



Uploaded to VFC Website ~ October 2012 ~

This Document has been provided to you courtesy of Veterans-For-Change!

Feel free to pass to any veteran who might be able to use this information!

For thousands more files like this and hundreds of links to useful information, and hundreds of "Frequently Asked Questions, please go to:

[Veterans-For-Change](#)

*Veterans-For-Change is a 501(c)(3) Non-Profit Corporation
Tax ID #27-3820181*

If Veteran's don't help Veteran's, who will?

We appreciate all donations to continue to provide information and services to Veterans and their families.

https://www.paypal.com/cgi-bin/webscr?cmd=_s-xclick&hosted_button_id=WGT2M5UTB9A78

Note: VFC is not liable for source information in this document, it is merely provided as a courtesy to our members.

Item ID Number: 00107

Author Anderson, Melvin E.

Corporate Author U.S. Air Force, Air Force System Command, Aerospace
Division, Air Force Aerospace Medical Research
Laboratory, Wright Patterson AFB, Ohio

Report/Article Title The Toxicity of Perfluoro-N-Decanoic Acid and 2,3,7,8-Tetrachlorodibenzo-P-Dioxin
in L5178Y Mouse Lymphoma Cells

Journal/Book Title

Year 1983

Month/Day March

Color

Number of Images 15

Description Notes Program 61102F, 2312, V1, 18

Anderson, M et al. 1982

Military
Toxicology

RECEIVED

SEP 01 1983

AFAMRL-TR-82-50

Special Projects Staff (1020)



AD 11783332

**THE TOXICITY OF PERFLUORO-N-DECANOIC ACID
AND 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN IN
L5178Y MOUSE LYMPHOMA CELLS**

MELVIN E. ANDERSEN, Ph.D.
MARILYN E. GEORGE

BIOCHEMICAL TOXICOLOGY BRANCH
TOXIC HAZARDS DIVISION

ANDREA M. ROGERS, Ph.D.

MICROBIOLOGICAL ASSOCIATES
3221 RIVER ROAD
BETHESDA, MARYLAND 20816

KENNETH C. BACK, Ph.D.

DEPARTMENT OF PREVENTIVE MEDICINE AND BIOMETRICS
UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814

MARCH 1983

Approved for public release; distribution unlimited

DTIC
SELECT
SEP 27 1983

REF. FILE COPY

AIR FORCE AEROSPACE MEDICAL RESEARCH LABORATORY
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT PATTERSON AIR FORCE BASE, OHIO 45433

NOTICES

When US Government drawings, specifications, or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever, and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data, is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

Please do not request copies of this report from Air Force Aerospace Medical Research Laboratory. Additional copies may be purchased from:

National Technical Information Service
8285 Port Royal Road
Springfield, Virginia 22161

Federal Government agencies and their contractors registered with Defense Technical Information Center should direct requests for copies of this report to:

Defense Technical Information Center
Cameron Station
Alexandria, Virginia 22304

TECHNICAL REVIEW AND APPROVAL

AFANRL-TR-62-50

This report has been reviewed by the Office of Public Affairs (OPA) and is releasable to the National Technical Information Service (NTIS). If NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication
FOR THE COMMANDER


ROSE C. INMAN, Colonel, USAF
Chief
Toxic Hazards Division
Air Force Aerospace Medical Research Laboratory

| REPORT DOCUMENTATION PAGE | | READ INSTRUCTIONS BEFORE COMPLETING FORM |
|--|-------------------------------------|---|
| 1. REPORT NUMBER AFAMRL-TR-82-50 | 2. GOVT ACCESSION NO. AD-A127354 | 3. RECIPIENT'S CATALOG NUMBER |
| 4. TITLE (and Subtitle) The Toxicity of Perfluoro-n-decanoic Acid and 2,3,7,8-Tetrachlorodibenzo-p-dioxin in L5178Y Mouse Lymphoma Cells | | 5. TYPE OF REPORT & PERIOD COVERED Technical Report January 80 - December 81 |
| | | 6. PERFORMING ORG. REPORT NUMBER |
| 7. AUTHOR(s) M. E. Andersen, A. M. Rogers*, M. E. George, and K. C. Back** | | 8. CONTRACT OR GRANT NUMBER(s) |
| 9. PERFORMING ORGANIZATION NAME AND ADDRESS AFAMRL, Toxic Hazards Division, AMD, AFSC, Wright-Patterson AFB, Ohio 45433 | | 10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 61102F, 2312, VI, 18 |
| 11. CONTROLLING OFFICE NAME AND ADDRESS | | 12. REPORT DATE MARCH 1983 |
| | | 13. NUMBER OF PAGES 14 |
| 14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office) | | 15. SECURITY CLASS. (of this report) UNCLASSIFIED |
| 16. DISTRIBUTION STATEMENT (of this Report) Approved for public release; distribution unlimited. | | 15a. DECLASSIFICATION DOWNGRADING SCHEDULE |
| 17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report) | | |
| 18. SUPPLEMENTARY NOTES * Microbiological Associates, 5221 River Road, Bethesda, MD 20816 ** Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814 AFAMRL Primary Investigator: Dr. M. E. Andersen, AFAMRL/THB, (513) 255-5150. | | |
| 19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Perfluoro-n-decanoic Acid 2,3,7,8-Tetrachlorodibenzo-p-dioxin Toxicology Polyfluorinated acids L5178Y Mouse Lymphoma Cells Cell Culture Erythrocyte Fragility | | |
| 20. ABSTRACT (Continue on reverse side if necessary and identify by block number) () Perfluoro-n-decanoic acid (PFDA) causes toxic sequelae in vivo very similar to those caused by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The toxicity of these two compounds, several other polyfluorinated fatty acids, and corresponding hydrogenated fatty acids have been studied in vitro in L5178Y mouse lymphoma cells. Below concentrations which cause cell lysis (≥ 500 $\mu\text{g/ml}$), PFDA did not affect suspension growth. After 24 hr treatment with concentrations between 1 and 100 $\mu\text{g/ml}$ treated cells no longer grew into clones when plated in semi-soft agar. This impairment of clone-forming ability was reversible after growth of | | |

