 mg/kg of Fisher-purified cacodylic acid were administered to groups of 30-80 animals intraperitoneally or intravenously and intraperitoneal LD_{50} values were calculated as 720, 520, 520, and 600 mg/kg for male and female rats and male and female rats, respectively, and 470 mg/kg for female rats dosed intravenously. In addition to pathological changes observed after inhalation exposure, loss of the righting reflex,

rigidity, and decrease in body temperature occurred; the thymus was reduced in size and appeared red and the adrenals appeared dark. All deaths occurred within 4 days of the injection. The authors concluded that the cacodylic acid aerosol produced some pulmonary irritancy that complicated interpretation of pulmonary toxicity data.

934. Stevens, J. T., Hall, L. L., Farmer, J. D., DiPasquale, L. C., Chernoff, N., and Durham, W. F. (1977) Disposition of ^{14}C and/or ^{74}As -cacodylic acid in rats after intravenous, intratracheal, or peroral administration. Environ. Health Perspect. 19:151-157.

The pharmacokinetics of cacodylic acid absorption, distribution, and elimination are described in the rat following administration by 3 routes. All studies were performed on male Sherman rats except for the experiment to identify sex differences which also included female Sherman rats and the study of placental transfer with pregnant CD rats. A dose of 78 mg/kg [^{14}C]-cacodylic acid was administered by tracheal cannula and the lungs and trachea were removed after 0-20 min. and assayed for remaining activity. A dose of 60 mg/kg [^{14}C]-cacodylic acid was administered perorally and after 4 hours the gastrointestinal tract was removed and assayed for remaining activity. The half-time for absorption was calculated to be 2.2 min. from the pulmonary route and 248 min. from the oral route. Tissue concentrations of cacodylic acid were calculated from the levels of radioactivity detected in various tissues of groups of 3-4 rats (rats weighed 280-380 g each). Tissue levels were determined 0.1 to 168 hrs. after 200 mg/kg [^{14}C]-cacodylic acid was administered intravenously, 5 min. to 60 days after 33 ug of [^{14}C]-cacodylic acid plus 3.5 ug of [^{74}As]-cacodylic acid were administered intravenously was, 104 days after 1 or 5 oral doses of 33 ug of [^{14}C]-cacodylic acid plus 6.9 ug of [^{74}As]-cacodylic acid were administered or 1 dose of 33 ug of [^{14}C]-cacodylic acid plus 13.8 ug of [^{74}As]-cacodylic acid was administered intratracheally. One hour after both the high and low intravenous doses about 11-15%, 1.2% and 0.8% of each dose was recovered in whole blood, liver, and kidney respectively and less than 0.5% remained in the lung, brain and spleen. The only differences in distribution between the 2 doses occurred at 15 minutes, with a higher level in the liver after the high dose than low dose and a higher level in the kidney after the low dose than high dose. No differences in tissues distribution of [^{14}C] compared to [^{74}As] occurred after intravenous administration. At 168 hr. after the high doses, 10% of the dose remained the blood and less than 0.5% remained in any other tissue. After 105 days, the concentration of [^{14}C] in the spleen and kidney were highest after intravenous dosing than by other routes and were highest in the liver and blood after oral dosing than by other routes while all other tissue levels were the same, regardless of route of administration. Comparing tissue distribution after 1 and 5 doses, the spleen, liver, and brain retained more of the administered doses after the multiple dosage regimen than after the single dosage regimen. Tissue distribution of 24-34 mg/kg of [^{14}C]-cacodylic acid administered intravenously was the same for male and female rats. Levels of [^{14}C] were analyzed in both

fetal and maternal tissues 24 hrs. after a pregnant rat was administered 33 ug [¹⁴C]-cacodylic acid plus [⁷⁴As]-cacodylic acid on day 21 of gestation. Levels were comparable for maternal tissues and the corresponding tissues in the fetus. Blood samples collected from rats from each treatment group described for the experiments on tissue distribution were separated into plasma and red blood cells for separate analysis of radioactivity and the results were analyzed pharmacokinetically. The data for plasma levels of [¹⁴C] after the high intravenous dose was administered were triphasic, with clearance half-times of 0.014, 0.22 and 3.42 hr. for the 3 phases. Peak plasma levels were reached 5 min., 10 min., and 1 hr. after the intravenous, intratracheal, and oral doses, respectively. Clearance from whole blood was 92, 76, and 90 days, respectively. Excretion of [¹⁴C] from the low intravenous dose and from doses given by the other routes were determined by analysis of radioactivity in urine, feces, expired air, and bile collected from cannulated bile ducts and the amount in the whole body after 24 hr. Recovery of administered doses were 88-93% and absorption was 66% for the oral dose and 92% for the intratracheal dose. After oral dosing, over 31% of the dose was recovered in feces and less by other routes. Biliary secretion was demonstrated. From 60-71% of the doses given by the other routes were excreted in urine and an insignificant amount was excreted as ¹⁴CO₂ by the lungs after dosage by any route. The authors concluded that cacodylic acid was absorbed slower after oral administration than after other routes were used and had a high affinity for erythrocytes. The authors also concluded that cacodylic acid was not converted to inorganic arsenic, that biliary secretion accounted for fecal excretion after intravenous administration of cacodylic acid, and that cacodylic acid readily crosses the placenta.

935. Strange, J. R., Allred, P. M., and Kerr, W. E. (1976) Teratogenic and toxicological effects of 2,4,5-trichlorophenoxyacetic acid in developing chick embryos. Bull. Environ. Contam. Toxicol. 15(6):682-688.

The embryoletality and teratogenicity of 2,4,5-T was studied in fertilized chicken eggs. The results, published elsewhere (Strange, J. and Kerr, W.E.; 1976), indicated that 2,4,5-T administered in DMSO altered hatchability with LD₅₀ values of 68 mg/kg when injected on day 0 and 62 mg/kg when injected on day 5. No malformations were observed in embryos exposed to 50 mg/kg 2,4,5-T, for 2 days. In this paper (Strange, J.R., Allred, P.M., and Kerr, W.E., 1976) an additional experimental group given 25-150 mg/kg 2,4,5-T in acetone and control groups given acetone alone were injected on day 0. The LD₅₀ for 2,4,5-T in acetone was 133 mg/kg while the maximum amount of acetone used as the 2,4,5-T vehicle produced no effect on mortality. The authors concluded that DMSO and 2,4,5-T acted synergistically, producing a lower LD₅₀ than 2,4,5-T in acetone. (The solubility of 2,4,5-T in each vehicle was not indicated.)

936. Strange, J. R., and Kerr, W. E. (1976) Teratogenic and toxicological examination of 2,4,5-trichlorophenoxyacetic acid in developing chick embryos. Toxicology 6:35-40.

The teratogenic and embryo-lethal effects of 2,4,5-T were studied in chick embryos. Fertilized eggs were injected with 12-125 mg/kg 2,4,5-T into the air space on day 0 or day 5 of incubation and the number of hatched eggs was recorded. 2,4,5-T contained less than 0.1 ppm dioxin contaminant and was administered in dimethyl sulfoxide (DMSO). Control included eggs that were untreated, eggs that were drilled only, and eggs that were injected with distilled water or DMSO. Teratologic effects were studied in 12 embryos administered 50 mg/kg 2,4,5-T and 12 control eggs (drilled only), which were incubated for 48 hours. Embryos were fixed in Bouin's fluid, stained, and examined for malformations. The LD₅₀ for eggs injected on day 0 was 62 mg/kg and on day 5 was 68 mg/kg. DMSO did not produce toxicity at levels below 100 mg/kg. The LD₅₀ for DMSO was 210 mg/kg. No abnormalities were observed in the eggs examined for teratologic effects. However, at the developmental stage examined, the kidney was not developed sufficiently to reveal the types of malformations reported in rodents. 2,4,5-T at 50 mg/kg prevented 37% of the eggs in the embryo lethality study from hatching, but all of the eggs examined for malformations were viable 2 days after exposure, suggesting that death occurred late in development. The authors estimated that 41 mg/sq. ft. of 2,4,5-T would be distributed on a field from application of 2,4,5-T at recommended agricultural rates, resulting in exposure of 4.1 mg per egg externally.

937. Strik, J. J. T. W. A. (1979) Porphyrins in urine as an indication of exposure to chlorinated hydrocarbons. Ann. N. Y. Acad. Sci. 320:308-310.

The results of urinalysis of TCDD-exposed people for porphyrins is reported. Twenty months after the explosion in Seveso, Italy which released a cloud of TCDD, urine was collected from people in the most polluted area. Total porphyrin values from this group were not significantly different from control groups from other countries. Thin layer chromatography of the porphyrins revealed an abnormal pattern indicating chronic hepatic porphyria type A. No other details of the group examined or the results were given. Other samples were analyzed for porphyrins that were collected from groups exposed to chlorinated hydrocarbons. The levels of urinary porphyrin excretion were not elevated in these groups but the patterns of porphyrins excreted in general was indicative of chlorinated hydrocarbons.

939. Stroo, W. E., McCormack, K. M., and Hook, J. B. (1979) Renal functional effects of 2,4,5-trichlorophenoxyacetic acid and silvex after acute and prolonged exposure. J. Toxicol. Environ. Health 5(5):845-854.

Renal clearance of organic ions in vivo and uptake of organic ions in vitro were compared after acute and chronic doses of 2,4,5-T and Silvex

were administered to rats. Sprague-Dawley rats were administered 99% pure herbicides with less than 0.05 ppm dioxin contaminant subcutaneously in 95% ethanol or orally in the diet. Renal cortical slices in vitro were incubated in a medium with ^{14}C -labeled organic ions and then uptake was determined by counting radioactivity in the solubilized tissue and in the medium. Clearance in vivo was determined in rats infused with saline containing [^3H]-inulin and [^{14}C] para-aminohippurate (PAH). Renal parameters were studied during several clearance periods in which herbicide-treated rats were given a saline load or high (1.14 mg/min) or low (0.114 mg/min) PAH infusion rates. The in vitro uptake of PAH or N-methyl nisotinamide chloride (NMN) was altered in renal slices from rats given 800 ppm Silvex orally but not in rats given 200 ppm silvex or 2,4,5-T. PAH and 2,4,5-T uptake was decreased to the same extent in slices of kidneys removed 24 hours after the last of 14 doses or 1 dose of 100 mg/kg 2,4,5-T. Decreases in uptake of PAH or tetraethylammonium were observed 4 hours after one or 14 doses of 20 mg/kg 2,4,5-T but not after 24 hours. These decreases also occurred 4 hr. after a single dose of 50 mg/kg of Silvex was given. The results from in vivo studies showed the same trends; a large (100 mg/kg) single dose or series of 14 doses of 2,4,5-T caused decreased PAH and TEA clearance and increased filtration fraction (i.e. PAH clearance/inulin clearance) 24 hours after administration. The effects of a low dose (20 mg/kg) of 2,4,5-T or (50 mg/kg) Silvex observed after 4 hours did not persist for 24 hours, even after 14 consecutive doses of 2,4,5-T had been administered. Alterations in electrolyte excretion occurred with changes in PAH clearance. The authors concluded that 2,4,5-T probably did not accumulate in vivo from chronic exposure and suggested that decreased anion transport reflected the presence of 2,4,5-T in the kidney but was not a manifestation of toxicity. To strengthen the conclusions pertinent to 2,4,5-T accumulation, the authors would have to demonstrate that the single dose of herbicide produced a submaximal effect that could potentially be increased by multiple doses (or a higher single dose) and that the lack of increased effect by repeated doses is not the result of compensatory mechanisms.

940. Stumm, W., and Morgan, J. J. (1970) Aquatic chemistry. [New York: John Wiley and Sons]. p. 69.

[Background material.]

941. Styles, J. A. (1974) Studies on the effects of paraquat and diquat on cells in culture. Viability of macrophages and fibroblasts incubated with paraquat and diquat. Br. J. Exp. Pathol. 55(1):71-77.

The viability of rat alveolar macrophages and cloning efficiency of mouse fibroblasts were studied in cells cultured in the presence of diquat. Stationary and suspension cultures of rat alveolar macrophages, rat peritoneal macrophages and mouse L929 fibroblast cells were cultured in medium that contained 10^{-2}M to 10^{-6}M diquat dibromide for up to 24 hr. Viability was evaluated by exclusion of trypan blue of suspended cells and by eosin exclusion for stationary cultures.

After 24 hr. of exposure of suspended cells to 10^{-6} M diquat, 60% of each type of macrophage survived and 0-15% survived 24 hr. exposure to 10^{-3} M diquat. Exposure of stationary cells to 10^{-6} M diquat for 30 min. resulted in a decrease in macrophage viability of 40% during the subsequent 2-3 days. Higher concentrations of diquat and long periods of exposure produced larger effects on viability. At 10^{-4} M, for 24 hr. diquat killed all fibroblasts and between 10^{-4} and 10^{-6} M, produced a dose-related decrease in fibroblast cloning efficiency. The cytotoxic effects of paraquat were also studied. The authors concluded that diquat toxicity to macrophages in vitro was equivalent to paraquat toxicity and to fibroblast cloning was substantially higher than paraquat toxicity and suggested that the reverse order of toxicity observed in vivo reflected different distributions of the two compounds to the lung.

942. Styles, J. A. (1973) Cytotoxic effects of various pesticides in vivo and in vitro. Mutat. Res. 21:50-51.

[Abstract, only.]

943. Sudak, F. N., Claff, C. L., and Cantor, M. (1966) Body temperature regulation in rats treated with 2,4-dichlorophenoxyacetic acid. Arch. Int. Pharmacodyn. 160(2):253-264.

The ability of 2,4-D treated rats to maintain a normal body temperature when exposed to the warm or cold was studied. Male Wistar rats were administered 2,4-D intraperitoneally or intracisternally and control rats were administered saline. After 45 minutes, skin and colonic temperatures were monitored, carbon dioxide (CO_2) in expired air was measured, and electrocardiograms and electromyograms were obtained from animals exposed to the cold, heat, or ambient (thermoneutral) temperatures. Some rats were acclimatized to the cold by exposure to 6°C for 4 weeks. 2,4-D produced a dose-related decrease in body temperature in rats placed in the cold (2°C) after treatment. Rats injected with 300 mg/kg 2,4-D were unable to maintain their body temperature in the cold and showed a steady decrease in metabolism (measured as CO_2 production) during 90 minutes in the cold, no increase in blood circulation to the skin (measured as the difference between skin and colonic temperatures), and no increase in shivering (measured as increased activity in the electromyogram). Statistically significant increase in CO_2 production over controls was observed for treated rats at ambient temperature, but only a small increase in colonic temperature occurred. At 35°C , colonic temperature and CO_2 production increased in 2,4-D treated rats causing death to all animals after 30-105 minutes of heat exposure. None of the controls died within 120 minutes. Cold-acclimatized rats treated with 2,4-D had significantly lower body temperatures and a smaller increase in CO_2 production than acclimatized saline controls. Rats given 3 mg/kg intracisternally under chloralose anesthesia showed the same extent of impairment of temperature regulation in the cold as rats given 300 mg/kg 2,4-D (without anesthetic). At colonic temperatures below 20°C ,

no heart activity was detected. After rewarming, all animals survived. The authors suggested that 2,4-D impaired heat regulation by acting on central mechanisms that control heat production and loss.

944. Sugar, J., Toth, K., Csuka, O., Gati, E., and Somfai-Relle, S. (1979) Role of pesticides in hepato-carcinogenesis. J. Toxicol. Environ. Health 5(2/3):183-191.

This report presents some of the same data as the reports by Toth et al., 1978, and Toth et al., 1979. Data for Swiss H/RIOP mice fed combinations of trichlorophenoxyethanol (TCPE) and TCDD were used; the data from mice fed TCDD alone were not reported here. The authors noted that significantly increased number of liver tumors in male mice was apparent in the two groups fed the highest dose of TCPE (67 or 70 mg TCPE/kg) and that the TCDD doses (0.112 ug/kg or 0.007 ug/kg) were apparently unrelated to the production of liver tumors. Another group given 7.0 mg TCPE/kg with 0.07 ug TCDD/kg did not show an increased liver tumor incidence. The authors also noted that this particular strain of mouse had an unusually high rate of spontaneous liver tumors (average incidence in controls of 26.33 percent). There was no predominance of any particular type of chemically induced liver tumor, nor did cirrhosis ever precede the formation of tumors. Because the incidence of liver tumors was significantly greater in male than in female mice, the authors considered a possible sex-linked effect on aryl hydrocarbon hydroxylase (AHH) induction by TCPE. Because of the contamination of TCPE by TCDD, TCDD's AHH inducing activity was also looked at using the fluorometric procedure described by Nebert and Belboin (1968) to determine AHH activity, it was found that in male mice activity increased over the course of the experiment, while in females it leveled off after the third day and then declined. Induction increased in direct proportion to TCDD content. The authors note that TCDD, which is a strong inducer of AHH, was also shown ineffective in producing tumors, while quite the reverse is the case for TCPE.

945. Sugiura, K., Matsumoto, N., Washino, T., Mihara, Y., and Goto, M. (1979) Ecological chemistry XVII. Accumulation of organochlorine compounds in fishes. Distribution of 2,4,5-T, alpha-HCH, beta HCH, gamma HCH and 2,4,6,2'4'6'-hexachlorobiphenyl in tissues. Chemosphere (6):365-368.

The authors studied the accumulation and elimination of ^{14}C -2,4,5-T and several other organochlorine compounds in carp using whole body autoradiography. In the accumulation experiment, a carp was placed in a 40 liter tank containing 0.056 ppm ^{14}C -2,4,5-T (purity unspecified) for 6 days after which the fish was processed for autoradiography. In the elimination experiments, the fish were removed from the treated water after six days and placed in 2,4,5-T free water for 2 days after which the fish were processed. Numbers of fish in each group were not reported. Accumulation of 2,4,5-T occurred only in the gall bladder and was almost entirely eliminated within 2 days.

946. Summary of Dioxin Planning Conference. (1974) Washington, DC. July 25-26. 15 pp.

[Background material.]

947. Summary of the Statement of Mr. Cleland before the Subcommittee on Medical Facilities and Benefits of the Veterans Affairs Committee, House of Representatives, February 25. (1980).

[Background material.]

948. Sundell, L., Rehn, M., Axelson, O. (1973) An epidemiological study concerning herbicides.

This study reports findings similar to those presented in Axelson et al.; Herbicide exposure and tumor mortality: an updated epidemiological investigation on Swedish railroad workers and Axelson et al., Herbicide exposure and tumor incidence: an epidemiological investigation on Swedish railroad workers, 1974.

949. Suschetet, M., and Causeret, J. (1973) Pathophysiologic and nutritional effects of amino-4-trichloro-3,5,6-picolinic acid (picloram) in rats. CR Acad. Sci. (Paris) 276(11):1775-1777.

[Foreign language.]