micro

SECURITY CLASSIFICATION OF THIS PAGE(When Data Entered)

treated cells in fresh medium for 36 hr. Perfluoro-n-octanoic acid did not impair clone-forming ability at any concentration; and neither did the straight-chain hydrogenated fatty acid analogs. All polyfluorinated acids tested (either perfluorinated or ω -hydro-analogs) with chain length 9 or greater caused impairment of clone-forming ability after treatment with concentrations that were non-toxic in suspension. TCDD (highest dose, 0.5 μ g/ml) had no effect on growth in suspension. After 48 hr treatment with TCDD concentrations of 0.01 μ g/ml or greater, plated cells formed clones with altered morphology. These clones were less discrete, lacking a clearly defined boundary. The effect on clone morphology required 36 hr treatment of cells with TCDD in suspension and was reversible following 48 hr growth in fresh medium. Cell division time in suspension was 10-12 hrs and was unaffected by PFDA or TCDD. In vivo PFDA treatment altered erythrocyte fragility in rats. It is suggested that the toxicity of PFDA and TCDD in vivo and in L5178Y cells in vitro may be due to an ability of these chemicals to interfere with normal structure and/or function of biological membranes.

SECURITY CLASSIFICATION OF THIS PAGE(When Data Entered)

PREFACE

This research was performed in the Biochemical Toxicology Branch, Toxic Hazards Division, Air Force Aerospace Medical Research Laboratory from January 1980 through December 1981. It was performed in support of Task 2312V1, "Toxicological Mechanisms of Air Force Chemicals and Materials;" Work Unit 2312V118, "Effects of Air Force Propellants and Chemicals on Metabolic Mechanisms." Portions of this work were presented at the 21st Annual Meeting of the Society of Toxicology, Boston, Massachusetts, 22-26 February 1982.

| | |
|--------------------|-------------------------------------|
| Accession For | |
| NTIS GRA&I | <input checked="" type="checkbox"/> |
| DTIC TAB | <input type="checkbox"/> |
| Unannounced | <input type="checkbox"/> |
| Justification | |
| By | |
| Distribution/ | |
| Availability Codes | |
| Avail and/or | |
| Dist | Special |
| A | |



INTRODUCTION

Perfluorinated fatty acids, perfluorinated sulfonic acids, and appropriate derivatives are used commercially in numerous applications which take advantage of their exceptional surfactant properties and extreme chemical and thermal stability (Guenther and Victor, 1962). Most commercially important derivatives are based on perfluoroalkyl chain lengths of 5 to 7. The acute and subchronic toxicity of ammonium perfluoro-n-octanoate (PFOA) has been described in detail in both rats and rhesus monkeys (Griffith and Long, 1980). Less is known of the toxicity of longer chain analogs.

In an abstract Andersen et al. (1981) described the acute toxicity of perfluoro-n-decanoic acid (PFDA; nonadecafluoro-n-decanoic acid; $C_{10}F_{19}O_2H$) in a variety of rodent species. This acid was significantly more toxic than PFOA and its toxicity differed both quantitatively and qualitatively from that of the shorter chain analog. Toxic signs and target organs for PFDA were similar to those seen with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The single dose oral LD_{50} ~ 30 days of PFDA in male rats was about 50 mg/kg and rats intubated with 90 mg/kg lost nearly 50% of their initial body weight before dying two to three weeks after intubation. As does TCDD, PFDA caused severe thymic atrophy.

As part of a comparison of the biological effects of PFDA and TCDD, we have evaluated the toxicity of these chemicals in several isolated cell systems. In part, this paper describes effects of various polyfluorinated fatty acids, hydrogenated fatty acids, and TCDD on growth characteristics of L5178Y mouse lymphoma cells, a T-cell derived lymphoma (Muller et al., 1981), which grows both in suspension and in semi-soft agar. A T-cell lymphoma was used because T-lymphocytes appear to be targets of PFDA and TCDD toxicity in rodents. This conclusion was based on the marked thymic cortical atrophy noted in animals treated with either of these chemicals. L5178Y cells are commonly used for mutagenicity testing and the mutagenic potential of these chemicals in L5178Y cells is reported elsewhere (Rogers et al., 1982). In addition, limited results of osmotic fragility studies of erythrocytes from rats treated with PFDA are described in an attempt to relate altered osmotic fragilities to the effects of PFDA on L5178Y cells.

MATERIALS AND METHODS

L5178Y Mouse Lymphoma Cells: L5178Y cells were originally obtained from Dr. C. F. Arlett, MRC, Cell Mutation Unit, Brighton, England. They were routinely screened for mycoplasma contamination. The routine methods for maintenance of L5178Y cells and the soft agar cloning technique were as described elsewhere (Cole and Arlett, 1976), except that McCoy's 5A medium (supplemented with penicillin, streptomycin, sodium pyruvate, and 10% horse serum) was used instead of Fischer's medium. For toxicity experiments, L5178Y cells were treated for 24 hrs with doses of PFDA ranging from 0.01 μ g/ml to 1 mg/ml, or for 48 hrs with doses of TCDD ranging from 0.001 μ g/ml to 0.50 μ g/ml. At the end of the treatment period, cells were centrifuged, washed in McCoy's 5A medium and resuspended in McCoy's 5A containing 20% horse serum. Cells were plated for growth in soft agar, and plates were examined for clones after 9-10 days incubation in a humidified CO_2

incubator. Horse serum was obtained from Gibco-Biocult. Penicillin, streptomycin, and sodium pyruvate were obtained from Sigma.

Chemicals: Fatty acids (all > 99% pure)¹, perfluoro-n-decanoic acid (>98%)¹, perfluoro-n-octanoic acid (>96%)¹, 11-H eicosafuoro-n-undecanoic acid (97-99%)², and 9-H hexadecafluoro-n-nonanoic acid³ were obtained commercially. The latter two compounds contain a single hydrogen at the omega position. Perfluoro-n-dodecanoic acid⁴ (71% C₁₁F₂₃CO₂H; 3% C₁₀F₂₁CO₂H; 2% C₉F₁₉CO₂H; remainder unidentified, nonfunctional fluorocarbon) and TCDD were gifts⁵. For L5178Y studies, TCDD was dissolved in acetone and the fatty acids and fluorinated analogs were dissolved in dimethylsulfoxide except perfluorinated dodecanoic was also dissolved in acetone.

Osmotic Fragility: Male Fischer 344 rats (200-300 g) were treated ip with 50 mg PFDA/kg. Propylene glycol:water (50:50 v/v) was used as diluent with a final dosing volume of 2 ml/kg. Treated and diluent control rats in groups of four to five were killed at various times after injection. Blood was drawn from the inferior vena cava after opening the abdomen of anesthetized rats and erythrocytes harvested by centrifugation. Osmotic fragility was determined as described in Dacie and Lewis (1963). Curves were constructed for hemolysis at 10 saline concentrations between 0.25 and 0.85%. Data presented are percent hemolysis at a single intermediate saline concentration, 0.4%.

RESULTS

Fatty Acids: PFDA had little effect on L5178Y suspension growth at concentrations below 100 µg/ml (Fig. 1). At concentrations of 500 µg/ml or above, cells were dissolved by the surfactant action of the acid and neither cells nor debris were visible in suspensions at these concentrations. In comparison to the dose-response curve for suspension growth, the curve for clone-forming ability was shifted some 2.5 log units to the left: the ED₅₀-24 hr for impairing clone-forming ability was approximately 3×10^{-1} µg/ml. To our knowledge, this ability - dissociating the markers of suspension growth and clone-forming ability in these transformed cells - has not been reported for any other chemical. Perfluorinated-n-dodecanoic and 11-H-eicosafuoro-n-undecanoic acid caused a similar displacement of the two dose response curves (Table 1). On the other hand, PFOA which was slightly less toxic to cells in suspension than was PFDA did not show the differential toxicity with respect to suspension and clonal growth. The ω-H-hexadecafluoro-n-nonanoic acid displaced the dose response curves for suspension and clone forming ability, but the displacement was less than that seen with PFDA (Table 1). With hydrogenated fatty acid analogs from C₈ to C₁₁, toxicity was equal both in suspension and in agar (Table 2).

¹ Aldrich Chemical Company, Milwaukee, WI 53233.