950. Suskind, R. R. (1980) TCDD contamination in the United States case study. Presented at the National Academy of Sciences International Workshop of Areawide Chemical Contamination, March 17. 13 p.

[Review article.]

951. Suskind, R. R. (1979) A study of workers involved in the manufacture of 2,4,5-T. Protocol for Nitro, West Virginia Study, June 1979. Prepared by the Department of Environmental Health, Kettering Laboratory, Cincinnati, Ohio. 29 pp.

[Background material.]

952. Suskind, R. R. (1978) Chloracne and associated health problems in the manufacture of 2,4,5-T. Report to the Joint Conference, National Institute of Environmental Health Sciences and International Agency for Research on Cancer, WHO, Lyon, France, January 11. 7 pp.

The health of workers at a plant in Nitro, West Virginia, is described. The plant manufactures 2,4,5-T and in 1949 an accident occurred in which a relief valve of a reaction vessel opened, releasing vapors into

the factory. Five years later, 117 persons had chloracne, which was traced to this incident. A total of 228 persons, including workers' family members and medical personnel, were affected 5 years after the manufacture of 2,4,5-T had been started. Following the explosion, the typical symptoms described included headache, dizziness, and vomiting within hours, a facial rash within 1 week, which developed into acne during the next week, and a tendency to feel tired. One month after the explosion, aching muscles were reported, leading to inability to walk; insomnia, and extreme irritability. Three months after the explosion, hyperpigmentation of the skin appeared. In three out of the four hospitalized cases, the liver was palpable. Nerve biopsies showed destruction of myelin sheaths and nerve fibers, although muscle biopsies showed no abnormal features. Total serum lipid values tended to be high, and prothrombin concentration was one-half of the normal value. Therapy was unsuccessful for acne or for pain, although tranquilizers were successful for treating nervousness and irritability. Hepatic changes were temporary, and resolved in a few months. At the time of the incident in this factory, TCDD was not identified as the causative agent, precluding its hasty removal from the work environment. Following the experimental application of 2.5-5.0 percent trichlorophenate to human subjects, acne developed and persisted for 3-4 months.

953. Sweeney, G. D., Jones, K. G., Cole, F. M., Basford, D., and Krestynski, F. (1979) Iron deficiency prevents liver toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Science 204(4390):332-335.

The toxic effects of TCDD in mice maintained on an iron deficient diet were ascertained. C57Bl mice were fed an iron deficient diet and 1.6 to 2.0 ml of blood was removed over a 4 week period, prior to TCDD treatment. Weekly intraperitoneal doses of 25 ug/kg TCDD in corn oil were administered for 11 weeks to mice continued on the iron deficient diet and to mice fed a normal diet. Urinary porphyrin levels were monitored during the TCDD treatment period. After the 11 week treatment period, mice were weighed and liver weights, iron levels, and enzyme levels were determined. Liver tissue was also examined histopathologically. Prior to TCDD treatment, hemoglobin levels dropped from 10.4 to 5.5 g/dl but recovered to 10.2 g/dl in mice exposed to TCDD and to 13.4 g/dl in mice fed the iron deficient diet but not exposed to TCDD. No mice died after the start of the TCDD exposure, but the mice exposed to TCDD on both diets weighed about 10% less than controls fed the corresponding diet. Non-TCDD-treated mice on iron deficient diets weighed about 20% less than controls. Liver hypertrophy occurred in both TCDD-treated groups, but only the TCDD-exposed mice on the control diet developed elevated hepatic and urinary porphyrins (primarily coproporphyrin), hepatic histopathology, a decrease in uroporphyrinogen decarboxylase, to 20% of controls, and a deterioration of the condition of the fur. Mixed function oxidase activity was induced by TCDD in both groups, but to a slightly smaller extent in the iron deficient group. The authors concluded that tissue iron in a form different from that of circulating iron is essential for TCDD to elicit toxicity.

954. Szocs, J., Dr., Molnar, V., Balogh, E., Ajtay, M., and Fulop, I. (1970) Experimental data on the toxic effects of 2,4-D (diclordon) herbicide. Revista Medicala 16:91-93.

The acute and subacute toxicities of 2,4-D were studied in the rat. Male rats (12-50 rats per group) were administered a single dose of 625 mg/kg 2,4-D sodium salt by stomach tube and were killed 2-48 hr. later; other groups of rats were administered doses of 125 mg/kg (total number of doses not indicated) and were killed 21 days later (did not specify whether this referred to 21 days after the first or last dose of the subacute regimen). Blood and liver samples were analyzed for various enzyme activities by histochemical analyses or unspecified methods and serum proteins were analyzed by paper chromatography. Serum and liver aldolase levels were decreased by 35-40% in all groups and all time points below the control value. Glutamic oxaloacetic transaminase levels were elevated maximally at 6 hours in the liver and 12 hours in the serum after acute exposure and slightly increased after subacute exposure. Fluctuations in both directions were observed for glutamic pyruvic transaminase, catalase, cholinesterase, and succinic dehydrogenase, activities, and in various protein fractions. Alkaline phosphatase activity in the cytoplasm increased and acid phosphatase activity decreased, as evidenced from histochemical staining. Fatty infiltration of the liver and dystrophias were also observed. The authors concluded that 2,4-D produced subacute hepatitis as well as neurotoxicity (reported by others) and recommended that safeguards be instituted for occupational handling.

955. Tarrant, R. F., and Allard, J. (1972) Arsenic levels in urine of forest workers applying silvicides. Arch. Environ. Health 24(4):277-280.

The levels of arsenic excreted by forest workers involved in applying cacodylic acid to trees is presented. Six men who applied cacodylic acid by 2 different methods (with an injection hatchet, 3 men; by squirt bottle, 3 men) provided urine samples at the start of each work week and at the end of each 5 day work week, over 9 weeks. Arsenic in the urine samples was analyzed by a colorimetric method. Clean clothing and eye protection were provided daily to the workers. A control group of 3 workers in the same area that were not exposed to chemicals provided urine samples on the same schedule as the exposed workers for arsenic analyses. The mean levels of arsenic at the start and end of the work week was 0.04 ppm and 0.07 ppm, respectively for controls and 0.08 and 0.41 ppm for workers using the squirt method of application for 5 weeks in which all subjects provided samples and 0.21 and 0.63 ppm respectively for the group that used the injection-hatchet, for the 2 weeks in which all subjects provided samples. The means for both exposed groups was 0.56 ppm arsenic. All levels for exposed groups were significantly higher than control levels at the end of each week, but the levels for the 2 exposure groups were not significantly different from each other. Arsenic levels were also presented for workers exposed to monosodium methanearsonate. The authors concluded that forest workers were occasionally exposed to excessive quantities of arsenic, in which total arsenic exceeded 0.3 ppm in urine.

956. Taylor, C. (1974) Chemical toxicity and mental disorder. [letter to the editor] Am. J. Psych. 731(5):609.

[Editorial.]

957. Taylor, J. S. (1979) Environmental chloracne: Update and overview. Ann. N. Y. Acad. Sci. 320:295-307.

[Review article.]

958. Taylor, J.S. (1974) Chloracne--a continuing problem. Cutis 13:585-591.

[Review article.]

959. Taylorson, R., and Kleisath, J. (1961) Cacodylic acid investigations. BL Technical Memorandum 9-24. Crops Division, Ft. Detrick, MD. DTIC No. AD254825L. 23 pp.

[Not available.]

960. Teitelbaun, P. J., and Poland, A. P. (1978) Studies of the hepatic uptake of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the mouse. Fed. Proc. Fed. Am. Soc. Exp. Biol. 37(3):692.

[Abstract, only.]

961. Telegina, K. S., and Bikbulatova, L. I. (1971) Affection of the follicular apparatus of the skin in workers occupied in production of butyl ether of 2,4,5-trichlorophenoxyacetic acid. Vestn. Dermatol. Venerol. 44(12):35-39.

[Foreign language.]

962. Tenchini, M. L., Crimaudo, C., Simoni, G., De Carli, L., Giorgi, R., and Nuzzo, F. (1979) Approaches to the evaluation of genetic damage after a major hazard in chemical industry: preliminary cytogenetic findings in TCDD exposed subjects after the Seveso accident. Part of a special project to investigate TCDD-exposed pregnancies. Task Force from Universita di Milano (Italy). Supported by the Assessorato alla Sanita, Regione Lombardia (Italy).

The authors report on the cytogenetic analysis of peripheral blood and aborted tissues obtained from 25 pregnant women who were exposed to TCDD during the Seveso accident. Peripheral blood cultures and explants from embryos, placentas, umbilical cords were established. Chromosome preparations were made from 24-72 hour peripheral blood cultures and from 9-40 day explant cultures. The number of metaphases of peripheral blood cells examined for each individual varied from 90-150. No control were reported. The numbers of chromosome aberrations observed were in the normal range according to the authors. In fetal tissues, larger numbers of chromosomal aberrations were observed. The chromosomal damage was higher in embryonic tissue than in placental or umbilical explants. No conclusion can be made on these results because no controls of unexposed fetal tissues were included in the experiment. It is possible that chromosomal effects observed in these tissues were a result of culturing conditions.

963. Tenchini, M. L., Giorgi, R., Crimaudo, C., Simoni, G., Nuzzo, F., and de Carli, L. (1977) Approaches to examination of genetic damage after a major hazard in chemical industry: preliminary cytogenetic findings on TCDD-exposed subjects after Seveso accident. Presented at the Expert Conference on Genetic Damage Caused by Environmental Factors, Oslo, Norway, May 11-13, 1977.

[Not available.]

964. Thiess, A. M., and Frentzel-Beyme, R. (1977) Mortality study of persons exposed to dioxin following an accident which occurred in the BASF on 13 November 1953. Presented at the Fifth International Conference of Medicchem - Occupational Health in the Chemical Industry, 9 pp.

The author performed a 20-year followup investigation of 75 BASF workers exposed to trichlorophenol and dioxin (the structures of these compounds were not given) during an industrial accident in 1953. The historical prospective design utilized both internal and external cohort groups for comparison of mortality and neoplastic incidence. Three populations were selected for use as external comparison groups. 1.) Total population of Ludwigshafen population 180,000 where approximately 25% of persons were workers at BASF, 2.) Total governmental district Rhinehessia Palatinate 3,700,000, 3.) Total Federal Republic of Germany (FRG) 60 million. The internal comparison group was derived from BASF matched by age and date of entry into the factory. An analysis of the 3 external and internal matched comparison groups showed that the dioxin exposed group had a lower mortality rate than Ludwigshafen, but a higher rate than the internal matched comparison population, the administrative district of Rhinehessia Palatinate and the Federal Republic of Germany as a whole. The dioxin exposed group demonstrated 6 neoplasms at a rate twice that of the total German Republic and a third greater than the internal controls. Four of the neoplasms were of the stomach which was greater than found in all comparison groups and significantly greater than 3 comparison groups. The statistical method utilized was the Poisson distribution. The author reported that the number of malignant neoplasms is increased above expectation in the age group 65-75.

965. Thigpen, J. E., McConnell, E. E., Moore, J. A., and Faith, R. E. (1977) Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on host resistance to infectious agents. Environ. Health Perspec. 20:245.

Abstract, only.]

966. Thigpen, J. E., Faith, R. E., McConnell, E. E., and Moore, J. A. (1975) Increased susceptibility to bacterial infection as a sequela of exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Infection and Immunity 12(6):1319-1324.

The effect of subacute administration of TCDD on resistance of mice to infectious agents is described. Male C57BL 16Jfh mice were administered TCDD (99+% purity) in acetone-corn oil (1:6) by gastric intubation. Doses of 0.5-20 ug/kg were administered once weekly for 4 weeks. Two weeks after the last dose of TCDD was given, mice were injected intraperitoneally with Salmonella bern or Herpesvirus suis (pseudorabies virus; PRV). During the following 14 days, general appearance of the animals, weight gains, mortality rates and time from infection to death were determined and necropsies were performed on some mice. The highest dosage group gained significantly less weight

than the non-TCDD-treated group. Mortality rates were 25% for vehicle controls, about 65% for the 1, 5 and 10 ug/kg dosage groups, and 95% for the 20 ug/kg dosage group. The time interval to death was shortened from 8.4 days for non-TCDD-treated mice to 3.0 days for the highest dosage group. No alterations in response to the PRV infection were observed in TCDD-treated mice, compared to non-TCDD-treated mice. Thymic atrophy and a hepatic necrosis were observed in mice that received the highest dose of TCDD. Lesions attributed to the bacterial infections were observed in animals infected with S. bern. The authors concluded that doses of TCDD which did not elicit clinical or pathological changes were able to affect host defense.

967. Thomas, J. A. (1974) Actions of pesticides and other drugs on the male reproductive system. U.S. Environmental Protection Agency Report, National Technical Information Service Publication, ISS No. PB-237-381. 30 pp.

The effect of 2,4,5-T on the biotransformation of ^3H -testosterone by the mouse prostate gland was studied. Three groups of rats were administered 6.25, 12.5, or 25.0 mg/kg 2,4,5-T by gastric intubation daily for 10 days. On the next day, ^3H -testosterone was administered intraperitoneally and the anterior prostate glands were excised 5 minutes later, weighed, and analyzed by thin layer chromatography for various radioactive metabolites of testosterone. For other experiments hepatic microsomes of the pretreated mice were incubated for 60 min. with ^3H -testosterone and then radioactivity associated with 6 beta gamma-, 7 gamma-, and /6 gamma-hydroxytestosterone were determined. The low, intermediate, and high doses of 2,4,5-T caused decreases of 14%, 19%, and 35% respectively, in the total amounts of radioactivity associated with the prostate. The proportions of the total radioactivity associated with each of the four metabolites isolated were not altered. The weights of the seminal vesicles and prostate glands were not altered by the treatment, and prostate fructose levels were not altered. Radioactivity (per mg liver tissue) associated with hydroxytestosterone derivatives was not altered by 2,4,5-T pretreatment. The authors concluded that 2,4,5-T did not alter blood levels of androgens (since organ weights were not altered) but decreased testosterone assimilation by the prostate possibly by a direct inhibition of hormone binding to target tissue. Other organochloride herbicides showed effects resembling those of 2,4,5-T while other classes of herbicides tested did not alter androgen metabolism.

968. Thomas, J. A., and Lloyd, J. W. (1973) Organochlorine pesticides and sex accessory organs of reproduction. Pestic. Environ: Continuing controversy, Pap. Inter. Am. Conf. Toxicol. Occup. Med. 8th pp. 43-51.

[Not available.]

969. Thomas, P., and Amor, O. F. (1968) A case of diquat poisoning in cattle. Vet. Rec. 83(26):674-676.

The toxic effects of diquat were described in cattle. A drum that originally contained diquat had been discarded on a farm near pastureland. Four years later, 3 cows that grazed this pasture died and the toxic symptoms and tissue analyses of one heifer for diquat indicated that the deaths were probably from acute oral ingestion of diquat. Tissue levels of the heifer were from 9 to 21 ppm for the liver, kidney, and abomasum and 2 ppm of brain. The total ingested dose was estimated from these data to be 50-100 ml. The viscera of a second cow that died contained no detectable diquat and death was assumed to have occurred after diquat had been excreted. Symptoms observed before death in the heifer included dehydration, sunken eyes, rapid respiration, and hind-leg incoordination. The animal became comatose and died the night after symptoms were recognized. The cow also displayed symptoms of hind-leg incoordination and sunken eyes on the day prior to death. Microscopic examination of tissues from a second heifer that died from the same farm revealed edema, congestion, and inflammation of the mucous of the gastrointestinal tract and cranial and cardiac hemorrhages. Only minor lesions were observed in the cow at autopsy. The authors did not state clearly whether the clinical symptoms, histopathology, and diquat tissue levels were all from the same heifer, as 2 heifers and one cow died, but diquat was confirmed in the tissues of one heifer, only and was the presumed cause of death of the other two animals.

970. Thomas, P. T., and Hinsdill, R. D. (1979) The effect of perinatal exposure to tetrachlorodibenzo-p-dioxin on the immune response of young mice. Drug and Chem. Toxicol. 2(1 & 2):77-98.

The reproductive effects of TCDD administration prior to mating and during gestation is described, and immune competence of offspring is evaluated. Female Swiss Webster mice were fed diets of 1-20 ppb TCDD for 4 weeks and then were bred with male Swiss Webster mice and were maintained on the experimental diet throughout gestation and for the first 3 weeks of lactation; uncontaminated feed was administered on the fourth week. Litters were reduced to 7-8 offspring 1 week after birth and mice were weaned at 4 weeks and tested for immunologic function starting 1 week after weaning. Fetal and newborn mortality, maternal appearance and newborn weights were recorded. Serum of offspring immunized twice with sheep red blood cells (SRBC) was assayed for hemolysin titers and the number of anti-SRBC plaque-forming spleen cells was determined in vitro. 2,4-Dinitro, 1-fluorobenzene (DNFB) was applied to 1 ear of DNFB-sensitized mice and the thickness of the ear 6-72 hours later was compared to the thickness of the other ear which received only vehicle. Spleen cells were cultured with concanavalin A (Con A) or E. coli lipopolysaccharide (LPS) and the proliferative response was measured as the incorporation of [³H]-thymidine into DNA. Some offspring were inoculated with Listeria monocytogenes or Salmonella typhimurium LPS and mortality over the subsequent 14 days was recorded. White blood counts, organ weights and histopathological

changes were determined. Only the highest dosage group of adult mice showed signs of toxicity. These signs appeared by 6 weeks of treatment and included facial alopecia and edema but not reduced body weights. No effects on conception rate or litter size occurred in treated groups, while survival during the first week after birth was decreased in the 2 highest groups (fed 10 and 20 ppb TCDD diets). Offspring showed the same signs of toxicity as adult mice. These signs appeared in the 3 highest dosage groups (5-20 ppb); decreased weights were also observed in some offspring by 4 weeks of age. A dose-related decrease in plaque-forming cells was observed in TCDD-treated offspring, but no decrease in lymphocyte counts or antibody levels occurred. TCDD treatment did not elicit changes in DNFB sensitivity, blastogenic response to mitogens, survival of Listeria inoculation, spleen or liver weights, or differential white cell counts. A marked increase in sensitivity to Salmonella endotoxin, thymic atrophy, and hepatic necrosis were produced by 1-5 ppb TCDD feeding regimens. The authors concluded that doses of TCDD that failed to elicit toxic signs on adult mice impaired immunologic functions of a specific subpopulation of T lymphocytes in offspring. The authors also indicated that the doses used in these experiments were relevant to chronic human exposure to environmental TCDD and their results suggest that these doses induce toxicity. Details of mating frequencies, rate of successful matings and other reproductive and teratogenic parameters that are not reported would have been useful to relate these doses and effects reported here with those of other studies.

971. Thomas, W. L. (1974) The effects of herbicides in South Vietnam. Part B. Working papers: Economic Stress and Settlement Changes. National Academy of Sciences-National Research Council. AD-779 021. 61 pp.