² PCR Research Chemicals, Inc., Gainesville, FL 32602.

³ Alfred Bader Library of Rare Chemicals, Division of Aldrich Chemical Company, Milwaukee, WI 53233.

⁴ Commercial Chemicals Division 3M, 3M Center, St Paul, MN 55144.

⁵ Dow Chemical USA, Midland, MI 46460.

24 HR. TREATMENT OF L5178Y CELLS WITH PFDA.

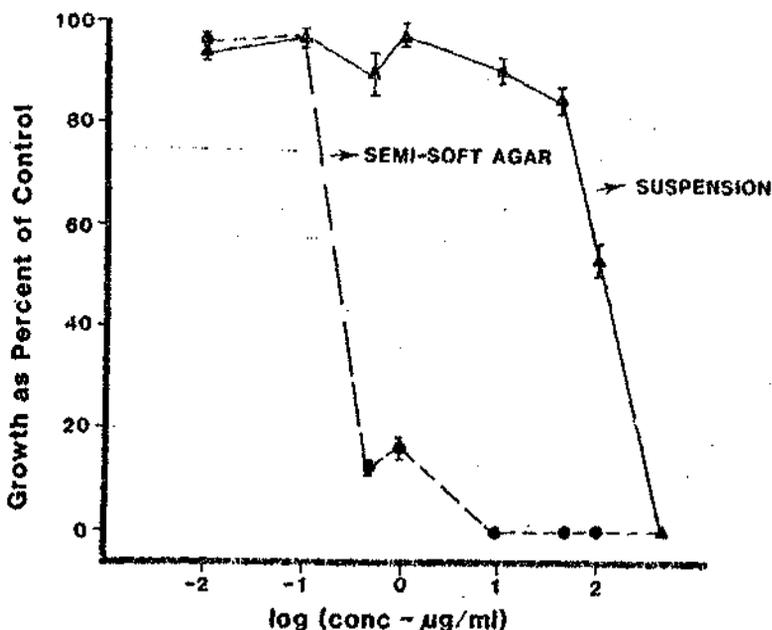


Figure 1. Toxicity of PFDA in L5178Y Cells. L5178Y cells were grown for 24 hr in the presence of varying amounts of PFDA (x-axis). Triangles are total growth in suspension as percent of control growth in the absence of PFDA. Aliquots of the cells treated with different concentrations were plated and grown for 8 to 10 days on semi-soft agar. Circles are percent of plated cells which gave rise to clones relative to similar values for control cells. Data points are mean and standard errors ($n = 3-7$).

Table 1

The Effects of Various Polyfluorinated Fatty Acids on Growth of L5178Y Mouse Lymphoma Cells in Suspension and on their Clone Forming Ability in Semi-Soft Agar

| Dose (µg/ml) | Perfluoro-n-octanoic Acid | | 9-H Hexadecafluoro-n-nonanoic Acid | | Perfluoro-n-decanoic Acid | | 11-H Eicosafluoro-n-undecanoic Acid | | Perfluoro-n-dodecanoic Acid | |
|-----------------|---------------------------|-------------|------------------------------------|-------------|---------------------------|-------------|-------------------------------------|-------------|-----------------------------|-------------|
| | Suspension Agar | (% Control) | Suspension Agar | (% Control) | Suspension Agar | (% Control) | Suspension Agar | (% Control) | Suspension Agar | (% Control) |
| 0.01 | 94 ^a | 98 | 94 | 97 | 94 ± 2 ^b | 96 | 96 | 100 | 87 | 97 |
| 0.1 | 100 | 96 | 83 | 95 | 97 ± 1.4 ^c | 97 ± 0.7 | 78 | 90 | 78 | 87 |
| 0.5 | | | | | 89 ± 4.7 ^b | 12 ± 1.1 | | | | |
| 1 | 89 | 98 | 94 | 95 | 97 ± 1.1 ^b | 16 ± 1.9 | 75 | 11 | 91 | 25 |
| 10 | 89 | 98 | 94 | 89 | 90 ± 2.2 ^b | 0 | 82 | 0 | 72 | 0 |
| 50 | 76 | 90 | 92 | 89 | 84 ± 2.3 ^b | 0 | 82 | 0 | 75 | 0 |
| 100 | 76 | 90 | 83 | 0 | 53 ± 3.6 ^b | 0 | 82 | 0 | 72 | 0 |
| 500 | 21 | 21 | --- ^d | --- | --- | --- | --- ^d | --- | --- ^d | --- |
| 1000 | --- ^d | --- | --- ^d | --- | --- | --- | --- ^d | --- | --- ^d | --- |

^a Result of single experiment. Numbers in each column are growth as percent of growth of control cells.

^b Mean ± standard error ($n = 3$).

^c Mean ± standard error ($n = 7$).

^d These concentrations dissolved cells in suspension.

Table 2

The Effect of Various Fatty Acids on Growth of L5178Y Mouse Lymphoma Cells in Suspension and on their Clone Forming Ability in Semi-Soft Agar

| Dose ($\mu\text{g/ml}$) | Nonanoic Acid Suspension Agar | | Decanoic Acid Suspension Agar | | Undecanoic Acid Suspension Agar | |
|------------------------------|----------------------------------|----|----------------------------------|----|------------------------------------|----|
| | (% Control) | | (% Control) | | (% Control) | |
| 0.01 | 85 ^a | 98 | 106 | 96 | 95 | 96 |
| 0.1 | 82 | 98 | 94 | 94 | 85 | 93 |
| 1 | 92 | 98 | 100 | 93 | 75 | 96 |
| 10 | 90 | 94 | 84 | 90 | 82 | 93 |
| 50 | 85 | 98 | 75 | 82 | 82 | 86 |
| 100 | 72 | 88 | 63 | 63 | 42 | 44 |
| 500 | 33 | 26 | -- ^b | -- | -- ^b | -- |
| 1000 | -- ^b | -- | -- ^b | -- | -- ^b | -- |

^a Numbers in both columns are growth as percent of growth of control cells.

^b These concentrations dissolved cells in suspension.

Time to Effect and Reversibility: Cell division time for L5178Y cells in suspension under growth conditions used in this study was 10 to 12 hours. An experiment was performed to see if cells required a period of treatment with PFDA before diminished clone-forming ability could be observed. Cells were grown in suspension containing 0.5 μg PFDA/ml and removed at various times for plating to observe loss of clone-forming ability (Fig. 2a). There was a lag of 8 hr before any appreciable effect was observed and the time of treatment required to reduce plating efficiency to 50% of control was about 12 hr, i.e., one cell generation.

Cells were also grown for 24 hr in the presence of 0.5 μg of PFDA/ml harvested by centrifugation and washed in fresh growth medium. These treated cells were resuspended for growth in fresh, uncontaminated medium and aliquots withdrawn after various times for plating (Fig. 2b). The decreased plating efficiency was reversible, but recovery was more prolonged than the time required to induce the diminished clone-forming ability. The time of growth in fresh medium necessary to restore 50% plating efficiency was nearly 36 hr, or about three cell generations. Cell division time of L5178Y cells in suspension was unaffected by pretreatment with 0.5 μg PFDA/ml.

Dioxin: In L5178Y cells TCDD did not dissociate growth in suspension from growth in soft agar at any concentration tested, up to 0.5 $\mu\text{g/ml}$. However, the morphology of the clones obtained after treating cells in suspension with concentrations of TCDD between 0.01 and 0.5 $\mu\text{g/ml}$, was markedly different from controls (Fig. 3). Instead of the well-circumscribed, circular clusters of control clones, those clones formed after dioxin-treatment were less-discrete and lacked a well-defined border. After growing cells in suspension for 46 hr in the presence of 0.005 μg TCDD/ml,

TIME TO RESPONSE/TIME TO RECOVERY OF L5178Y
CELLS TREATED WITH PFDA.