[Background material.]

972. Thomasson, W. A. (1979) Deadly legacy: Dioxin and the Vietnam veteran. The Bulletin pp. 15-19.

[Editorial.]

973. Thompson, D. J., Emerson, J. L., and Sparschu, G. L. (1971) Study of the effects of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) on rat and rabbit fetal development. Teratology 4:243.

[Abstract, only.]

974. Thompson, D. J., Emerson, J. L., Strebing, R. J., Gerbig, C. G., and Robinson, V. B. (1972) Teratology and postnatal studies on 4-amino-3,5,6-trichloropicolinic acid (picloram) in the rat. Food Cosmet. Toxicol. 10(6):797-803.

The effect of picloram on the fetal and neonatal development of the rat were studied. Groups of 35 pregnant rats were administered 500, 750 or 1000 mg picloram /kg by oral gavage from days 6-15 of gestation. A control group of 35 rats received the corn oil vehicle only. Up to 3/4 of each group of rats were killed on day 20 of gestation and corpora lutea, resorptions, live fetuses, fetal weights and gross malformations were determined. Fetuses were fixed in Bouin's fixative or stained with alizarin red and observed for visceral or skeletal malformations, respectively. The remaining litters were allowed to deliver and fetal growth weights and viability were recorded to 21 days of age. The skeletal development of two male and two female weanling rats from each group was examined. Maternal toxicity and mortality occurred from the two highest dosages of picloram. No reductions in litter size or pup weights or increases in the numbers of resorptions or corpora lutea occurred in any treatment group. Malformations with statistically significant higher incidences in treatment groups compared to controls were unilateral hydroureter, unossified fifth sternbrae, and accessory ribs. The gestation period, duration of labor, viability, lactation indices, body weights, and skeletal development was normal for the two lowest dose groups. The viability and lactation indices from birth to day 5 was decreased in the highest dose group, which included a litter of 12 pups with 10 deaths, reported to be from maternal neglect. No other parameters were abnormal for the high dosage group. The authors concluded that picloram was not teratogenic or detrimental to postnatal development under their experimental conditions.

975. Thunberg, T., Ahlborg, U. G., and Johnsson, H. (1979) vitamin A (retinol) status in the rat after a single oral dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Arch. Toxicol. 42(4):265-274.

Retinol levels in the serum and livers of TCDD-treated rats were measured. Male Sprague-Dawley rats were administered 10 ug/kg TCDD in corn oil by oral gavage. Rats were fed diets containing 3.6 mg of retinol per kg feed and food consumption and body weights were recorded twice per week. From 2 days to 8 weeks after TCDD treatment, rats were killed and the livers and serum were removed. Liver homogenates and sera were extracted with ether, dissolved in methanol, and analyzed for retinol by liquid chromatography. The body weights of TCDD-exposed rats were 61 g less than controls after 8 weeks and food consumption was also decreased, corresponding to 1.0 mg of retinol less consumed in 8 weeks by treated rats. Serum levels of retinols varied widely and showed no consistent trends while hepatic retinol concentrations steadily increased in controls over 8 weeks and remained constant in treated rats. Retinol storage in livers of treated rats, expressed as a percentage of storage in control rats, decreased steadily to 30% of controls after 8 weeks. The authors concluded that this difference represented an increase in retinol storage by control rats that was

blocked after TCDD treatment which the authors suggested was caused by an enzyme-induced increase in retinol turnover. No systemic toxicological significance or indications of hepatic toxicity seem to be associated with the altered vitamin A concentrations and storage levels in liver reported in this study.

976. Tinsley, I. J. Chemical concepts in pollutant behavior. (New York: Wiley-Interscience, 1979), 265 p.

977. Todd, R. L. (1962) A case of 2-4D intoxication. J. Iowa Med. Soc. 52:663-664.

A case involving dermal exposure of a 52-year-old man to 2,4-D is described. Two exposures, one on the arm and one on the leg were followed by episodes of nausea and vomiting during the following 5-10 days. The second episode also produced a low-grade fever. A general feeling of weakness noted after the second exposure developed into paralysis of the leg muscles, weakness of the arm and hand muscles, and losses of deep pain sensations, vibratory sensations, and deep tendon reflexes. Severe pain in the posterior portion of the thigh was treated with analgesics. After 6 months of physical therapy the patient was able to walk aided by crutches, and after 2 years was able to walk unaided. A transient bone-marrow depression was also observed accompanied by leukopenia and granulocytopenia of the peripheral blood.

978. Tognoni, G. (1977) Health survey at Seveso. NATO Ecotoxicology Workshop, Univ. of Surrey, Guilford, Surrey, U.K.

[Not available.]

979. Toth, K., Somfai-Relle, S., Sugar, J., and Bence, J. (1979) Carcinogenicity testing of herbicide 2,4,5-trichlorophenoxyethanol containing dioxin and of pure dioxin in Swiss mice. Nature 278(5704):538-549.

This report describes the results of a feeding study, the preliminary results of which were reported in Toth et al., 1978. Twelve groups of 10-week-old Swiss/H/Riop mice weighing 24-30 g were given 2,4,5-trichlorophenoxyethanol (TCPE) and TCDD, TCDD alone, or only the vehicle. The groups receiving a combination of TCPE and TCDD consisted of 100 males and 100 females, while those receiving TCDD or the vehicle alone consisted of 45 males. The study was conducted in three phases, consisting of groups 1-3, 4-8, and 9-12; the first two phases considered animals receiving various combinations of TCPE and TCDD; controls received carboxymethylcellulose as the vehicle. The third phase dealt with animals receiving TCDD only; controls received sunflower oil as the vehicle. TCPE containing TCDD was suspended in a salt solution of 0.5 percent carboxymethylcellulose, and TCDD given alone was dissolved in sunflower oil. The animals were given various

doses by gastric tube once a week for a year and observed for their lifetimes. The TCPE administered to group 1 was 99.7 percent pure. The LD₅₀ of TCPE containing .1 ppm TCDD is 1,320 mg/kg¹ when an acute, peroral dose is given. The maximum tolerated dose was determined to be 70 mg/kg; this was the largest dose that produced no noticeable tissue lesions in the mice during 6 months of treatment. Moribund animals and those died spontaneously were autopsied and their organs were histologically examined. Sections stained with haematoxylin and eosin were examined by light microscopy. Electron microscopy and polarized light were used to identify amyloid deposits stained with Congo red. Statistical analysis was made of the pathological findings. The cumulative data indicate that the only significant difference between treated groups and controls was in the incidence of liver tumors in males. Groups 1, 2, and 10 showed the highest incidence of liver tumors (48, 58, and 48 percent, respectively)--about twice that of their control groups (3 and 12). Group 1 received TCPE at 67 mg/kg and TCDD at .112 ug/kg, in the vehicle given at 50 mg/kg; group 2 received 70.0 mg TCPE per kg and .007 ug TCDD per kg, in the vehicle given at 50 mg/kg; and group 10 received TCDD at .7 ug/kg in the vehicle given at 10 mg/kg. Smaller doses of TCPE and TCDD caused less frequent tumors; groups 5 and 6 showed insignificant differences from control groups 7 and 8. TCDD when given alone was found to have a significant liver tumor-enhancing effect only at the second highest dose (group 10 - .7 ug/kg: 48 percent incidence compared to 18% in controls). Group 9, which received the largest dose of TCDD (7.0 ug/kg) showed a considerable decrease in the average life span, and severe, chronic, ulcerous skin lesions followed by generalized lethal amyloidosis. Skin lesions and amyloidosis were observed in all groups fed TCDD alone. The authors concluded that both TCPE and TCDD enhance liver tumors in male mice, and that this effect is dose-dependent. Severe skin lesions resulting from TCDD may eventually lead to amyloidosis.

980. Toth, K., Sugar, J., Somfai-Relle, S., and Bence, J. (1978) Carcinogenic bioassay of the herbicide 2,4,5-trichlorophenoxyethanol (TCPE) with different 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin) content in Swiss mice. Progress in Biochemical Pharmacology 14:82-93.

The authors studied the hepatic effects in mice of 2,4,5-trichlorophenoxyethanol (TCPE) and TCDD. Five groups of 200 Swiss H/RIOP mice, half of them male and the rest female were treated with a variety of combinations of 2,4,5-trichlorophenoxyethanol (TCPE) and TCDD. Three additional groups receiving only TCDD consisted of 50 males each. The mice were 10 weeks old and weighed 25-30 g at the beginning of the experiment, and they received the compound through gastric intubation once a week for 1 year. They were observed for 1 year following cessation of treatment. Moribund mice, those that died spontaneously, and a healthy mouse from each treatment group were autopsied, and their organs were examined histologically. Tissue samples were fixed in formol and set in paraffin, stained with hematoxylin and eosin, and examined by light microscopy. Amyloid deposits were stained with congo red, and examination under the polarizing microscope showed greenish birefringence. Liver specimens were fixed in a 2.5 percent solution of

glutaraldehyde and then in 1 percent osmium tetroxide. They were then dehydrated in alcohol and embedded in durcupan, and stained with uranyl acetate and lead citrate. These specimens were examined under electron microscope. TCPE containing TCDD was suspended in a salt solution of .05 percent carboxymethyl cellulose, and TCDD alone was dissolved in sunflower oil. The LD₅₀ of TCPE containing 0.1 ppm TCDD was found to be 1,320 mg/kg when an acute peroral dose was given to 6 mice. The maximum tolerable dose was determined to be 70 mg/kg. Three groups of 100 male and 100 female mice served as controls; 1 received only the carboxymethyl cellulose vehicle, and 2 were untreated. A fourth control group of 50 male mice received the sunflower oil vehicle. The doses given to the treated animals were as follows: Groups 1 and 2 were fed 70 mg TCPE/kg and 7×10^{-4} or 7×10^{-6} mg TCDD/kg, respectively; Group 4 mice received 7.0 mg TCPE/kg with 7×10^{-7} mg TCDD/kg; Group 5 received 0.7 mg TCPE/kg with 7×10^{-8} mg TCDD/kg; Group 6 received 7.0 mg TCPE/kg with 7×10^{-3} mg TCDD/kg; and Groups 9, 10, and 11 received only TCDD in doses of 7×10^{-3} mg/kg, 7×10^{-4} mg/kg or 7×10^{-6} mg/kg, respectively. The male mice in groups 1 and 2 showed twice the incidence of liver tumors (43% and 54%) as did the controls (15-28%) after the 2d year of observation. Decrease in the dose of TCPE resulted in a lesser incidence of liver tumors (15-21%) that was comparable to the controls. The dose of TCDD given alone did not appear to be related to the development of liver tumors in any of the mice. There were noticeable similarities between the tumors of control and treated animals on both a macro- and microscopic₃ level. In one group which was administered only TCDD, at a dose of 10^{-3} mg/kg, following autopsy of 19 mice, there were 8 cases of dermatitis associated with amyloidosis involving at least one organ. Three of these animals had post-necrotic liver cirrhosis, and in some cases focal necrosis was apparent. Lower doses of TCDD did not have these effects. The observed effects of TCDD alone were not examined in depth, as this portion of the test was still in progress.

981. Townsend, J. C., Bodner, K. M., VanPeene, P. F. D., Olson, K. D., and Cook, R. R. (1981) Survey of reproductive events of wives of employees exposed to chlorinated dioxins. Unpublished draft report of Dow Chemical Co., Inc. 39 p.

982. Toxic liquid wastes now biodegradable. (1981) Research News, (USDA) April 1981, 2 p.

[Editorial.]

983. Toxicology Information Response Center. (1979) Health effects and environmental fate of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) - A Bibliography, 1976-1979. 40 pp.

[Bibliography.]

984. Truhaut, R., Pham-Hau-Chanh, Van Haverbeck, G., Azum-Gelade, M. C., Saint Ruf, G., and Lareng, L. (1974) Acute toxicity of tetrachlorodibenzo-p-dioxin in rats; structural, ultrastructural, and enzymological study of the liver. C. R. Hebd. Acad. Sci. Ser. D. 279:1565-1569.

[Not available.]

985. TRW Inc. (1978) At sea incineration of Herbicide Orange on board the M/T Vulcanus. (1973) Prepared for the US-EPA, Industrial Environmental Research Laboratory, Office of Energy, Minerals, and Industry, Research Triangle Park, NC. NTIS Publication No. PB281690.

[Background material.]

986. Tsapko, V. P. (1966) The herbicide 2,4-D as a health hazard in agriculture. Gig. Sanit. 31:449-450.

Clinical symptoms that developed in farmers exposed to 2,4-D and the toxicity of 2,4-D in mice were reported. Farmers (number not given) who started to work in a field sprayed with 1 kg/hectare of 2,4-D 1 hour previously complained of general weakness, headache, vomiting, chest pain, and loss of consciousness after 40 minutes. Three days after another field was sprayed with 2,4-D from a tractor, farmers that worked in the field for up to 1 hour complained of headache, burning of the mouth, vertigo, vomiting, general weakness, and fever. A single intragastric dose of 370 mg/kg of 2,4-D produced 100% mortality in mice, while 182.5 mg/kg was not lethal to any mice (numbers of mice tested were not reported). The animals died 1-2 days after they received 2,4-D and exhibited paresis of the limbs, clonic-tonic spasms, shallow respiration, and began comatose prior to death. The authors concluded that the commercial preparation they tested was more toxic than other 2,4-D preparations reported elsewhere because it contained toxic impurities and recommended standardizing the compositions of these preparations as well as requiring that protective clothing be worn by workers in fields 1-2 days after 2,4-D spraying.

987. Tschirley, F. H. (1969) Defoliation in Vietnam. Science 163:779-786.

[Review article.]

988. Tuchmann-Duplessis, H. (1978) Pollution de l' environnement et descendance. A propos de l' accident de Seveso. Med. et Hyg. 36:1758-1766.

[Review article.]

989. Tucker, D. P. (1978) Bromacil and diuron residue levels in Florida citrus soils. Pest. Monit. J. 12(2):47-50.

The authors present data on bromacil and diuron residues in citrus soils following herbicide application for 7-8 years. Two paired blocks of citrus, 10 acres each, were selected as sampling sites. In one block weeds were controlled by tillage while in the block herbicides were used. Herbicide application generally occurred once per year. Soil types differed in each sampling location. The soil in the Polk County grove was Astatula fine sand; the soil at the Hardee County grove was Mayakka fine sand. Each site averaged 114-127 cm rainfall per year. The Hardee County site had permanent supplemental irrigation (30-50 cm/yr), while the Polk county grove recieved only supplemental irrigation. Herbicides were sprayed as wetttable powder formulations of bromacil (3.61 kg/ha) and diuron (1.8 kg/ha). Soil samples from 0-15 cm and 15-30 cm depths were collected in rows between trees, at the drip line or under the tree canopy. Each sample was a composite of 10 subsamples. Samples were collected three times in Polk County and in Hardee County, and were analyzed by microcoulometric gas chromatography (bromacil) or colorimetrically after chromatographic cleanup (diuron). Limits of detection of these analytic methods were not reported. Residue levels expressed as percentages of total herbicide applied over 7-8 years ranged from 0.3-3.9% for bromacil and from 3.7-13.1% for diuron. As percentages of only the last application, the residue levels range from 2.5-31% for bromacil and from 33.6-84.6% for diuron. According to the authors, this is an indication that a large part of the herbicide application remains after 1 year. Bromacil was more evenly divided among the soil layers sampled than diuron, probably a reflection of the greater solubility of bromacil in water. Overall residues were higher in Mayakka fine sand than in Astatula fine sand. The authors conclude that the data indicate that levels of these herbicides that would be toxic to citrus fruit would not accumulate in the soils tested.

990. Tulp, M. Th. M., and Hutzinger, O. (1978) Rat metabolism of polychlorinated dibenzo-p-dioxins. Chemosphere 9:761-768.

Metabolites of polychlorinated dibenzo-p-dioxins were identified. Male Wistar rats were administered a single oral dose of 250 mg/kg of 1 of 8 dibenzo-p-dioxins. Urine and feces were collected for the subsequent 7 days and were analyzed for metabolites. Metabolites were isolated by thin layer chromatography and identified by gas chromatography-mass spectroscopy. The dibenzo-p-dioxins administered included 1 non-chlorinated, 2 mono-chlorinated, 2 di-chlorinated, 1 tri-chlorinated, 1 tetra-chlorinated and 1 octa-chlorinated compound; 2,3,7,8-TCDD was not tested. The monochlorinated compounds and non-chlorinated compound were excreted as sulfur-containing metabolites. All compounds, except the octa-chloro compound, were metabolized to mono- and dihydroxy derivatives. Primary hydroxylation occurred only at the 2,3,7 or 8 position. None of the compounds were metabolized by cleavage of a carbon-oxygen bond. The octa-chloro ompound was not metabolized. The authors concluded that dibenzo-p-dioxin metabolism resulted exclusively

by formation of 2,3-epoxides and that in the 2,3,7,8-TCDD the relevant carbons are chlorinated, blocking this metabolic pathway and preventing its metabolism.

991. Tung, T. T. (1973) Primary cancer of the liver in Viet Nam. Chirurgie 99(7):427-436.

The authors reported that between 1955 to 1961, 159 patients with primary liver cancer out of a total of 5,492 cancer patients were admitted to hospitals in Hanoi, North Vietnam. Between 1962 and 1968, the prevalence of liver cancer cases increased to 791 patients out of a total of 7,911 cancer patients. The increased prevalence of liver cancer in North Vietnam was attributed to the spraying of herbicides in South Vietnam which began in 1961. The appearance of liver cancer in the north was believed to be the result either of population mixing between the north and south regions, or by transport of the herbicide to the north by wind or contaminated mammals, birds, or insects. The report also described methods of diagnosis and treatment of liver cancer. A viral origin of the liver cancer was considered unlikely following analysis of the patients' blood for the presence of antigen which was found in 2.05% of the patients. The authors provided no rationale as to why this particular test eliminated the possibility of a viral etiology. No other possible etiology was considered (i.e., changes in diet or environment) and no proof was provided to support the possible relationship between the prevalence of liver cancer in North Vietnam and herbicide spraying in South Vietnam.

992. Tung, T. T. (1973) Primary carcinoma of the liver in Viet Nam. Pesticide Abstracts 7(6):4-1355.

[Abstract, only.]

993. Tung, T. T., Anh, T. K., Tuyen, B. Q., Tra, D. X., and Huyen, N. X. (1971) Clinical effects of massive and continuous utilization of defoliants on civilians. Vietnamese Studies 29:53-81.