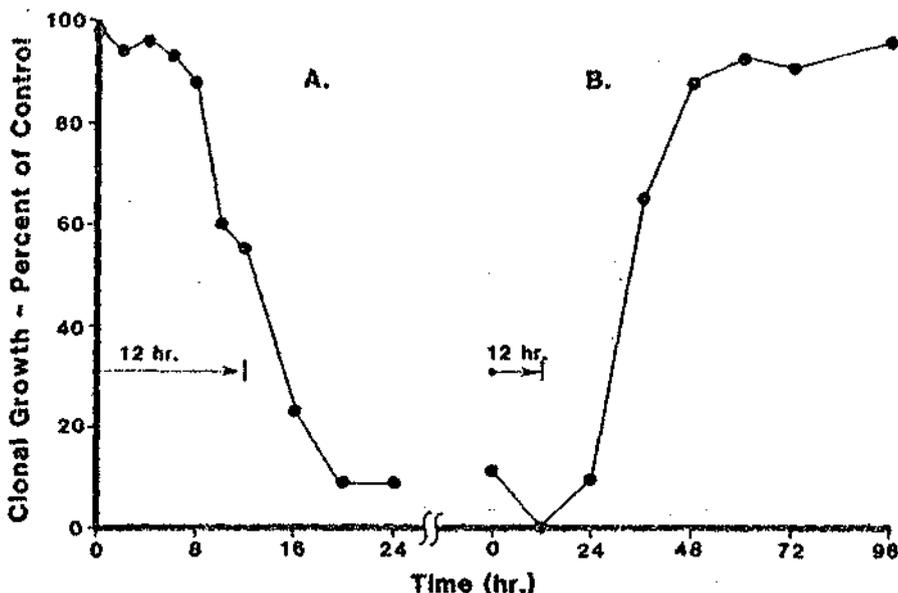


Figure 2. Time course of impairment and recovery of cloning ability in L5178Y cells treated with PFDA in suspension. A: Time to effect: cells were grown for various lengths of time (x-axis) in suspension in a medium containing 0.5 µg PFDA/ml and plated in semi-soft agar. Growth is expressed as percent of plated cells forming clones after treatment relative to percent of untreated cells which give rise to clones. B: Time to recovery: cells were treated in suspension with 0.5 µg PFDA/ml for 24 hr, and harvested by centrifugation. Aliquots were removed and grown in fresh, uncontaminated medium for various lengths of time (x-axis). Cells were then plated to observe recovery of the ability to form clones.

all clones formed after plating were normal; at 0.01 µg/ml, most clones formed were abnormal; and by 0.5 µg/ml, all clones had altered morphology. By inspection of the plates, the ED₅₀, that is the concentration of dioxin required to produce alterations affecting 50% of the formed clones when cells were initially maintained in suspension with dioxin for 48 hr before plating, was about 0.01 µg/ml, i.e., about 3×10^{-8} M.

In time-course experiments analogous to those in Fig. 2, but conducted with 0.01 µg dioxin/ml, the time of treatment in suspension required to produce 50% of maximum response in altering clone morphology was about 36 hr. A time to recovery of normal growth characteristics was also estimated for cells grown initially for 48 hr in the presence of 0.01 µg TCDD/ml. The time of growth in fresh medium required to give a 50% return to normal clonal morphology was about 48 hr. As noted with PFDA, effects on clone growth were reversible, but recovery and expression times for the effects were longer with TCDD than with PFDA.

Red Blood Cell Fragility: Rat red blood cells were obtained from rats killed at various times after ip injection of 50 mg PFDA/kg. There was increased resistance to hemolysis after treatment with PFDA (Fig. 4) and the time course of decreased fragility was similar to the time course of weight loss in treated rats (Andersen et al., 1981).

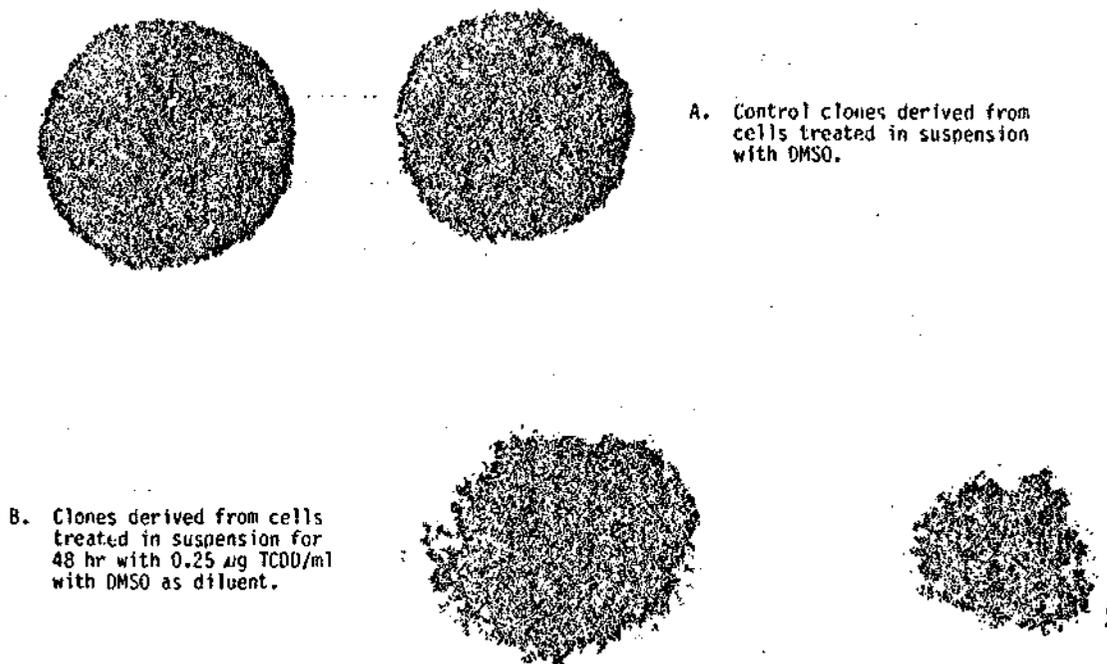


Figure 3. Altered clone morphology after treating L5178Y cells in suspension with TCDD.