Health effects of South Vietnamese refugees to North Vietnam are presented. A group of 179 refugees out of a total of 903 refugees in Hanoi were selected for this study. (The basis for the selection was not described.) The refugees lived in sprayed areas for 2 months to 5 years. The refugees described the effects of spraying that were elicited immediately after exposure as well as symptoms that persisted for months or years. Chromosomal aberrations were investigated in peripheral blood smears. The content of the spray or the frequency of exposures for individuals reporting symptoms was not addressed. Immediately after spraying, refugees experienced rhinorrhea, continuous sneezing, vomiting, headache and asthenia. These symptoms do not last past 4 days. Other patients experienced swelling of the eyelids, and a sensation of burning on the skin. Prolonged symptoms included asthenia, visual difficulties in reading, and chromosomal aberrations.

For each patient, 300 cells were analyzed and a total of 1600 cells of spray victims (apparently from 5-6 patients) were analyzed. These cells had a higher anomaly rate (5.9 per 100 cells) than the control group rate of 1.1 per 100 cells). The control population was not described. Three cases of Trisomy 21 were described; in only 1 case the time of exposure was considered - the mother left South Vietnam 7 months before the premature birth of the Trisomy 21 child. Other information needed to determine whether the mother or father was exposed to any spray at relevant times was not provided. One case of malformations was described, also without accounting for when exposure occurred relative to conception. The authors concluded that defoliant spraying in Vietnam produced ocular lesions and genetic defects. The correlation between herbicide exposure and adverse health effects in this report is not convincing. The authors do not document exposure to herbicides specifically nor have they demonstrated a causal effect between spraying and chromosomal aberrations or illnesses in the exposed group (which does not appear to be selected in an unbiased manner).

994. Tung, T. T., Lang, T. D., and Van, D. D. (1980) The problem of mutagen effects on the 2nd generation after exposure to herbicides.

The reproductive effects of North Vietnamese populations were studied. Former South Vietnamese soldiers who subsequently migrated to North Vietnam and married women from North Vietnam villages that were not sprayed with chemicals were interviewed regarding the numbers and types of birth defects among their children. Information for some locations were obtained from hospital obstetric records which did not include information on the military service of the father. Half of the 30 birth defects among couples from Yen-Bai were in children whose father was a veteran from South Vietnam and whose mother was from this northern region (the proportion of parents fitting this description for all of the births during 1975-78 in this province was not disclosed). Of these 15 children, five children had limb defects, 6 had anencephaly (usually with other deformities) and the remaining children had cleft palate (1), other cranial abnormalities (1) or abdominal abnormalities (2). Birth defects in the 15 children, whose fathers were not southern veterans included 4 cases of hydrocephaly, 8 cases of Batrachian abdomen, 3 cases of harelip, 1 each of cleft palate, club foot, and anal imperforation. In Quy Mang district, all 9 birth defects of the 233 births between 1976-1978 were in children whose fathers were southern veterans. Thirty families of the 4500 inhabitants (number of families not specified) had fathers that were southern veterans. The birth defects occurred in 6 families and included 3 anencephalies (all in the same family), 1 cleft palate, 1 hydrocephaly, 3 hare lips and 5 cases of limb abnormalities. No other information, including descriptions of families, herbicide exposure potential, sources of information or clinical conditions were presented for these people. The incidence of birth defects, abortions and premature deliveries and sterility were all reported to be higher among families of veterans from the south than the north. The authors concluded that the southern veteran was more likely to have offspring with birth defects than other

Vietnamese fathers. The types of records examined (and their reliability), the clinical definitions of malformations, the family histories, ages of parents, any evidence that herbicide exposure was different among fathers, other concomitant exposures and many other factors, important in evaluating the etiology of these birth defects were not reported.

995. Ulanova, I. P. (1975) Toxicometry and prophylactic toxicology. In Methods Used in the USSR for Establishing Biologically Safe Levels of Toxic Substances. Paper presented at a World Health Organization meeting held in Moscow, December 12-19, 1972; 45-55.
- [Review article.]
996. U.S. Air Force. (1979) Aircraft Sampling Westover AFB MA. USAF OEHL Technical Report: OEHL 79-59. USAF, OEHL, AFSC, Brooks AFB, Texas, 5 p.
- [Background material.]
997. U.S. Air Force (1979) Herbicide Orange site treatment and environmental monitoring. USAF OEHL Report: OEHL TR-79-169. USAF, OEHL, Brooks AFB, Texas. 37 p.
- [Background material.]
998. U.S. Air Force Armament Laboratory. (1968) Biological effectiveness of still bifluid and orange. Technical Report No. AFATL-TR-68-122.
- [Background material.]
999. U.S. Air Force Armament Laboratory. (1969) Comparison Test of Defoliants. Vol. II. Appendix III. Biological effectiveness of still bifluid and Orange No. ADTC-TR-69-30.
- [Background material.]
1000. U.S. Air Force Armament and Development Test Center. (1969) Comparison of Defoliants Vol. I. Authors. No. ADTC-TR-69-30.
- [Background material.]
1001. U.S. Air Force. (1966) An ecological study on the effects of certain concentrations of cacodylic acid on selected fauna and flora. No. APGC-TR-66-54. 23 p.
- [Background material.]
1002. U.S. Air Force. (1975) Studies of the ecological impact of repetitive aerial applications of herbicides on the ecosystem. AD-A032 773. 127 p.
- [Background material.]

1003. U.S. Department of Health, Education, and Welfare. (1978) Bioassay of picloram for possible carcinogenicity. National Cancer Institute Carcinogenesis Report Series No. 23. 91 pp.

In this report, the carcinogenicity of technical grade picloram (90% pure) was evaluated in mice and rats. Osborne-Mendel rats (50 animals of each sex per dosage group) or B6C3F1 mice (80 animals of each sex per dosage group) were fed picloram for 80 weeks. Only 10 animals of each sex were used as matched controls. The low-dose rats were fed 10,000 ppm picloram for 39 weeks, then 5,000 ppm for 41 weeks for a time weighted average of 7,437 ppm. The high-dose group of rats received 20,000 ppm picloram for 39 weeks and 10,000 ppm for 41 weeks. Animals were observed for another 33 weeks before termination of the study. In mice, the low-dose group was fed 5,000 ppm for 1 week and 2,500 ppm for 79 weeks, while the high-dose group was fed 10,000 ppm for 1 week and 5,000 ppm for 70 weeks. Time weighted averages were 2,531 and 5,062 ppm for the low- and high-dose groups, respectively. Animals were observed for 10 weeks after treatment was terminated. Picloram was mixed with the standard lab diet with acetone and corn oil each at 2% of the final feed weight. Treated feed was kept at 17°C for no longer than 1 week. Samples of formulated feed were analyzed for picloram concentration at intervals through the experimental period. The mean analytical concentrations for the tested samples was within 2.5% of the calculated concentration; the coefficient of variation did not exceed 6.7%. Food and water were supplied to the animals ad libitum. During the course of the study all animals were observed for signs of toxicity, tumor formation, and body weight gain. No blood chemistry of liver enzyme analyses were performed. Pathological evaluation of animals who were killed or died during the study included gross and microscopic examination of major organs and tissues, and all lesions. Signs of toxicity in rats began to be observed during the second 6 months of treatment and included diarrhea, hematuria, and rough coats. During the second year of the study, additional signs of toxicity occurred: pale mucous membranes, dermatitis, alopecia, tachypnea, discolored urine, diarrhea, and vaginal bleeding. However, no significant differences in survival were observed between control and treated groups. A relatively high incidence of follicular hyperplasia, c-cell hyperplasia, and c-cell adenoma of the thyroid was observed in both sexes. However, statistical tests did not show evidence for association of these conditions with picloram treatment. There was some evidence of liver effects associated with picloram treatment. Females were more effected than males. Increased incidences of hepatic neoplastic nodules, a benign lesion, were observed in treated animals of both sexes compared with untreated controls, which was statistically significant ($p=0.016$) in females but not males. In addition, both males and females possibly developed a treatment related lesion, foci of cellular alteration, that is frequently associated with induction of hepatic carcinoma and neoplastic nodules in rates. No statistics on this lesion were presented. In mice, signs of toxicity associated with picloram treatment were not observed until the second year of the study. These included slight hyperactivity, rough hair coat, and abdominal distention. No differences in survival occurred. No tumors associated with

picloram treatment were found in either male or female mice. From this bioassay, the report concluded that picloram was not carcinogenic in male Osbourne-Mendel rats or B6C3F1 mice. In female rats, however, incidence of benign tumors was associated with picloram treatment.

1004. U.S. Department of Health and Human Services. [Fourth progress report of Interagency Work Group made public.] News Release, August 1, 1980. 5 pp.

[Background material.]

1005. U.S. Environmental Protection Agency. (1979) Preliminary report of assessment of a field investigation of six-year spontaneous abortion rates in three Oregon areas in relation to forest 2,4,5-T spray practices. Prepared by the Epidemiologic Studies Branch Benefits and Field Studies Division OPP, OTS, EPA.

The report describes a retrospective study of 2,4,5-T exposure and the incidence of spontaneous abortion. Three cohort groups were selected from a highly sprayed control forest region (Study) a bordering non-forested agricultural region (Urban), and a control area with little reported use of 2,4,5-T. To estimate exposure rates information or when, where, and how much 2,4,5-T was sprayed was obtained. Incidence data were abstracted by hospital personnel and staff epidemiologists at hospitals in each of the three areas. Physician interview data supplemented abstracted data. A spontaneous abortion index with a 5-month moving average was used to describe incidence. Statistical methods used in the covelational analysis included: analysis of variance, frequency tables by chi-square, power spectrum analysis, cross-correlation analysis, both parametric and non-parametric statistics and linear multiple regression fitted to a sine wave model. Data show the following: significantly higher spontaneous abortion rate for the study group than either the control or urban group; a statistically significant seasonal cycle in the abortion index with approximate 4-month peaking period observed in the study group in June; a significant cross-correlation between the study group spontaneous abortion index rates and exposure by month with an lag of 2 or 3 months.

1006. U.S. Environmental Protection Agency, Office of Pesticides Programs. (April 21, 1978) Rebuttable presumption against registration and cont'd registration of pesticide products containing 2,4,5-T. Federal Register 43:78. pp. 6500-6501.

[Background material.]

1007. U.S. Environmental Protection Agency. (1978) Pesticide programs - Rebuttable presumption against registration and continued registration of pesticide products containing 2,4,5-T. Federal Register 43(78):17116-17147.

[Review article.]

1008. U.S. General Accounting Office, The Use of Herbicides and Other Chemicals in Vietnam. Letter and attachments to R. H. Metcalfe, U. S. House of Representatives, on August 16, 1978. 14 pp.

[Background material.]

1009. Van Logten, M. J., Gupta, B. N., McConnell, E. E., and Moore, J. A. (1980) Role of the endocrine system in the action of 2,3,7,8-TCDD on the thymus. Toxicology 15(2):135-144.

The toxic effects of TCDD were assessed in adrenalectomized rats and hypophysectomized rats. Female Frischer rats were administered one dose of 10-20 ug/kg TCDD in corn oil by oral gavage. Some rats underwent adrenalectomy or hypophysectomy at least one week prior to TCDD treatment and some of the hypophysectomized rats were administered 0.25 mg growth hormone subcutaneously daily for 11 days, beginning 1 day prior to TCDD treatment. Three or 10 days after TCDD was given to experimental rats or corn oil was given to controls, rats were killed, hematological analyses were performed in blood samples, and tissues were removed, weighed, and examined histologically. After 3 and 10 days, body weights and thymus weights relative to body weights for both adrenalectomized rats and normal rats given 10 ug/kg TCDD were significantly below corresponding rats that did not receive TCDD. After the higher dose was administered, these effects were more pronounced and were accompanied by decreased ulcerine weight and increased liver weight, both relative to body weight, thymic involution, and hepatic necrosis. Hypophysectomy even accompanied by growth hormone administration failed to prevent weight loss or thymic involution induced by TCDD treatment. Upon histologic evaluation, the thymus of hypophysectomized rats given TCDD was more affected than the thymus of normal rats treated with TCDD and rats given growth hormone and TCDD. No increases in peripheral blood lymphocytes or polymorphonuclear leukocytes occurred in TCDD-treated rats. The authors concluded that the adrenal-thymus axis was not involved in the TCDD effect because the spleen and adrenals were not affected. Thymic atrophy from TCDD treatment was also not mediated by the adrenals or the pituitary gland.

1010. Van Miller, J. P., and Allen, J. R. (1977) Chronic toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats. Fed. Proc. Fed. Am. Soc. Exp. Biol. 36:396.

[Abstract, only.]

1011. Van Miller, J. P., Lalich, J. J., and Allen, J. R. (1977) Increased incidence of neoplasms in rats exposed to low levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Chemosphere (9):537-544.

The authors investigated the carcinogenicity of TCDD in rats. Male Sprague-Dawley rats (10 per dose level) weighing about 60 g each were fed a diet containing 0, 1, 5, 50, or 500 ppt or 1, 5, 50, 500, or 100 ppb TCDD. Diets were formulated by adding TCDD in suspended acetone and dissolved in corn oil to standard lab chow. No analysis of the food was made to determine if the formulations were correct or if formulations degraded under storage conditions. The animals were fed the TCDD containing diet for 78 weeks, at 65 weeks laparotomies were performed on surviving animals to determine if tumors were present.

Animals were observed up until 95 weeks. Complete necropsies were performed on all animals and microscopic examination of major tissues was performed. Animals receiving 50, 500, and 1000 ppd died during the first 4 weeks of the experiment. Gross pathological examination revealed atrophy of the thymus and spleen, dilation of common bile ducts, and gastrointestinal tract hemorrhage. Microscopic examination of tissues detected severe liver necrosis and cellular proliferation of the common bile ducts. Decreased spermatogenesis was also observed in many of the animals. All of the animals in the 1 and 5 ppb groups died by the 90th week of the experimental, while 4-8 animals died per group at the 0-500 ppt dose levels. No death-related dose effect was observed. The overall incidence of neoplasms in the 5ppb was 38%. No tumors were found in the 1 ppt on the control groups. The number of neoplasms or the number of animals with neoplasms detected did not increase with increasing TCDD dose. Liver tumors occurred only in animals receiving 1 or 5 ppb TCDD. While tumor incidence of treated groups was higher than control groups, the experiment uses too few animals to prove conclusively that TCDD is carcinogenic. The authors concluded that the data suggest that TCDD may be carcinogenic.

1012. Van Miller, J. P., Marlar, R. J., and Allen, J. R. (1976) Tissue distribution and excretion of tritiated tetrachlorodibenzo-p-dioxin in non-human primates and rats. Fd. Cosmet. Toxicol. 14:31-34.

The distribution of TCDD in tissues of adult and infant Rhesus monkeys and in rats is presented. Single intraperitoneal doses of 400 ug/kg [³H]-TCDD in corn oil were administered to 3 adult female monkeys, 4 male infant monkeys (2-4 months of age) and 5 adult male Sprague-Dawley rats. Urine and feces were collected for 7 days. Body fluids and tissues were analyzed for radioactivity and tissue samples were prepared for light microscopy and liver samples for light microscopy. Seven days after exposure, the adult monkeys, infant monkeys and rats lost 11%, 21%, and 11% respectively of the initial body weights. Thymic atrophy and fatty infiltration of the liver were observed in the rats, hypertrophied hepatocytes were observed in adult monkeys, and proliferation of the smooth endoplasmic reticulum was observed in all livers. The percentages of the dose of radioactivity excreted in urine were 1.1, 2.0, and 0.5%, in adult monkeys, infant monkeys and rats, respectively, and in feces were 4, 1, and 5% respectively. Total recovery of radioactivity was 59-72% for the 3 groups. Differences in tissue distribution of TCDD were observed for the 2 species. Over 40% of the administered dose was retained in rat liver at 7 days and 10% was retained in the liver of monkeys. The fat, skin and muscle of monkeys had relatively higher levels of radioactivity than these tissues from rats. The authors concluded that the higher rat hepatic levels were in accordance with smooth endoplasmic reticular proliferation and hepatotoxicity in the rat and the higher skin levels for monkeys correlated with dermal lesions seen in monkeys but not rats.

1013. Verhulst, H. L., and Crotty, J. J. (1968) Deaths from chlorinated phenoxyacetic acids (2,4-D, 2,4,5-T; MCPA). National Clearinghouse for Poison Control Centers, Bulletin. Atlanta, Communicable Disease Center. pp. 1-4.

A brief review of reports of poisoning by 2,4,5-T and 2,4-D to the National Clearinghouse for Poison Control Centers is presented. The bulletin mentioned that several cases of skin rash and of peripheral neuritis were reported after exposure to these herbicides. After two sisters played in a heavily sprayed area for several hours, shortly after the spraying was done, inflammation of the mouth, lips, eyelids, and mucous membranes and a skin rash developed; poison oak was also a suspected cause of these illnesses. A Michigan farmer repeatedly unplugged an herbicide (specific compound was not mentioned) sprayer and developed peripheral neuropathy 4 days later. Other symptoms associated with unspecified chlorinated phenoxyacid derivatives were listed. A fatal suicide case of a 48-year-old man was described. The victim had ingested "a cupful" of weed killer which contained 20% 2,4-D and 40% 2,4,5-T. He vomited within 1 hour and became dazed. After 1 day, the patient was febrile, had low blood pressure, was hyperventilating, and had elevated blood urea nitrogen level. Drug therapy failed to reverse the clinical systems and an erythema and anuria developed. The patient died 46 hours after the ingestion, of cardiac arrest. Symptoms were compared to a case of MCPA poisoning. The authors concluded that some controversy has arisen regarding the toxicity of 2,4-D and 2,4,5-T.

1014. Verrett, J. (April 7 and 15, 1970) Effects of 2,4,5-T on man and the environment. Hearings of the Committee on Commerce (U.S. Senate). (Washington, DC: U.S. Gov't. Printing Office). p. 190.

[Not available.]

1015. The Veterans Administration. (1981) Advisory Committee on Health-Related Effects of Herbicides - Transcript of Proceedings. Feb 4, 1981. 158 pp.

[Background material.]

1016. The Veterans Administration. (1980a) Agent Orange Bulletin, December 1(1). 4 p.

[Background material.]

1017. The Veterans Administration. (1980b) Advisory Committee on Health-Related Effects of Herbicides - Transcript of Proceedings (Third Meeting, Dec. 12, 1979), 123 pp.

[Background material.]

1018. The Veterans Administration. (1980c) Advisory Committee on Health-Related Effects of Herbicides - Transcript of Proceedings (Fourth Meeting, April 23, 1980), 123 pp.

[Background material.]

1019. The Veterans Administration. (1980d) Advisory Committee on Health-Related Effects of Herbicides - Transcript of Proceedings (Fifth Meeting, Aug. 6, 1980), 136 pp.

[Background material.]

1020. The Veterans Administration. (1980e) Advisory Committee on Health-Related Effects of Herbicides - Transcript of Proceedings (Sixth Meeting, Nov. 6, 1980), 144 pp.

[Background material.]

1021. The Veterans Administration. (1980f) "Agent Orange" A Selected Bibliography. 2nd ed. 16 pp.

[Bibliography.]

1022. The Veterans Administration. (1980h) Proceedings from the 2d Continuing Education Conference on Herbicide Orange. Washington, DC, May 28-30.

[Background material.]

1023. The Veterans Administration. (1979) [Agent Orange] News Release, June 1, 1979. 4 pp.

[Background material.]

1024. The Office of the White House Press Secretary. (1979f) [Agent Orange]. News Release, Dec. 11, 1979. 1 p.

[Background material.]

1025. The Veterans Administration. (1979b) [Decision to study exposed Vietnam veterans] News Release, May 29, 1979. 2 pp.

[Background material.]

1026. The Veterans Administration. (1979c) [Health related effects of herbicides.] News Release, June 8, 1979. 3 pp.
[Background material.]
1027. The Veterans Administration. (1979d) [Health related effects of herbicides.] News Release July 22, 1979. 3 pp.
[Background material.]
1028. The Veterans Administration. (1979e) [Testing of Agent Orange carried in body fat.] News Release Feb. 7, 1979. 2 pp.
[Background material.]
1029. The Veterans Administration. (1979g) Transcript of Proceedings - In the Matter of: Advisory Committee on Health-Related Effects of Herbicides. June 11, 1979. 143 pp.
[Background material.]
1030. The Veterans Administration. (1979h) Transcript of Proceedings - In the Matter of: Advisory Committee on Health-Related Effects of Herbicides (Second Meeting, Sept. 24, 1979), 69 pp.
[Background material.]
1031. Veterans Administration Office of the Administrator of Veterans' Affairs: "Agent Orange and Veterans' Health." [Statement of Administrator of Veterans' Affairs] Washington, DC. 3 pp.
[Testimony.]
1032. Vinopal, J. H., and Casida, J. E. (1973) Metabolic stability of 2,3,7,8-tetrachlorodibenzo-p-dioxin in mammalian liver microsomal systems and in living mice. Arch. Environ. Contam. Toxicol. 1(2):122-132.

Biotransformation of TCDD by hepatic microsomes from rabbit, rat, and mice in vitro and by mice in vivo are described. Liver microsomes were prepared from male New Zealand rabbit, male Sprague-Dawley rat and male Swiss-Webster mouse tissue and were incubated in the presence of [³H]-TCDD. In the absence or presence of added NADPH, all of the radioactivity was recovered by extraction into organic solvents (i.e., as the unmetabolized compound). No metabolites were identified after the incubation period by thin-layer or gas chromatographics. Male mice (2-6 per group) were administered 130 ug/kg [³H]-TCDD in olive oil,

intraperitoneally. Urine and feces collected for 3 days and liver and kidneys removed 1-20 days after exposure were analyzed for radioactive metabolites by thin-layer and gas chromatographics. No metabolites were detected in any samples. Trace amounts of radioactivity were detected in the urine and kidney and 13% of the dose was recovered in the feces. About 15% of the dose was recovered in the liver after 1 and 4 days, 27 and 22% after 8 and 11 days, respectively, and 10% at 15 and 20 days. The microsomal fraction had the highest concentration of radioactivity, of the various subcellular fractions of hepatic tissue. The authors concluded that TCDD was preferentially localized in the endoplasmic reticulum was resistant to biotransformation, and was excreted primarily in feces, potentially from biliary excretion.

1033. Vogel, E., and Chandler, J. L. R. (1974) Mutagenicity testing of cyclamate and some pesticides in *Drosophila melanogaster*. Experientia 30:621-624.

The authors tested 2,4-D and 2,4,5-T Na⁺ salt in a sex linked recessive lethal test in *Drosophila melanogaster*. Adult 2-day-old Berlin K males were fed 2,4-D (4.5 or 9.0 mM) or 2,4,5-T (3.6 or 7.2 mM) for 3 days. Following treatment the males were mated to 2 females for 3 days (brood 1). Males were mated with new females for an additional 3-day brood (brood 2) and a 4²-day brood (brood 3). Results of the study were analyzed by the X² test with criteria for a positive result being p less than 0.01. Neither 2,4-D nor 2,4,5-T induced increased numbers of recessive lethals compared to controls. However, a decline in fertility in broods 2 and 3 was observed in flies treated with 7.2 mM 2,4,5-T.

1034. Vos, J. G. (1978) 2,3,7,8-Tetrachlorodibenzo-para-dioxin: Effects and mechanisms. In Chlorinated Phenoxy Acids and Their Dioxins, C. Ramel, ed. (Ecol. Bull. No. 27, Stockholm: Swedish Natural Science Research Council, 1978) p. 165-176.

[Review article.]

1035. Vos, J. G. (1977) Immune suppression as related to toxicology. CRC Critical Reviews in Toxicology 5(1):67-101.

[Review article.]

1036. Vos, J. G., Kreeftenberg, J. G., Engel, H. W. B., Minderhoud, A., and Van Noorle Jansen, L. M. (1978) Studies on 2,3,7,8-tetrachloro-dibenzo-p-dioxin induced immune suppression and decreased resistance to infection: Endotoxin hypersensitivity, serum zinc concentrations and effect of thymosin treatment. Toxicology 9:75-86.

The effects of TCDD on thymic atrophy in thymosin-treated mice, on endotoxin hypersensitivity and on macrophage function are described. Swiss mice were administered 1.5-100 ug/kg (98.6% purity) in

acetone-arachis oil (1:9) orally. After 2-5 days E. coli endotoxin was administered and serum zinc levels and mortality were assessed 48 hours after endotoxin administration. Listeria monocytogenes migration to the spleen was assessed in male mice inoculated 4 days after 4 weekly doses of 50 ug/kg TCDD had been administered. Peritoneal macrophages were harvested from male mice after 4 weekly doses of 50 ug/kg TCDD was administered; glucose oxidation rates of macrophages were estimated by a nitro-blue tetrazolium dye technique. Mice were treated with 10 ug/kg TCDD at 1, 4, 8, 11, 15 and 18 days of age. Thymosin was administered daily for 3 weeks. Thymus cells were isolated, and their response to mitogens was determined in vitro by assessing [³H]-thymidine incorporation. Thymogen treatment did not increase mitogenic responsiveness of thymic cells or alter thymic weight or serum zinc concentrations in TCDD-treated mice. Susceptibility to endotoxin was enhanced by TCDD treatment, while macrophage oxidation and Listeria phagocytosis and transport to the spleen remained unaltered. The authors concluded that immunosuppression by TCDD resulted from an unknown effect on T-lymphocytes, since no changes in macrophage phagocytosis viability or metabolism occurred which could account for observed endotoxin hypersensitivity.

1037. Vos, J. G., and Moore, J. A. (1974) Suppression of cellular immunity in rats and mice by maternal treatment with 2,3,7,8 tetrachloro-dibenzo-p-dioxin. Int. Arch. Allerg. Appl. Immunol. 47:777-794.

The effects of perinatal exposure of rats and mice to TCDD on cellular immunity is described. Female Fisher 344 rats and C57Bl/6 Sch mice were administered 1-5 ug/kg TCDD in acetone-corn oil (1:6) by oral intubation. Maternal treatments were administered to rats on days 11 and 18 of gestation and on postnatal days 4, 11 and 18, or only postnatally, on days 0, 7 and 14. Maternal treatments to mice were administered on days 14 and 17 of gestation and postnatal days 1, 8 and 15. Male 1-month-old mice were administered 4 weekly doses of 1, 5 or 25 ug/kg TCDD and male 4-month-old mice were administered 6 weekly doses of 1, 5 or 25 ug/kg TCDD. Spleen and thymus lymphocytes were removed from treated animals and cultured in the presence of phytohemagglutinin (PHA) or concanavalin A (Con A) mitogen and [³H]-thymidine incorporation into DNA was measured to assess the proliferative response of cultures to mitogens. Other lymphocytes were exposed to TCDD in vitro only. Graph versus host assays were performed by injecting C57Bl/6 donor mouse spleen cells into the right hind foot pad of (C57Bl/6 x DBA-2) hybrid recipient mice. The ratio of the weights of the right to left popliteal lymph nodes was used to determine GVH activity. For rat GVH studies, Fisher-344 donor cells were administered to (F-344 x BN) hybrid recipients. Tail skin grafts from DBA-2 mice or (F-344 x BN) hybrid rats were grafted to C57Bl/6 mice and F-344 rats, respectively; autografts were also performed. Allograft rejection times were measured. In some experiments hematological parameters and histopathology were evaluated. Rats in the 5 ug/kg TCDD treatment group pre- and postnatally or postnatally only had reduced spleen, thymus and body weights and high fetal mortality; decreased organ weights, but no mortality occurred in the 1 ug/kg

dosage group. Postnatal TCDD treatment resulted in reduced responsiveness of spleen and thymus cells to PHA and decreased GVH activity in rats and mice. Peripheral lymphocyte counts and levels of serum proteins were not significantly altered by postnatal treatment. Skin graft rejection times in mice that were treated pre- and post-natally with 2 and 5 ug/kg doses of TCDD were prolonged. Lymphocytes from one-month-old mice treated with 25 ug/kg TCDD showed decreased responsiveness to PHA and decreased thymus weights, while the 4-month-old mice treated with this dose had decreased thymus weights, only. No decrease in GVH activity or in other organ weights occurred in 1- or 4-month-old mice treated with any dose of TCDD. In vitro treatment of lymphocyte with up to 0.02 ug TCDD per ml. medium had no effect on proliferative response to mitogens. Thymus atrophy was confirmed histopathologically in treated mice and rats, although liver damage was not observed. Decreased eosinophilia of acidophilic cells of the adenohypophysis was observed in treated rats. The authors concluded that TCDD-induced suppression of cell-mediated immune responsiveness was an age-related phenomenon which, in the neonate, resembled the effects of thymectomy. The authors postulated that cellular-immunosuppression may be the cause of death in mouse and rat neonates and in adult guinea pigs, all of which fail to develop major liver pathology after TCDD treatment.

1038. Vos, J. G., Moore, J. A., and Zinkl, J. G. (1974) Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in C57Bl/6 mice. Toxicol. Appl. Pharmacol. 29:229-241.

The acute and subchronic toxicities of TCDD were evaluated in the mouse. Male C57Bl/6 mice were administered TCDD (99+% purity) in acetone-corn oil (1:6) by gastric intubation. Single doses of 100, 150 or 200 ug/kg TCDD or vehicle only were administered to 58 mice in an acute study and weekly doses of 0.2-2.5 ug/kg TCDD were administered for 2-6 weeks to 100 mice in a subacute study. Body weights and some organ weights were recorded and necropsies, histologic, and hematologic examinations were performed. Liver sections were stained with periodic acid-Schiff reagent for lipids and iron and was examined by fluorescence microscopy for porphyrins. The oral LD₅₀ was 114 ug/kg. The mean survival time was about 3 weeks for mice treated with 150 or 200 ug/kg TCDD. Body weight losses occurred, but were followed by terminal increases which were attributed to fluid accumulation. The mean body weight of survivors was 11% below controls 2 months after treatment. Ocular lesions, atrophy of the spleen and thymus, hemorrhagic and distended intestines, hepatic necrosis, lipid vacuoles, and evidence of excessive amounts of hepatic porphyrins were reported after acute exposure. After subacute exposure to 5 or 25 ug/kg doses of TCDD, significant losses in body weight, increases in liver to body weight ratios, and decreases in thymus to body weight ratios occurred. Increases in neutrophils, hemoglobin, mean corpuscular hemoglobin, and total serum protein concentrations occurred in the highest dosage group. Tissue changes after subacute exposure resembled those that occurred after acute exposure. Lipid accumulation in the liver showed a dose-related pattern that occurred from even the lowest dose of

0.2 ug/kg TCDD. The authors suggested that the toxic dose of TCDD to C57B1/6 mice was age-dependent based on their unpublished observations.

1039. Vos, J. G., Moore, J. A., and Zinkl, J. G. (1973) Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the immune system of laboratory animals. Environ. Health Perspec. 5:149-162.

The effects of TCDD on cell-mediated and humoral immunity were studied in the guinea pig, rat, and mouse. Female Hartley guinea pigs (10 per group) were administered 8 weekly doses of 0.008-1.0 ug/kg TCDD in acetone-corn oil, orally. Humoral immunity was determined by measuring serum tetanus-antitoxin concentrations by radial immunodiffusion following 2 subcutaneous injections of tetanus toxoid to guinea pigs. Delayed hypersensitivity to tuberculin was measured as the thickness of the skin at the site of tuberculin injections to sensitized guinea pigs. Pooled serum samples were also analyzed for cortisol and corticosterone levels. Delayed hypersensitivity was also tested in female CD rats (10 per group) administered 6 weekly doses of 0.2-5 ug/kg TCDD, orally. Donor spleen cells were prepared from male C57B1/6 mice administered 4 weekly doses of 0.2-25 ug/kg TCDD, orally. Donor cells were injected into the right hind foot pads of C57B1/6 x DBA-2 hybrid mice. The ratio of weights of the right to left the popliteal lymph nodes of the recipient after 7 days was used as a measure of cell-mediated immunity. Thymic atrophy was evident in guinea pigs administered 0.2 ug/kg TCDD, while the higher dose produced 100% mortality and this group was not studied further. Corticosteroid levels were not altered by TCDD and serum tetanus antitoxin levels were decreased in the 0.2 ug/kg treatment group, only. Skin reactions to tuberculin showed a TCDD dose-related decrease in response in guinea pigs but no effect was seen in the rat. The highest dose of TCDD administered to rats produced thymic atrophy, but no deaths occurred. Severe thymic atrophy was observed in mice treated with 5 and 25 ug/kg and at 5 ug/kg a highly significant decrease in graft versus host activity was observed; spleens of the 25 ug/kg treatment group were too small to perform the experiment. The authors concluded that sublethal doses of TCDD suppressed cell-mediated immunity in the guinea pigs and mice which may have been the cause of death in these species; TCDD also caused a slight suppression in humoral immunity in guinea pigs.

1040. Vos, J. G., et al. (1978) TCDD accident at a chemical factory in the Netherlands. Working Papers. IARC, Lyon - Joint NIEHS/IARC working group report.

[Not available.]

1041. Wade, N. (1979) Viets and vets fear herbicide health effects. Science 204:817.

[Editorial.]

1042. Wagner, S. L., and Weswig, P. (1974) Arsenic in blood and urine of forest workers as indices of exposure to cacodylic acid. Arch. Environ. Health 28(2):77-79.

The levels of exposure of 5 forest workers to cacodylic acid and the corresponding urinary excretion of arsenic are reported. The numbers of hours of exposure and amounts of cacodylic acid used by 5 white men were recorded for an 11-week period. Prescribed clothing and plastic masks were worn by the workers most of the time (the level of compliance was not estimated). Blood samples and 24-hour urine samples were collected at the end of every 5-day work week and were analyzed for cacodylic acid. Blood and urine were analyzed from a control group of 5 forest workers not exposed to cacodylic acid. Physical examinations and blood chemistries, hematological and urine analyses performed just prior to and following the 11 week exposure period revealed no unusual findings. No workers lost time from work for health reasons and the only clinical symptoms reported were nausea, diarrhea and general aches for 2 days by 1 worker. Average duration of exposure was 23.7 hours per week to 817 grams (dry) cacodylic acid per man per week. Both blood and urine levels rose during the first weeks of exposure. Urine levels dropped on the ninth week, when exposure ended. Blood levels dropped after week 4 and the only change that correlated with this drop was a severe heat wave which was suggested to have resulted in higher arsenic excretion in the sweat. No correlation coefficients were calculated for individual cacodylic acid exposure levels and urine or blood arsenic levels. The correlation between urine and blood arsenic levels was low (coefficient = 0.14). The maximum level reached for exposed workers was 0.27 ppm of arsenic in blood and for controls was 0.08 ppm. The maximum urine levels for exposed workers was 519 ppm and for controls was 201 ppm. The authors concluded that cacodylic acid did not cause intoxication when proper handling methods were used. The authors suggested that a strong garlic odor in sprayed areas which was reported by all workers indicated that forest workers may be exposed to the toxic metabolite, arsine gas after spraying cacodylic acid.

1043. Walker, A. E., and Martin, J. V. (1979) Lipid profiles in dioxin-exposed workers. Lancet: 446-447.

[Abstract, only.]

1044. Walker, A., and Smith, A. E. (1979) Persistence of 2,4,5-T in a heavy clay soil. Pestic. Sci. 10:151-157.

The authors report on the persistence of 2,4,5-T in a heavy clay soil when temperature and soil moisture are varied. Regina heavy clay soil,

0-5 cm layer, was collected and stored for 3 months. In the laboratory experiments, ^{14}C -2,4,5-T (2ug/g soil) was used; in field experiments unlabelled 2,4,5-T (5 mg/20x20 cm plot) was used. In the laboratory, degradation of 2,4,5-T (2 ug/g soil) was measured at 8-34% moisture at 10-35 C for up to 70 days. Duplicate samples were analyzed for 2,4,5-T at 7-14 day intervals. Regardless of temperature, at 8% soil moisture little to no 2,4,5-T was degraded. The half life of 2,4,5-T in this soil sample varied from 4 days at 35C and 34% moisture to 60 days at 10C and 20% moisture. Under field conditions the soil has 40% moisture. In field experiments in the spring, 90% degradation of 2,4,5-T occurred in 50 days when the temperature averaged 16C and rainfall was high (amounts not specified). Under dry summer conditions, degradation was slower even though temperatures were higher. In the fall, when rainfall was low and temperatures averaged 10C, degradation was also slow. These data are consistent with trends observed in laboratory models. The authors concluded that when field and laboratory experiments were compared, the laboratory models generally overestimated the degradation patterns of 2,4,5-T. From these experiments, it appears that degradation of 2,4,5-T is dependent on temperature and moisture content of the soil. The authors did not discuss the importance of biological, chemical, and physical losses which may explain these patterns.

1045. Walker, E. M., Gadsden, R. H., Atkins, L. M., and Gale, G. R. (1972) Some effects of 2,4-D and 2,4,5-T on Ehrlich ascites tumor cells in vivo and in vitro, Industr. Med. 41(1):22-27.