DISCUSSION

Knutson and Poland (1980) studied the effects of TCDD on 23 cultured cell types and found no toxicity in any of these mammalian cell lines at treatment concentrations of up to 10^{-7}M and contact times of up to two weeks. Markers for toxicity included (1) alterations in the morphology of cells or the cell cultures, (2) percentage viable cells, and (3) growth rate. Among the 23 cell lines were five lymphoid cell types derived from thymic cortex - three were murine and two were virally transformed human leukocytes. All these cell types were tested for growth in suspension and cell viability by trypan blue exclusion. Beatty et al. (1975) found that TCDD had no effect on growth or morphology of normal human lymphocytes in suspension. Our results are similar to the extent that TCDD did not affect growth or cell viability of L5178Y cells in suspension. The altered growth characteristics observed in this paper are more subtle and only apparent when cells are grown in semi-soft agar, where they are constrained to grow in close proximity to each other. The concentration dependence of the effect with TCDD is such that a 48 hr treatment with 0.01 $\mu\text{g}/\text{ml}$ (i.e., about $3 \times 10^{-8}\text{M}$ TCDD) causes the effect in most of the treated cells. This concentration is reasonable for physiological significance since the mouse LD₅₀ is about 300 μg TCDD/kg, or about 1 $\mu\text{moles}/\text{kg}$ (McConnell et al., 1978).

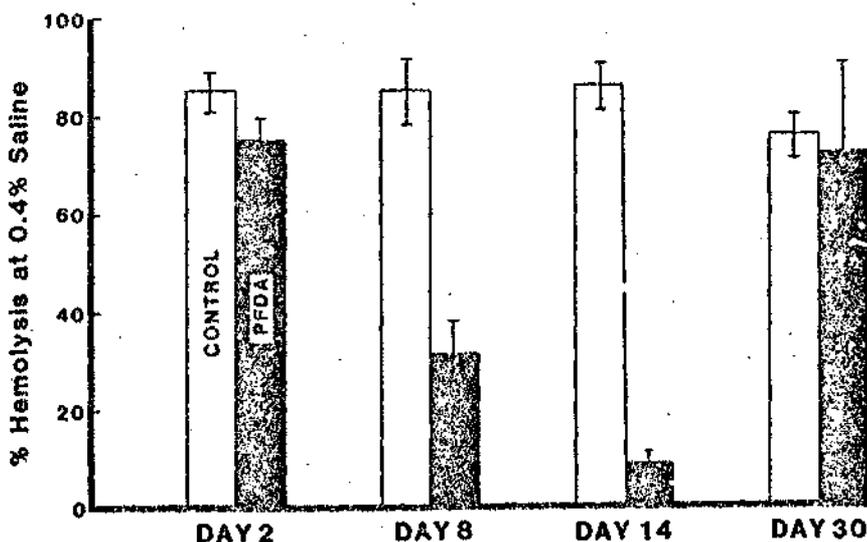


Figure 4. Relative osmotic fragilities of red blood cells from rats injected ip with 50 mg PFDA/kg and killed at various times after injection. Data are mean and standard deviation (n = 4-5). From the overall curves with 10 salt concentrations, the concentration at which 50% hemolysis occurred was 0.43, 0.38, 0.34, and 0.43%, respectively, in treated rats at 2, 8, 16, and 30 days. Control groups at these sampling times had 50% hemolysis at 0.45, 0.44, 0.45, and 0.43%, respectively.

Alterations in clone morphology seen after TCDD are striking, but estimations of concentration dependence are essentially qualitative, i.e., the percentage of abnormal clones is estimated by inspection and making a distinction between normal and slightly abnormal clones is difficult. We have maintained a restrictive definition of what constitutes an abnormal clone and scoring was done solely by Dr. A. M. Rogers. For these reasons, the estimated ED₅₀ for the effects with TCDD are probably high. More quantitative determinations of these TCDD dose response curves await determination of the biochemical basis of the altered morphology and methods to unequivocally identify altered clonal units.

With PFDA, results are readily quantified since treated cells no longer proliferate in semi-soft agar. The ED₅₀ - 24 hr for the loss of clone-forming ability was 0.3 µg/ml (i.e., about 6×10^{-7} M); this contrasts to a single dose LD₅₀ in mice of about 100-150 mg PFDA/kg or 0.2-0.3 mmoles/kg (Andersen et al., 1981; Van Rafelghem and Andersen, unpublished experiments, 1981).

With the polyfluorinated acids examined, this toxicity is present with acids of chain length greater than 8. The differences in single cell toxicity between the fluorinated octanoic and decanoic acids are striking, but consistent with the different acute toxicity reported for these two acids in rats. The hydrogenated fatty acids are without differential effect on clone-forming ability of L5178Y cells. In terms of cell lysis, expressed

as toxicity in suspension, hydrogenated and polyfluorinated fatty acids were about equipotent (Tables 1 and 2).