The effects of 2,4-D and 2,4,5-T on Ehrlich ascites tumor growth and synthesis of DNA, RNA, and protein by tumor cells were evaluated. Ehrlich ascites tumor cells were maintained in BALB/c mice and daily intraperitoneal doses in dimethyl sulfoxide of 45-75 mg/kg 2,4-D (99% purity) or 62-85 mg/kg 2,4,5-T (99% purity) were administered for 5-6 days. Controls received vehicle only. Average change in body weight, mortality, total packed tumor cell volume, and survival time were recorded. The effects of 2,4-D and 2,4,5-T on de novo RNA purine synthesis were evaluated in tumor cells in vivo. [^{14}C]-Formate was administered intraperitoneally 24 or 48 hours after a single dose of 25-100 mg/kg of 2,4-D and 2,4,5-T in DMSO was administered intraperitoneally. Three hours after formate was administered, the cells were removed and radioactivity associated with adenine and guanine were determined. Incorporation of ^3H -uridine, ^3H -thymidine and ^{14}C -leucine into RNA, DNA, and protein, respectively, was determined in vitro after a 20 minute pulse period by radioactive analysis of TCA-precipitable cell contents. 2,4-D and 2,4,5-T (10^{-4}M each) were introduced into the cultures 0-2 hours before the precursors. Both 2,4-D and 2,4,5-T produced ion inhibition of tumor growth in vivo, with 30-73% inhibition of total packed cell volume for all doses of both compounds tested. Survival time was increased by 26% for 2,4-D and 41% for 2,4,5-T. Effects of both compounds on RNA synthesis in vivo were variable and slight increases in RNA, DNA, and protein synthesis were observed for treated cultures. The authors concluded that an appreciable inhibitory effect on Ehrlich ascites tumor growth occurred from 2,4-D and 2,4,5-T

treatments but the changes observed in the remaining biochemical parameters were inadequate to explain the growth inhibition.

1046. Wallis, W. E., Van Poznak, A., and Plum, F. (1970) Generalized muscular stiffness, fasciculations, and myokymia of peripheral nerve origin. Arch. Neurol. 22:430-439.

A clinical description is made of a 21-year old male who suffered from a syndrome of generalized muscular stiffness, fasciculations and myokymia. He developed these symptoms after having sprayed 2,4-D. For one year, the patient had been employed as a farmhand to spray sugar cane fields with 2,4-D herbicide. During spraying, he utilized no protective mask or clothing and handled herbicide freely. Paresthesia of the hands and feet were the first symptoms to develop, followed by painful muscular stiffness in all four limbs. The pain disappeared after a week, but the muscular stiffness gradually progressed over the next two years, causing impairment of gait and loss of manual dexterity. Upon clinical examination, the patient exhibited abnormalities in skeletal muscle function and motility, deep tendon reflexes and posture. Passive flexion of the limbs met with a steady uniform resistance that gradually increases as the flexion continued. The facial, masseter, trunk and extremity muscles all displayed myokymia when at rest. The patient was treated with 1,500 mg of diphenylhydantoin (DPH) given intravenously. The clinical symptoms improved dramatically and continued treatment with 0.3 gm of DPH daily alleviated practically all of the muscular stiffness, myokymia and postural deformities. To determine the physiologic origin of this neuromuscular disorder, electromyography, peripheral nerve block, nerve conduction velocities, treatment with curare, and muscle and nerve biopsies were performed on the patient prior to treatment with DPH. Based on results of these tests, the authors concluded that the cause was not in the central nervous system since the muscular stiffness was unchanged by sleep, spinal anesthesia, and deep barbiturate anesthesia. The authors also concluded that the myokymia did not originate in the post-synaptic neuromuscular junctions since curare eliminated the muscle tetany and all electromyographic activity. The fact that DPH, which acts specifically on motor nerve terminals, alleviated the syndrome led the authors to conclude a peripheral motor nerve origin. Depressed motor nerve conduction velocities and degenerative changes in the biopsy of the sural (sensory) nerve supported the authors' conclusions. They stated that the actual cause of the patient's affliction was unknown, but damage to peripheral nerves from exposure to chemicals such as 2,4-D may play an etiologic role.

1047. Walsh, J. (1977) Seveso: The questions persist where dioxin created a wasteland. Science 197:1064-1067.

[Review article.]

1048. Ward, C. T., and Matsumura, F. (1978) Fate of 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD) in a model aquatic environment. Arch. Environ. Contam. Toxicol. 7:349-357.

The authors describe the persistence and metabolic degradation of TCDD in lake sediment and water under laboratory conditions. Four sediment and lake water samples were used in the study, three from Lake Mendota and one from Lake Wingra in Wisconsin. Samples were prepared by filtering portions of sediment and water to obtain damp sediment and clear lake water. Five grams of sediment and about 18 ml of water were placed in glass tubes to provide an anaerobic environment. TCDD was added to the tubes at 0.71, 1.0, or 1.83 ppm of ^{14}C -TCDD (label position unspecified) for one hour to 589 days. The authors also reported that 2,4,5-T (100 ppm) was added to some samples, however, no results were presented. Analysis of ^{14}C -TCDD and metabolites present in sediment or lake water was by thin-layer chromatography. Most of the radioactivity, 94-96%, was recovered from the sediment. TCDD disappeared faster in sediment and lake water together than in lake water alone. The half-life of TCDD in Lake Mendota sediment was calculated to be about 600 days. If TCDD was added to lake water without sediment, approximately 71% of the radioactivity was recovered after 589 days. Nearly all of the TCDD recovered was the parent compound. Percent recovery of radioactivity was related to water loss, suggesting that water-mediated evaporation of TCDD takes place. Of the small amount of radioactivity present in lake water from the sediment plus lake water samples, 33-98% of the radioactivity recovered was metabolites of TCDD. However, of the total original TCDD added, metabolites accounted for only 1-4%. When samples were exposed to low levels of illumination, no significant photolysis of TCDD occurred. The author explained this by the fact that most of the ^{14}C -TCDD was associated with the sediment and was not exposed to light. The authors concluded that under the laboratory conditions of this study, TCDD is a persistent chemical in lake water sediment and is resistant to microbial attack.

1049. Wassom, J. S., Huff, J. E., and Loprieno, N. (1977/1978) A review of the genetic toxicology of chlorinated dibenzo-p-dioxins. Mutat. Res. 47:141-160.

[Review article.]

1050. Watson, A. P., Van Hook, R. I., and Reichle, D. E. (1976) Toxicity of organic and inorganic arsenicals to an insect herbivore. Environ. Sci. Tech. 10(4):356-359.

The authors report on the toxicity of two cacodylic acid formulations to meadow katydids. Fourth instar nymphs of Conocephalus fasciatus were collected in the field and placed in experimental cages (10 per cage) with fresh fescue clippings and water containing the herbicide. Cacodylic acid (65% dimethyl arsenic acid, 35% elemental arsenic) was introduced into drinking water at 1.5, 15, 150, 1,500, and 15,000 ppm.

Phytar 560 (4% free cacodylic acid, 23% sodium cacodylate, 73.5% inert ingredients, 12.7% elemental arsenic) was tested at 0.15, 15, 150, and 1,500 ppm. Chronic oral toxicities of both compounds were calculated at 7 and 14 days. LD₅₀ values obtained were 34.4 ppm (7 days) and 7.5 ppm (14 days) for cacodylic acid, and 10 ppm (7 days) and 3.5 ppm (14 days) for Phytar 560. At 14 days, there was no difference between the two compounds in the elemental arsenic dose required to produce the LD₅₀ value. The authors concluded that toxicity of these compounds was due to digestive assimilation of elemental arsenic. The authors also concluded that at recommended applications rates, Phytar 560 would be toxic to herbivorous insects, such as the katydid.

1051. Way, J. M. (1969) Toxicity and hazards to man, domestic animals, and wildlife from some commonly used dioxin herbicides. Residue Rev. 26:37-62.

[Review article.]

1052. Weakley, B. S. (1977) How dangerous is sodium cacodylate? Journal of Microscopy 109, Pt. 2:249-251.

[Editorial.]

1053. Weber, J. B. (1972) Interaction of organic pesticides with particulate matter in aquatic and soil systems. In: Fate of Organic Pesticide in the Aquatic Environment. Advan. Chem. Ser. No. 111; Am. Chem. Soc., Washington, DC: pp: 55-120.

[Background material.]

1054. Weber, J. B., Monaco, T. J., and Worsham, A. D. (1973) What happens to herbicides in the environment? Weeds Today 4:16-22.

[Background material.]

1055. Weimer, J. T., Ballard, T. A., Owens, E. J., and McNamara, B. P. (1970) Toxicological studies on herbicide "White" in animals. US NTIS Technical Report No. AD 712 317. 28 pp.

The inhalation, dermal, and oral toxicities of single and repeated exposure of rabbits, and rats to herbicide White are described. A commercial preparation of herbicide White and 2 samples from a military source (which were found to be identical chemically and toxicologically to the commercial sample) were used and contained 11.7% triisopropanolamine salt of 4-amino-3,5,6-trichloropicolinic acid, 44.9% triisopropanolamine salt of 2,4-D and water; other ingredients were not revealed by the manufacturer. Compounds were applied under temperate

conditions of moderate temperature and humidity, and tropical conditions of high temperature and humidity. Undiluted White was applied as a single dose or 5 successive daily doses to clipped, undamaged rabbit skin and to sateen cloth taped to clipped rabbit skin for 48 hr. White was applied to one eye of rabbits and in some cases procedures to prevent infection were implemented. The sites of application were examined twice daily for 30 days. Rats and rabbits were administered undiluted White by oral gavage and LD₅₀ values were calculated from mortality rates at 48 hr. and 5 days. Rats and rabbits were exposed to 1230-1419 mg. White aerosol per cubic meter air from 120 to 270 min. and mortality rates were calculated. Erythema resulted from single exposure of bare skin to 0.03-0.50 ml of White, while necrosis appeared after 5 doses and cleared within 6 days of the last dose. Doses of 0.02 ml, applied under tropical conditions, produced necrosis by the third day that had not cleared in 10 days. Many rabbits succumbed to the tropical conditions (total number was not reported and this experiment was terminated). Applications to clothed skin under temperate conditions resulted in necrosis only after the highest dose was administered 5 times and occurred in 1 of the 6 rabbits. Single doses of 0.01 ml or higher to the eye, under temperate or tropical conditions, produced eye irritation that was diminished (tropical blepharitis) or disappeared in 10 days. At 0.05 ml or above, bilateral corneal opacity occurred, which cleared in 21-28 days. The oral LD₅₀ was 1.67 ml/kg for rabbits and 4.17 ml/kg for rats; all deaths occurred within 24 hours and inactivity was the only observed sign of toxicity. The LC₅₀ was 150,982 mg-min/cubic meter in rabbits. Deaths occurred up to 14 days after exposure. Signs of toxicity included inactivity during the exposure and blepharitis 4-5 days after exposure. Rats were also inactive during the inhalation exposure, but no deaths or other toxic signs resulted. The authors concluded that White is not likely to produce toxicity by oral, dermal, or inhalation exposures, except for temporary eye irritation and corneal opacity after direct contact to the eye and temporary local skin damage from repeated exposures.

1056. Weirich, J. (1969) Intoxication with diquat (reglone). Dtsch. Gesundheitsw. 24(42):1986-1988.

A case report of diquat poisoning is described. A 43 year old man sprayed 4 liters of diquat in 600 liters water per hectare for 6 hours (at the rate of 2 hectares per hour) under windy conditions. On the following day, he experienced fever, dizziness, head and eye pain, and diarrhea, and polyuria. Although the headaches remained 5 days later, he again sprayed diquat (20 liters over 3 hours) under the same conditions. The other symptoms returned and 4 days after the second exposure, these symptoms were alleviated, except for headaches and a feeling of general weakness. These conditions persisted for an additional week, at which time the patient was admitted to the hospital. Disturbances in liver and kidney function and in EKG stimulation were noted and were all reversible over the next 23 days (without treatment). Respiratory effects were never observed. The authors concluded that the windy weather conditions resulted in higher

than usual exposure to diquat, which had rarely been reported to produce signs of toxicity in workers.

1057. Weiss, S. U., and Beckert, W. H. (1975) Herbicide effects on cultured animal cells. Abstracts 15th Annual Meeting Am. Soc. Cell Biol. p. 451a.

[Not available.]

1058. Weissberg, J. B., and Zinkl, J. G. (1973) Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin upon hemostasis and hematologic function in the rat. Environ. Health Perspect. 5:119-123.

Hematologic parameters were evaluated in rats 10 and 14 days after daily administration of TCDD began. Female CD rats were orally administered 10 ug/kg TCDD in acetone-corn oil daily for 2 weeks. After 10 and 14 days, blood was drawn by cardiac puncture under methoxyflurane anesthetic from four treated and four vehicle-control rats. Smears of bone marrow preparations were stained for megakaryocyte identification. Bleeding times were estimated by lacerating an ear and observing the time required for bleeding to stop with periodic blotting. Clot extraction, platelet factor III activity, platelet aggregation, prothrombin times, factor X assay, and fibrinogen degradation products were among the hematologic parameters that were determined. The parameters that were significantly elevated in TCDD-treated groups were cell volumes, erythrocyte counts, reticulocyte count (at 14 days only, due to a low control value), neutrophil counts, and times for prothrombin consumption assay with and without inosithin. Significantly lowered values were obtained for TCDD-treated groups than controls for mean corpuscular volume and mean corpuscular hemoglobin at day 10 only, platelet counts and clot retraction. Other parameters were comparable in control and treated animals. The authors suggested that the observed hemoconcentration was consistent with dehydration; alterations in leukocytosis and red cell indices were nonspecific, indicative of widespread toxicity; depression of blood platelets was suggested to result from an antibody response, as normal marrow megakaryocyte levels and absence of fibrinogen degradation products eliminated other possibilities. The authors were unable to explain the prolonged prothrombin consumption time in the absence of altered prothrombin times. Depression of hematopoiesis in monkeys fed toxic fat (reported by others) was concluded to indicate species variability or causative agent other than TCDD. No other systemic effects in the treated rats were mentioned in relation to the hematologic findings and no discussion of the appropriateness of the selected time points was provided.

1059. West, I. (1979) Toxicity of the herbicide 2,4,5-T. [letter to the editor] Western J. Med. 131(4):335-336.

[Editorial.]

1060. Westing, A. H. (1979) The safety of 2,4,5-T. [letter to the editor] Science 206(4423):1135-1136.

[Editorial.]

1061. Westing, A. H. Ecological considerations regarding massive environmental contamination with 2,3,7,8-tetrachlorodibenzo-p-dioxin. In Chlorinated Phenoxy Acids and Their Dioxins, C. Ramel, ed. (Ecol Bull. No. 27, Stockholm: Swedish Natural Science Research Council, 1978) p. 285-294.

[Review article.]

1062. Westing, A. H. (1972a) Herbicidal damage to Cambodia. In Harvest of Death, J. B. Neilands, ed. (Free Press, 1972) pp. 177-201.

[Review article.]

1063. Westing, A. H. (1972b) Herbicides in war: Current status and future doubt. Biolog. Conserv. 4(5):322-327.

[Review article.]

1064. Westing, A. H. (1971) Ecological effects of military defoliation on the forests of South Vietnam. Bioscience 21(17):893-898.

[Review article.]

1065. Wheeler, J. A. (1968) Herbicides in the perspectives of 20 months and 20 years. Science 161:255-256.

[Background material.]

1066. Whitehead, C. C. and Pettigrew, R. J. (1972a) The effect of 2,4-dichlorophenoxyacetic acid on laying hens. Br. Poult. Sci 13:191-195.

The effect of 2,4-D on reproduction was studied in hens. Hens (10 per group) were administered 6.2 or 18.7 mg (acid equivalents) of 2,4-D butoxyethyl ester in ethanol orally in a gelatin capsule daily from 28 to 34 weeks of age. Controls (10) received capsules only. Egg production, egg and yolk weights and shell thickness were recorded from 22 weeks of age. From 34 to 48 weeks of age, hens were artificially inseminated and hatchability, gross malformations, and 21 day survival of chicks were observed. None of the hens died, no adverse effects were observed for any parameter examined and no malformations occurred. The authors concluded that the doses used for these experiments, equivalent

to 50 and 100 mg/kg of 2,4-D, were below the doses that cause reproductive or teratogenic effects in chickens, but are doses likely to be encountered in normal agricultural practice.

1067. Whitehead, C. C., and Pettigrew, R. J. (1972b) The subacute toxicity of 2,4-dichlorophenoxyacetic acid to chicks. Toxicol. Appl. Pharmacol. 21:348-354.

The subacute oral toxicity of 2,4-D and of 2,4,5-T are described for chicks. Broiler chickens (4 weeks of age) in groups of 2-10 were administered single peroral doses of 250-900 mg/kg of 2,4-D butoxyethyl ester or 2,4,5-T butoxyethyl ester in ethanol (agricultural grade of both herbicides were used). Mortality, body weights and food intake were monitored during the subsequent week. Mortality was high (40-50%) for only the groups given the highest dosage of each herbicide. Transient decreases in food consumption were observed for all groups. Groups of 10 chicks (1 day of age) were fed diets with 10-7,500 mg per kg feed of either phenoxy ester for 3 weeks. In addition to the parameters observed after acute exposures, necropsies were performed. Chicks fed at least 2,000 mg/kg 2,4-D or 1,000 mg/kg 2,4,5-T had reduced food consumption and body weights. Levels of 5,000 mg/kg or higher of 2,4,5-T caused 90-100% mortality. Levels of 5,000 mg/kg 2,4-D did not cause any deaths but histological changes including swollen kidneys and mottled spleens occurred. Histological changes after 2,4,5-T treatment were not mentioned. Groups of four chicks (2 weeks of age) were administered diets with 1,000 or 5,000 mg/kg of feed of either herbicide for 1 week. Three control groups were used, including two groups that were pair-fed with the groups that received the higher dosage of each herbicide. Plasma calcium and magnesium levels were measured at the end of the exposure period. Groups of chicks fed 5,000 mg/kg of either herbicide for 1 week were fed control diets for 3 subsequent weeks and body weights were recorded. No alterations in calcium or magnesium levels were observed between exposed groups and their corresponding control groups. All chicks fed high levels of herbicide for 1 week recovered and resumed normal growth when they were administered control diets. When offered a choice, chicks consumed control diets rather than diets contaminated with 5,000 mg/kg of either herbicide. The authors concluded that chicks were able to tolerate high dosages of 2,4-D or 2,4,5-T administered subacutely and toxic symptoms that occurred were reversible.

1068. Williams, R. F., Inman, Q. S., and Ulberg, L. C. (1979) Development of isolated mammalian embryo techniques for toxic substance screening. U.S. Environmental Protection Agency, Research Triangle Park, NC.: Report No. EPA-600/1-79-007. 72 p.

[Background material.]

1069. Willis, G. H., Rogers, R. L., and Southwick, E. M. (1975) Losses of diuron, linuron, fenac, and trifluralin in surface drainage water. J. Environ. Qual. 4(3):399-402.

The authors studied the loss of diuron in surface drainage water from agriculture plots. Diuron was applied by ground spraying 0.84 kg/ha on two experimental cotton plant plots. The plots were 7.3 by 61 m with a slope of 0.2% and were composed of Mhoon silty clay loam soil. Three annual applications were made. Surface runoff was analyzed for diuron by gas chromatography (detection limit 10 ppb) for 3 months after application. Diuron was present in surface runoff from the plots but at concentrations less than 10 ppb. Two to 3 months after application, no diuron was detected in surface runoff. The authors conclude that the data suggest that diuron properly applied poses little threat to aquatic areas adjacent to sprayed areas.