The molecular basis of the impairment of clone-forming ability is unknown. Subtle changes may have occurred in cell membranes to inhibit growth of cells when maintained in close contact. In this regard, the osmotic fragility results suggest a biological membrane more resistant to hypoosmotic insult. Increased resistance can be due to a variety of causes, one of which is altered membrane composition (Kuiper et al., 1971). Preliminary studies in our laboratory have now shown that erythrocytes from PFDA-treated animals also show increased membrane fluidity (M. George and M. E. Andersen, unpublished studies, 1982), and that the total fatty acid composition of the liver lipid pool in these rats shows a dramatic shift toward increasing unsaturation, especially in the stearic to oleic acid ratio (Olson et al., 1982). While indirect, these results suggest compositional and functional alterations in membranes subsequent to PFDA exposure in the rat in vivo.

There is no unifying hypothesis explaining the toxicity of TCDD and materials causing similar toxic effects, i.e., certain polyhalogenated biphenyls (Sleight et al., 1981; Biocca et al., 1981) and long-chain perfluorinated fatty acids of chain length 9 or above (Andersen et al., 1981). It may be that these various chemicals, including PFDA and TCDD, are toxic due to effects on cell membranes resulting in interference with cell-cell or cell-mediator interactions. These effects could either be direct or mediated by interference with some endogenous hormonal control of membrane composition/function. Toxicity would not be a result of cell necrosis or grossly visible organellar alterations, but from more subtle structural alterations of biomembranes and attendant disturbances in intercellular communication. This hypothesis is under active investigation in our laboratory.

The cell line used for this research was so-called TK ^{+/+} with regard to the gene locus for the enzyme thymidine kinase (TK). Our stock of these cells, brought to Dayton from England by Dr. Rogers, was destroyed during a malfunction of the deep freeze storage unit. We have not observed differential effects on suspension and clonal growth with L5178Y TK^{+/-} cells - a cell line more commonly used in mutation research and, therefore, much more readily available. It appears that future work on this phenomenon will have to be restricted to the TK^{+/+} cells.

REFERENCES

- Andersen, M.E., Baskin, G., and Rogers, A.M., 1981, "The acute toxicity of perfluoro-n-decanoic acid: Similarities with 2,3,7,8-tetrachlorodibenzo-dioxin," The Toxicologist, 1, 16.
- Beatty, P.W., Lemback, 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, 309-312.

Biocca, M., Gupta, B.N., Chae, K., McKinney, J.D., and Moore, J.A., 1981, "Toxicity of selected symmetrical hexachlorobiphenyl isomers in the mouse," Toxicol. Appl. Pharmacol., 58, 461-474.

Cole, J. and Arlett, C.F., 1976, "Ethyl methanesulphonate mutagenesis with L5178Y mouse lymphoma cells. A comparison with ouabain, thioguanine, and excess thymidine resistance," Mutation Res., 34, 507-526.

Dacie, J.V. and Lewis, S.M., Practical Hematology, 3rd ed., J.A. Churchill, Ltd., London, 1963.

Griffith, F.D. and Long, J.E., 1980, "Animal toxicity studies with ammonium perfluorooctanoate," Amer. Ind. Hyg. Assoc. J., 41, 576-583.

Guenther, R.A. and Victor, M.L., 1962, "Surface active materials from perfluorocarboxylic and perfluorosulfonic acids," Industrial and Engineering Chem. Prod. Res. & Dev., 1, 165-169.

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.

Kuiper, P.J.C., Livne, A., and Meyerstein, N., 1971, "Changes in lipid composition and osmotic fragility of erythrocytes of hamster induced by heat exposure," Biochem. Biophys. Acta, 248, 300-305.

McConnell, E.E., Moore, J.A., Haseman, J.K., and Harris, M.W., 1978, "The comparative toxicity of chlorinated dibenzo-p-dioxins in mice and guinea pigs," Toxicol. Appl. Pharmacol., 44, 335-356.

Muller, W.E.G., Zahn, P.K., Maidhof, A., Schroder, H.C., and Umezawa, H., 1981, "Bestatin, a stimulator of polysome assembly in T-cell lymphoma (L5178Y)," Biochem. Pharmacol. 30, 3375-3377.

Olson, C.T., Andersen, M.E., George, M.E., Van Rafelghem, M.J., and Back, A.M., 1982, "The toxicology of perfluorodecanoic acid in rodents," presented at the Thirteenth Annual Conference on Environmental Toxicology, Dayton, Ohio.

Rogers, A.M., Andersen, M.E., and Back, K.C., 1982, "Mutagenicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin and perfluoro-n-decanoic acid in mouse lymphoma cells," Mutation Res. 105, 445-449.

Sleight, S.C., Rander, J.A., Akoaso, B.T., Aust, S.D., and Nachreiner, R., 1981, "Comparative toxicopathology of firemaster BP-6, 2,2,4,4',5,5'-hexabromobiphenyl (HBB) and 3,3',4,4',5,5'-HBB after 10 and 30 days of dietary administration to rats," The Toxicologist 1, 12.

END

DATE
FILMED

5-83

DTIC