1070. Wilson, J. G. (1977) Teratogenic effects of environmental chemicals. Fed. Proc. 36(5):1698-1703.

[Not available.]

1071. Wilson, J. G. (1972a) Abnormalities of intrauterine development on non-human primates. WHO Research and Training Centre on Human Reproduction, Stockholm. The use of non-human primates in research on human reproduction. WHO Symposium. ed. E. Diezfolvsy and C. C. Standley. pp 261-292.

Data from an experiment on the teratologic effects of 2,4,5-T on Rhesus monkeys is presented, along with a review of teratology observed in non-human primate studies. Seventeen pregnant Rhesus monkeys (4-5 per group) were orally administered doses of between 5 and 40 mg/kg 2,4,5-T three times per week between days 20 and 48 of gestation. On day 100, fetuses were removed by hysterotomy and were weighed and observed for gross, internal, and skeletal malformations, methods were not described. One abortion occurred, on day 61 in the highest treatment group. Compared to fetuses from the control group of 7 monkeys, fetuses from groups treated with 10 mg/kgations were not reported. No conclusions were stated by the authors related to the teratogenicity of 2,4,5-T.

1072. Wilson, J. G. (1972b) Report on treatment of pregnant Rhesus monkeys with 2,4-D and CMPA. Report to Swedish Poisons and Pesticides Board.

[Not available.]

1073. Wilson, J. G. (1972c) Teratological potential of 2,4,5-T. Proc. South Weed Sci. Soc. 25:26-30.

[Not available.]

1074. Witschi, H. (1977) Environmental agents altering lung biochemistry. Fed. Proc 36(5):1631-1634.

[Review article.]

1075. Witschi, H., Kacew, S., Hirai, K. I., and Cote, M. G. (1977) In vivo oxidation of reduced nicotinamide-adenine dinucleotide phosphate by paraquat and diquat in rat lung. Chem. Biol. Interactions 19(2):143-160.

Male Sprague-Dawley rats were administered 40 mg/kg [¹⁴C]-diquat and tissue concentrations of diquat were determined from 0.1 to 24 hrs. later. All tissue levels fell rapidly with no selective pulmonary uptake observed. The ratio of NADPH/NADP in control lung was determined by an enzymatic assay procedure to be 3.4-4.8 and this ratio dropped substantially in the presence of diquat, indicating that oxidation occurred. Incorporation of [³H]-glycine by lung in vivo was determined as a measure of de novo synthesis of adenine-containing compounds, including pyridine nucleotides, and was not elevated 4 hours after diquat was administered. (The specific activity of free glycine was not altered by diquat administration). In the presence of 100% oxygen, the ratio of pulmonary NADPH/NADP was decreased for low doses of diquat (compared to the ratio for diquat treatment to rats in air) but no effect was observed when high doses of diquat were used. Light microscopy of pulmonary tissue from diquat-treated rats revealed no lesions, while electron microscopy revealed damage to type I alveolar cells. The effects of paraquat were also described. The authors concluded that a cause-and-effect relationship between NADPH oxidation (and the resulting biochemical consequences) and diquat mediated lung damage was not clearly established.

1076. Wojtalik, T. A., Hall, T. F., and Hill, L. O. (1971) Monitoring ecological conditions associated with wide-scale applications of DMA 2,4-D to aquatic environments. Pestic. Monit. J. 4(4):184-203.

In this study, the concentrations of 2,4-D and biological response to its application at the Nickajack and Guntersville Reservoirs were measured. In June-April 1969, the reservoirs, located on the Tennessee River were sprayed with about 170,000 gallons of dimethylamine salt of 2,4-D containing 4 lb of 2,4-D acid equivalent per gallon to control Eurasian watermilfoil. The herbicide was applied by helicopters at rates of 20 to 40 lb acid equivalent per acre on about 18,000 acres. Four areas on the Guntersville Reservoir that were treated and one area that was not treated were sampled before and after treatment. Water samples, plankton, chlorophyll, carbon-14, Eurasian watermilfoil and other plant samples were collected. Temperature, dissolved oxygen, alkalinity and other physical parameters were sampled to determine the efficacy of the herbicide. Organisms were collected in the reservoir and adjacent riverbed to determine the toxicity and accumulation of 2,4-D. Two weeks after spraying, the 2,4-D concentrations ranged from less than 0.001 mg/l to 0.72 mg/l. No living watermilfoil were found

in the area 1 year after treatment but there were no negative effects on most of the other aquatic plants. No fish kills were observed and there were no indications of acute toxicity. Macro-invertebrate samples did not indicate toxicity but did show evidence of population changes. The authors attribute this to the collapse of the milfoil. Mussel samples taken below the reservoirs shortly after application showed levels of less than 0.050 mg/kg to 0.97 mg/kg net weight with accumulations decreasing downstream. The authors indicate there is no known source of the high concentration of 2,4-D in a pretreatment mussel sample. Samples taken from raw and treated water at water treatment plants indicated that the facilities did not remove significant amounts of 2,4-D. Plankters adsorbed or absorbed 24% of 2,4-D 1 hour after application and almost 100% 24 hours after application. The authors conclude that reduced amounts of liquid 2,4-D are effective in controlling watermilfoil if flow and habitat characteristics are considered. Underwater injection of 2,4-D near the rootcrowns could achieve effective control.

1077. Wolfe, W. H. (1980b) Human health effects following exposure to the phenoxy herbicides and TCDD. Presented at the Educational Conference on Herbicide Orange, U.S. Veterans Administration, Washington, DC, May 28-30.

[Review article.]

1078. Wong, A. S., and Crosby, D. G. (1978) Decontamination of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) by photochemical action. Monograph of the Giovanni Lorenzini Foundation. 1:185-189.

[Review article.]

1079. Wood, A. E. (1972) Interrelations of humans, dogs, and rodents. Science 176:437.

[Editorial.]

1080. Wood, T. E., Edgar, H., and Salcedo, J. (1976) Recovery from inhalation of diquat aerosol. Chest 70(6):774-775.

The clinical symptoms of human exposure to diquat aerosol were described. The subject, a 45-year-old man, had been spraying diquat when the nozzle clogged and then suddenly discharged a cloud of aerosol into his face. Myalgia, headaches, a cough that produced thick red sputum, fever, and neck stiffness developed. The patient was admitted to a hospital 4 days after the accident and was then observed to be in a confused state and showed consolidations of the lung. An erythematous rash developed on one arm. After prednisone therapy was initiated, all clinical symptoms disappeared. Oxygen, which was administered before exposure to diquat was recognized, was considered to have been potentially detrimental.

1081. Woods, J. S. (1973) Studies on the effects of 2,3,7,8-tetrachloro-dibenzo-p-dioxin on mammalian hepatic α -aminolevalinic acid synthetase. Environ. Health Perspec. 5:221-225.

The porphyrinogenic activity of TCDD was studied in rats by measuring hepatic delta-aminolevulinic acid (ALA) synthetase activity after exposure in vivo and in vitro. Male rats, pregnant rats 3 days prior to expected delivery, and newborn rats (3 and 11 days of age) were administered a dose of 5-25 ug/kg TCDD in corn oil-acetone (6:1) 1-28 days prior to being killed. One group of control rats received vehicle only and the second group received 400 mg/kg allylisopropylacetamide (AIA) subcutaneously. Hepatic ALA synthetase activities of adult, newborn and fetal liver homogenates and of mitochondrial and extra-mitochondrial fractions of liver cells were determined. ALA synthetase activity was also assayed after in vitro exposure of liver homogenates, isolated mitochondria, and ALA synthetase from prophyrin rat liver to 10^{-9} - 10^{-6} M TCDD. No change in ALA synthetase activity occurred in liver homogenates prepared from adult male rats 1-28 days after TCDD exposure. Twenty-four hours after ALA exposure, ALA synthetase activity was increased seven-fold and returned to normal 2 days later. TCDD exposure did not result in altered ALA synthetase activity in liver of rats of any age, in any subcellular fraction or after in vitro exposure. The authors concluded that the induction of ALA synthetase activity observed in embryonic chick liver (reported elsewhere) and lack of induction in rats in the current study represents a major difference in species susceptibility.

1082. Woolson, E. A. (1977a) Fate of arsenicals in different environmental substrates. Environ. Health Perspec. 19:73-81.

[Review article.]

1083. Woolson, E. A. (1977b) Generation of alkylarsines from soil. Weed Sci. 25:412-416.

The author measured alkylarsines generated from soils treated with cacodylic acid. Flasks containing 100 g of silt loam soil and 6 g of ground soybean meal were treated with 13.33 moles cacodylic acid, which included 1.1 (u)Ci 14 C-cacodylic acid (specific activity 1.2 (u)Ci/mg). Both aerobic and anaerobic soils were studied in duplicate. Gases coming from soil samples were analyzed for 160 days. Volatile organo-arsenicals were formed most rapidly under aerobic conditions. Gases trapped from aerobic soils contained 18% of the activity of the original treatment, while gases from anaerobic soils contained only 7.8% of the activity. Volatile gases were identified as dimethyl arsine and trimethylarsine. In addition, organo-arsenicals remaining in the soil were also measured. In both treatments, most of the extractable arsenic was present as a trimethyl arsenical. The authors concluded that volatilization of organo-arsenicals in soil is an important part of the arsenic cycle in soils. The formation of 14 CO₂ was not measured in this study.

1084. Woolson, E. A. Organoarsenical herbicides. In Herbicides: Chemistry, degradation and mode of action. Vol 2 eds., P. C. Kearney and D. D. Kaufman (New York: Marcel Dekker Inc., 1976) pp. 741-776.

[Review article.]

1085. Woolson, E. A., Ensor, P. D., Reichel, W. L., and Young, A. L. (1973) Dioxin residues in lakeland sand and bald eagle samples. Advan. Chem. Ser. 120:112-118.

The authors report on TCDD residues in soil profile samples collected at Eglin AFB in an area where massive herbicide spraying occurred and in bald eagle samples collected from 15 States. Sample analysis was conducted by gas chromatography (detection limit, 1 ppb). Twelve soil cores 36 in long were collected in 1970: 2 cores from an area receiving 2,4-D and 2,4,5-T at 947 lb/acre for each herbicide (1962-1964); 4 cores from an area receiving 584 lb/acre of each herbicide (1964-1966); and 6 cores from an area receiving 160 lb/acre 2,4,5-T and 183 lb/acre 2,4-D (1968-1969). Small amounts of 2,4-D (0.8 - 15.4 ppb) and 2,4,5-T (8.0-8.4 ppb) were detected in the cores samples. However, no TCDD was detected in any of the samples. No TCDD was detected in any of the bald eagle samples (detection limit 0.05 ppm).

1086. Woolson, E. A., and Isensee, A. R. (1981) Soil Residue accumulation from three applied arsenic sources. J. Weed Sci. 29:17-21.

The authors report on the accumulation of arsenic in the soil often repeated applications of cacodylic acid. Herbicide was applied to Matapeake silt loam soil at 11.2, 22.4, and 112 kg/ha, which correspond to 1x, 2x, and 10x the recommended application rate. To devise a "worst possible case" situation, cacodylic acid was sprayed on bare ground, which was then rototilled, and soybeans and radishes were planted to indicate phytotoxicity. Under normal application conditions, herbicide is sprayed on vegetation for weed control and planting does not occur until the following year. Twelve core soil samples (0-15 cm and 15-30 cm) were collected from each plot and composited at the end of the growing season. Arsenic (As) residues in the samples were determined by colorimetric methods. Each year total As losses from the soil were about 15%, probably as a result of volatilization of alkylarsines, according to the authors. Soil As levels increased with increasing application rates. Accumulation of arsenic in the soil was gradual. The authors calculated that at the maximum recommended rate of cacodylic acid application, equilibrium would be reached after about 22 years of application. Soybeans and radishes were adversely affected by the highest application rate of cacodylic acid but not by the two lower rates. The authors concluded that use of recommended application rates of arsenical herbicides should not adversely affect plants because of soil accumulation.

1087. Woolson, E. A., and Kearney, P. C. (1981) Brief overview of cacodylic acid in soils and man. Public Statement by U. S. Dept. of Agriculture, 3 p.

[Background material.]

1088. Woolson, E. A., and Kearney, P. C. (1973) Persistence and reactions of ^{14}C -cacodylic acid in soils. Environ. Sci. Technol. 7:47-50.

The authors studied the persistence and degradation of cacodylic acid in three soil types, Lakeland loamy sand, Hagerstown silty clay loam, and Christiana clay loam. The authors also examined the distribution of cacodylic acid into water soluble, iron, aluminum, and calcium fractions in the three soils. ^{14}C -cacodylic acid (0.2uCi) was added to each air-dried soil sample at 1, 10, and 100 ppm. One set of soils was brought to 75% of field moisture capacity to measure aerobic degradation. Both sets of soil samples were incubated at 25C for 32 weeks. The rate of disappearance of ^{14}C -cacodylic acid was dependent on the soil type. After 32 weeks, 23% of the original ^{14}C remained in Christiana soil, 53% in Hagerstown soil, and 62% in lakeland soil. The rate of application, however, did not have any appreciable effect on cacodylic acid disappearance. Under aerobic conditions, approximately 35% of the original cacodylic acid was converted to a volatile organo-arsenical and 41% converted to $^{14}\text{CO}_2$ and AsO_4^{3-} in 24 weeks. Under anaerobic conditions, 61% of the cacodylic acid was converted to an organo-arsenical. According to the authors, the degradation was probably due to microbial activity.

1089. Workers' exposure to 2,4-D studied. (1981) Research News, (USDA), April 1981, 3 p.

[Editorial.]

1090. Wright, T. C. (1979) Agent Orange Dioxin: TCDD. L C Science Tracer Bullet No. TB 79-10. 9 pp.

[Bibliography.]

1091. Yamauchi, H., and Yakamura, Y. (1979) Urinary inorganic arsenic and methylarsenic excretion following arsenate-rich seaweed ingestion. Jap. J. Ind. Health 21:47-54.

[Abstract, only.]

1092. Yang, K. H., and Peterson, R. E. (1976) Effect of 2,3,7,8-tetra-chlorodibenzo-p-dioxin (TCDD) on plasma disappearance and biliary excretion of Ouabain in rats. Pharmacologist 18(2):246.
1093. Yockim, R. S., Isensee, A. R., and Jones, G. E. (1978) Distribution and toxicity of TCDD and 2,4,5-T in an aquatic model ecosystem. Chemosphere (3):215-220.

The authors evaluated the toxicity of TCDD and the environmental fate of TCDD and 2,4,5-T in an aquatic model ecosystem. Silt loam soil (pH 5.3, 4009) was treated with either ring-labeled ^{14}C -TCDD (460 mCi/g) at 0.1 ppm or carboxy-labeled ^{14}C -2,4,5-T (280 mCi/g) at 0.1, 1.0 or 10.0 ppm. The compounds were added to the soil in benzene, after which the soil was air-dried. Treated soil in triplicate was added to ecosystem tanks which were then flooded with 16 liters of water. Untreated soil was used as a negative control. The possible effects of the benzene solvent were not discussed. Each tank received 100 water fleas, 15 snails, 1g algae, and 15 mosquito fish, which were isolated from the other species to prevent predation. Water and tissue samples were removed for scintillation counting on days 1, 3, 7, 15, and 32). Radioactivity in the water from TCDD-treated tanks reached equilibrium by day 1 (2-4 ppt), while 2,4,5-T levels did not reach equilibrium during the experiment. Bioaccumulation ratios for organisms in TCDD treated tanks were $2\text{-}6 \times 10^3$ while ratios of less than 50 were observed in 2,4,5-T treated tanks. The first group of fish added to the TCDD tanks died by day 15 having tissue levels of 7.2 ppb TCDD. A second group of 15 fish were then added to the TCDD tanks. These fish all died by day 32 with TCDD tissue levels of 4.4 ppb. Nasal hemorrhaging and listless swimming were observed in the dying fish. Three pregnant females added to the TCDD treated tanks with the second group lost external signs of pregnancy and hemorrhaged shortly before their deaths on day 15. TCDD at the concentration tested had no apparent effect on any other organism tested. No toxicity induced by 2,4,5-T was observed. In addition, in a single trial experiment, the authors added 105 ppb TCDD to aquatic soils and measured TCDD persistence. Levels of TCDD decreased by 18% at the end of the 180-day experimental period. The TCDD concentration used in these experiments was 10^4 - 10^6 times higher than what would occur in a real world situation. The behavior of 10^4 - 10^6 lower levels of TCDD cannot be assessed by this experimental design.

1094. Yoder, J., Watson, M., and Benson, W. W. (1973) Lymphocyte chromosome analysis of agricultural workers during extensive occupational exposure to pesticides. Mutat. Res. 21:335-340.

The authors performed chromosomal analysis on lymphocytes from persons occupationally exposed to herbicides and insecticides. The study was part of an ongoing survey by the Idaho Community Study on Pesticides. Peripheral blood samples were drawn from male Caucasians only. Persons with recent histories of viral infections, chemotherapy or x-ray treatment or histories of malignancies were rejected. Controls were matched for age and physical characteristics (undefined) as closely as possible. The high exposure group was defined as spraying with pesticides 4 hours per day on 3 or more days per week during the peak spraying season of July 15 through August 30th. The control group was composed of 16 persons with no known pesticide exposure, mean age 39.8. The exposed group was composed of 26 persons employed by weed control agencies with a mean age 36.5 and a mean herbicide exposure of 6.3 years. These workers were exposed primarily to 2,4-D, amitrole and atrazine. Peripheral blood samples were obtained from each person during the peak spraying season and during the midwinter "off season." Lymphocyte cultures were prepared from each blood sample and cultured for 48 hours after which chromosome spreads were made. Scoring for chromatid gaps and breaks was performed on coded samples to avoid bias. Twenty-five metaphase spreads from each person were examined for chromosomal aberrations. Chromosomal aberrations in the unexposed group did not differ markedly when off season and spraying season samples were compared. During the peak spraying season the exposed group had nearly 4 times as many gaps per person and 25 times as many breaks per person compared to the mid winter sampling. However, the winter sampling of the herbicide exposed group had approximately half the number of gaps and one fourth the number of breaks compared to the unexposed group. The authors speculated that enhanced chromosome repair may be taking place at this time as compensatory protection. No statistical analysis of the results were presented. Ranges of chromatid gaps as well as chromatid breaks overlapped in all groups tested. No conclusions can be reached on the effects of 2,4-D alone because exposure was to unknown quantities of several herbicides.

1095. Yoshida, T., and Castro, T. F. (1975) Degradation of 2,4-D, 2,4,5-T and picloram in two Philippine soils. Soil Sci. Plant Nutr. 21(4):397-404.

The authors report on the degradation of 2,4-D, 2,4,5-T, and picloram in two Philippine rice field soils. Samples of Maahas clay soil (pH 6.6, 2.0% organic matter) and Louisiana clay soil (pH 4.7, 3.2% organic matter) were collected from rice paddies, air dried, passed through a 2 mm sieve and placed in test tubes at 20 g per tube. Herbicides were added to soil in acetone: 20 ppm 2,4-D, 10 ppm 2,4,5-T or 1 ppm picloram. The solvent was evaporated before incubation began. One half the samples were submerged to a depth of 3 cm to mimic flooding conditions, the other samples (Upland samples) were maintained at 80% field moisture capacity. Amounts of herbicides were measured at 0, 2,

4, and 6 weeks for 2,4-D; 0, 2, 4, 8, and 12 weeks for 2,4,5-T; and 0, 3, and 6 months for picloram. Soil samples were analyzed for herbicide residues by gas chromatography. In upland Maahas soil, 2,4-D was degraded nearly completely in 6 weeks. Submerged samples did not degrade 2,4,-D as rapidly. However, by 4 weeks over 50% of the herbicide had disappeared. In Louisiana soil, 2,4-D degraded more slowly. More than 60% 2,4-D had degraded after 6 weeks. 2,4,5-T also degraded rapidly in upland Maahas soil, and had disappeared by 12 weeks. In submerged samples, slight amounts of 2,4,5-T residues were being detected at 12 weeks. Degradation of 2,4,5-T in upland Louisiana soil was complete by 8 weeks. In submerged samples, degradation was about 60% in 12 weeks. Picloram was the most persistent of the herbicides studied. The amount of picloram recovered in upland or submerged Maahas soil was less than 50% in 3 months. However, on Louisiana soil, degradation did not begin for 3 months. By 6 months, less than half of the original picloram remained in the soil. In order to determine if microbial activity was responsible for herbicide degradation in the soils tested, the authors sterilized soil samples by autoclaving or cobalt irradiation. 2,4,5-T was added to autoclaved and unautoclaved samples of all 4 soil types at 9-10 ppm. By 12 weeks, the nonautoclaved samples had completely or nearly completely degraded the herbicides. Little to no degradation was observed in nonautoclaved samples. Similar results were observed with irradiated and non-irradiated samples using both 2,4-D and 2,4,5-T. The authors concluded that the major agent for degradation of these pesticides in soil is microbial activity.

1096. Young, A. L. (1980) Direct testimony before the U.S. Environmental Protection Agency, FIFRA, Docket No. 415 et. seq., June 1980.

[Testimony.]

1097. Young, A. L. (1980) Use of herbicides in South Vietnam, 1961-1971. Presented at the 2d Continuing Education Conference on Herbicide Orange, Washington, DC, May 28-30.

[Background material.]

1098. Young, A. L. (1974) Ecological studies on a herbicide-equipment test area (TA-C-52A), Eglin AF Base Reservation, Fla. US NTIS Publication ISS No. 780517/9GA. 141 pp.

[Background material.]

1099. Young, A. L., Calcagni, J. A., Thalcken, C. E., and Tremblay, J. W. (1978) The toxicology, environmental fate, and human risk of herbicide orange and its associated dioxin. USAF Occupational and Environmental Health Laboratory Report No. USAF OEHL - 78 -92. 262 pp.

[Review article.]

1100. Young, A. L., Thalke, C. E., Arnold, E. L., Cupello, J. M., and Cockerham, L. G. (1976) Fate of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the environment: Summary and decontamination recommendations. Headquarters Air Force Logistics Command Report No. AFA-TR-76-18. 41 pp.

[Background material.]

1101. Young, A. L., and Wolverton, B. C. (1970) Military herbicides and insecticides. Technical Notes AFATL-TN-70-1. Air Force Armament Laboratory, Eglin AFB, Florida. 59 pp.

[Not available.]

1102. Young, J. F., and Haley, T. J. (1977) Pharmacokinetic study of a patient intoxicated with 2,4-dichlorophenoxyacetic acid and 2-methoxy-3,6-dichlorobenzoic acid. Clin. Toxicol. 11(5):489-500.

A pharmacokinetic evaluation was described based on blood and urine data from a woman who ingested 100 ml (estimated) of a mixture of 2,4-D (20.1 gm) and 2-methoxy-3,6-dichlorobenzoic acid (1.9 gm; Dicamba). The clinically stabilized patient underwent lavage and had been treated with 3 drugs the nature of whose interactions with the herbicides were unknown. The concentrations of the 2 chemicals in blood and urine collected during the acute phase of intoxication were reported in a separate publication. These data were analyzed kinetically on an analog computer interfaced to a digital computer. The model that generated data that provided the best fit for the experimental data for 2,4-D was a one-compartment model. Clearance from the single compartment representing the blood content was described by three rate constants, 2 related to urinary elimination and 1 related to fecal elimination (although fecal levels of 2,4-D were not measured). Initially Dicamba was cleared by a urinary excretory route that became available to 2,4-D only after the relative concentrations of the 2 compounds favored 2,4-D. This pathway was hypothesized to be glycine conjugation. The plasma half life of 2,4-D was 59 hours initially and 17 hours when the route originally saturated by Dicamba was available to 2,4-D. The volume of 2,4-D distribution was estimated to be 10.2 liters.

1103. Young, J. F., and Haley, T. J. (no date) Simultaneous human pharmacokinetics of 2,4-dichlorophenoxyacetic acid and 2-methoxy-3,6-dichlorobenzoic acid. Fed. Proc. 38:679.

[Abstract, only.]

1104. Zack, J. A., and Suskind, R. R. (1980) The mortality experience of workers exposed to tetrachlorodibenzodioxin in a trichlorophenol process accident. J. Occup. Med. 1-4.

The mortality experience of 121 plant workers exposed to TCDD in a 1949 TCP process accident was studied. Data were obtained from plant safety, medical, and workmens compensation records. The development of chloracne was the criteria used to select the study population. Followup of the population verified 89 living and 32 dead. Death certificates were obtained on deceased cohorts and with the underlying cause of death coded using the 8th revision of the ICDA. Analyses were performed by the modified life table method using the Monson program. A standard mortality ratio (SMR) of .69 for all deaths was found with 32 observed deaths with 46.41 expected. This was the only statistically significant difference found.

1105. Zandvoort, R., Van Den Born, G. W., Braber, J. M., and Smelt, J. H. (1980) Leaching of the herbicide bromacil after application on railroads in the Netherlands. Water Air Soil Pollut. 13:363-372.

The authors measured the movement of bromacil through railroad-bed soils one year after application. A commercial preparation of bromacil (Hyvar X) was sprayed at 2.2 and 4.4 kg/ha yearly from 1972 until 1976 or at 2.2 kg/ha in alternate years. Soil core samples were collected down to a depth of 80 cm and analyzed for bromacil by gas chromatography. The highest concentrations of bromacil were found in the top 40 cm of the soil column. After 4 yearly sprayings of 2.2 kg/ha, residues of bromacil in the 0-80 cm soil columns were 3.1, 1.7, 2.5, and 1.4 for 1974 through 1977. Similar results were observed at the other close levels. It appears that bromacil does not accumulate in the soil to any great extent. In addition, the authors also studied the leaching of bromacil through soil columns under laboratory conditions. Two soil columns were used: one made of untreated air-dried soil from the railroad bed and the other made of untreated air-dried soil of the 20-90 cm layer of soil from the railroad bed. Bromacil (1.24 mg Hyvar X) mixed in sand was then added to the top of each column which was then wetted to field capacity. Elutions of 10 cm of water were applied for 2 days. Both the eluate and 5 cm sections of each of the columns was analyzed for bromacil. A large quantity of bromacil was found in the leachate. In the top layer soil column 34% of the recovered bromacil was found in the leachate and in the deep layer soil column 74%. Most of the bromacil remaining in the soil column was found in the application layer or in the 15-30 cm depths. From these experiments the authors concluded that bromacil is highly mobile in soil and can possibly leach below 100 cm or even into groundwater in one year. They did not verify these conclusions experimentally.

1106. Zelikov, A. K., and Danilov, L. N. (1974) Occupational dermatoses (acne) in workers engaged in production of 2,4,5-trichlorophenol. Sov. Med. 7:145-146.

A brief case report of chloracne occupational exposure to 2,4,5-trichlorophenol is given. The patient presented with clinical symptoms of chloracne 3 months after working in the production of 2,4,5-trichlorophenol. No internal pathology was detected, and blood urine analyses gave normal results. Based on the histological skin findings and the history of contact with 2,4,5-trichlorophenol, the authors diagnosed chloracne. The worker was removed from exposure to the chlorinated compound and received treatment. Sanitary and engineering changes were in the plant to prevent other workers from being exposed. No new cases were observed in the subsequent 1-1.5 years.

1107. Zepp, R. G., Wolfe, N. L., Gordon, J. A., and Baughman, G. L. (1975) Dynamics of 2,4-D esters in surface waters. Hydrolysis, photolysis and vaporization. Environ. Sci. and Technol. 9(13):1144-1150.

[Background material.]

1108. Zetterberg, G. (1978) Genetic effects of phenoxy acids on microorganisms. In Chlorinated Phenoxy Acids and Their Dioxins, C. Ramel, ed. (Ecol. Bull, No. 27, Stockholm: Swedish Natural Science Research Council, 1978) pp. 193-204.

The authors studied the mutagenicity of 2,4-D and 2,4,5-T in a haploid strain of yeast, Saccharomyces cerevisiae RAD18, which is auxotrophic and requires histidine for growth. Upon exposure to a mutagen RAD18 reverts from histidine dependence to histidine independence. To test the sensitivity of the yeast cells to 2,4-D, several growth stages of yeast cells, pH levels and concentrations of 2,4-D were used. 2,4-D caused increased mutations in log phase cells, but not in stationary cells. Increased mutation frequencies were observed only at concentration of 0.2 mg/ml or higher and pH less than 4.5. In addition, no controls or numerical data reported, and no statistical analysis performed. Criteria for a positive result was not presented by the authors. Under these conditions survival of the yeast cells was extremely low, 1% or less. In order to prove that the revertants observed in these experiments were "true" revertants and not 2,4-D resistant spontaneous revertants, the authors performed reconstruction experiments. Ten revertant colonies and 10 his- colonies were inoculated in growth medium and then treated with 2,4-D. Both types of cells had similar sensitivities to 2,4-D (0.1-0.25 mg/ml) proving that the increased mutation frequency was due to a mutagenic effect. The mutagenicity of 2,4,5-T was also dependent on pH. At pH values greater than 4.5, no mutagenic effects were observed. However, at pH 4.3 and cell survival less than 1%, mutation frequencies increased.

1109. Zetterberg, G., Busk, L., Elovson, R., Starec-Nordenhammar, I., and Rytman, H. (1977) The influence of pH on the effects of 2,4-D (2,4-dichlorophenoxyacetic acid, Na salt) on Saccharomyces cerevisiae and Salmonella typhimurium. Mutat. Res. 42:3-18.

The authors studied the genetic effects induced by 2,4-D on Saccharomyces cerevisiae and Salmonella Typhimurium in vivo and in vitro. Two strains of S. cerevisiae were employed: D4, a diploid strain heteroallelic at the ade 2 and trp 5 loci requiring adenine and tryptophan for growth and D5, another diploid strain requiring adenine for growth. Strain D4 detects mitotic gene conversion while D5 detects mitotic crossing over. In addition, S. typhimurium strains TA1530, TA1535, TA1531, and TA1538, which detect point mutations, were included in the test battery. TA1530 and TA1535 revert from histidine requiring to prototrophy by base pair substitutions, while TA1531 and TA1538 revert by frameshift mutations. In order to assess the genetic effects of 2,4-D in vivo, a host mediated assay in mice was performed using male CBA mice (309 each) treated orally with 2,4-D. Ethyl methane sulfonate was used as a positive control in all the in vitro experiments. In S. cerevisiae D4, 2,4-D (0.6 mg/ml) at pH 4.5 significantly increased the frequency of mitotic gene conversion for the ade 2 locus (p less than 0.05), but not at the trp 5 locus. A pH greater than 4.6 no increase in mitotic gene conversion was observed. From this the authors speculated that yeast cells can only take up the undissociated form of 2,4-D which occurs at pH 4.5 or lower. In S. cerevisiae strain D5, at pH 4.3, 0.3 mg 2,4-D/ml increased the frequency of mitotic recombination. However, in S. typhimurium 2,4-D (0.5 mg/ml) did not induce an increase in the number of revertants at either pH 4.3 or pH 6.8. No exogenous metabolic activation was included in the experiment. In the host mediated assay, 6 mg 2,4-D was administered in a single oral dose to each of 4-8 CBA mice that had been injected intraperitoneally with S. cerevisiae D4, TA1531 or TA1530. The dose of 2,4-D was close to the maximum tolerated dose according to the authors. After 3 hours exposure, the indicator organisms were recovered from the peritoneal cavity and plated on minimal medium. No increase in mitotic gene convertants of histidine independent revertants were observed. This, however, cannot be taken as a conclusive result. The compound may not have reached the indicator cells or the near neutral pH conditions in the peritoneal cavity prevented 2,4-D from being taken up by the cells. Furthermore, no positive control was included in the experimental design to ensure that the test systems were working properly.

1110. Zielinski, W. L., Jr., and Fishbein, L. (1967) Gas chromatographic measurements of disappearance rates of 2,4-D and 2,4,5-T acids and 2,4-D esters in mice. J. Agr. Fd. Chem. 15:841-844.

The rates of elimination of 2,4-D, 2,4-D butyl ester, 2,4-D isooctyl ester and 2,4,5-T were studied in the mouse. Female C57BL/6 mice were administered 100 mg/kg of 2,4-D acid or ester or 2,4,5-T in dimethylsulfoxide and were killed (6 mice per group) from 0 to 24 hours later. The amount of herbicide in the whole body was determined by

analyzing an aliquot of a whole-body homogenate by gas chromatography. Some mice were pretreated with 5 daily doses (100 mg/kg each) of herbicide prior to the test dose on the sixth day. Herbicide levels were expressed as a percentage of the last administered dose (100 mg/kg in each case). The percentage of the dose recovered at time 0 was 111% for the 2,4,5-T pretreated group and ranged from 77.1% to 81.8% for all other groups. The rates of disappearance were highest for the 2,4-D esters and lowest for 2,4,5-T. The rates for 2,4-D esters were faster after pretreatment and for 2,4,5-T were slower after treatment. The half times were not calculated from the same interval for each compound. The values calculated for 2,4-D butyl ester were 1.1 hours (0.9 after pretreatment) 0-2 hours after treatment; for 2,4-D isooctyl ester were 3.5 hours (3.2 after pretreatment) 0-4 hours after treatment; for 2,4-D was 4.1 hours between 0-16 hours; for 2,4,5-T was 14.1 hours (31.8 after pretreatment) for 0-24 hours. No 2,4-dichlorophenol metabolite was detected after administration of any of the 2,4-D compounds. The authors concluded that 2,4-D was excreted at a slower rate than the 2,4-D esters but much more rapidly than 2,4,5-T and pretreatment (with the same herbicide) enhanced 2,4-D butyl ester clearance only.

1111. Zimmerman, F. K. (1971) Induction of mitotic gene conversion by mutagens. Mutat. Res. 11:327-337.

[Review article.]

1112. Zingeser, M. R. (1979) Anomalous development of the soft palate in rhesus macaques (Macaca mulatta) prenatally exposed to 3,4,7,8-tetrachlorodibenzo-p-dioxin, (sic). Teratology 19(2):54A-55A.

[Abstract, only.]

1113. Zinke, P. J. (1974) The effects of herbicides in South Vietnam. Part B. Working papers: Effect of herbicides in soils of South Vietnam. National Academy of Sciences-National Research Council. AD-779 024. 39 pp.

[Not available.]

1114. Zinkl, J. G., Vos, J. G., Moore, J. A., and Gupta, B. N. (1973) Hematologic and clinical chemistry effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin in laboratory animals. Environ. Health Perspec. 5:111-118.

The effect of TCDD on serum enzyme levels, biochemistries, and hematology was evaluated in the rat, mouse, and guinea pig. Daily oral doses of 0.1 -10 ug/kg of TCDD were administered to female CD rats for 30 days. Blood was collected between 3 and 31 days from the start of the feeding regimen. A single oral dose of 1-50 ug/kg TCDD was

administered to CD-1 mice and blood was drawn 1-5 weeks later. Oral weekly doses of 0.008-1.0 ug/kg TCDD were administered to female Hartley guinea pigs for 8 weeks. At 8 weeks or when the animals in the highest dosage group became moribund, blood was collected. Half of each treatment group was also administered tetanus toxoid and the other half, mycobacterium tuberculosis. Blood was analyzed for hematologic parameters, blood chemistries, and serum enzymes. Serum bilirubin, cholesterol, glutamic pyruvate transaminase, and glutamic-oxaloacetic transaminase activities were elevated in rats that received the highest dosage, after 7-31 days and lactic dehydrogenase was elevated after 24 days. Alkaline phosphatase levels were normal in all groups of rats. Blood glucose concentrations were depressed between days 10-24. Serum protein levels fluctuated. The only significant dose-related responses were decreased blood glucose levels on days 10-24 and cholesterol elevations on days 17 and 24. Hemoconcentration was observed in the high dosage group on days 17 and 24 and thrombocytopenia occurred in all treatment groups. In guinea pigs and in mice, dose-related decreases in leukocyte and lymphocyte counts were observed 1 week after treatment. Mouse blood chemistries were not analyzed. The authors suggested that hemoconcentration (which occurred just prior to death) resulted from shock and dehydration; other blood parameters reflected liver damage. The authors concluded that the main toxic effect of TCDD in the rat was hepatocellular necrosis and was accompanied by an important platelet effect. In mice and guinea pigs, TCDD was immunosuppressive, as evidenced by lymphopenia in these animals after treatment.

1115. Zitko, V. (1972) Absence of chlorinated dibenzodioxins and dibenzofurans from aquatic animals. Bull. Environ. Contam. Toxicol. 7(2/3):105-110.

The authors reported on the absence of TCDD residues in tissues from aquatic animals. Muscle and liver of white shark, eggs of double crested cormorants and herring gulls, muscle of eel and chain pickerel and commercial samples of herring oil and groundfish-herring fishmeal were analyzed for TCDD by gas chromatography (detection limit 0.04 ug/g). The authors did not specify whether any of the animals had been exposed to herbicides.

1116. Zitko, V., Hutzinger, O., and Choi, P. M. K. (1972) Contamination of the Bay of Fundy-Gulf of Maine area with polychlorinated biphenyls, polychlorinated terphenyls, chlorinated dibenzodioxins, and dibenzofurans. Environ. Health Perspec. 1:47-50.

The authors analyzed an unspecified number of fish and bird samples from the Bay of Fundy for the presence of TCDD. Muscle and liver of white shark, eggs of cormorants and gulls, commercial herring oil, and groundfish herring fishmeal were analyzed by gas chromatography (detection limit 0.04 ug/g wet weight). No TCDD was found in any of the samples.

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