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(BSC) are divided as to its needs and implementation. Most BSC members endorse health surveillance especially when meaningful parameters of clinco-laboratory tests can be applied and evaluated. They are less certain of the value of general physical work-ups and batteries of laboratory tests performed merely for the sake of gathering statistical information. This is especially true when the lag period between a causative event and effect may be decades.

However, if pathogenic microorganisms are involved in a project, the minimum requirement should be to obtain and store base-line serum specimen. Confirmation of a work-connected illness could be made by comparing pre and post-exposure antibody titers. This serum sampling program is especially appropriate for primate animal handlers in addition to periodic TB tests.

Prompt reports of accidents, personal injury or massive spills, resulting from handling hazardous material or from animal contact, should be mandatory for all persons connected with the project.

In addition to the general comments above, I would add specific recommendations for Dr. Evenson's study on effect of feeding Dioxin to monkeys (See enclosure 2). Attached to his BMF form are evaluations and recommendations for this project.

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A. Physico-chemical and Toxic Properties of Substances used in Project.

1. Toxic Chemicals Utilized

TCDD (Dioxin)-(full name-2,3,7,8 tetrachlorodibenzo-p-dioxin). Solid, non-volatile; insoluble in most solvents except those which are themselves hazardous chemicals - pyrolysis at approx. 700-900°F.

2. Dosages and how delivered to animal

Commercially prepared diet pellets containing 25 parts/trillion (PPT).

3. Experimental Toxicology

TCDD has been shown to be an extremely toxic chemical with a variety of embryotoxic, fetotoxic, teratogenic, carcinogenic effects in animal studies. Chloracne is the major sign reported in cases of human exposure to the chemical.

Monkeys fed TCDD in doses 20 times greater than present project, levels after 9 months showed marked hematologic and epithelial tissue changes. At present level (25 PPT), considered by the PI to be a maintenance toxic diet, other investigators have demonstrated little or no toxic effect in a variety of animals (Ref 1).

4. Metabolic Fate

Accumulated in liver or fat but most excreted unmetabolized in feces or urine (Ref 1).

B. Hazard Assessment

This substance has known or suspected toxic, carcinogenic, or mutagenic properties at dosages in excess of those in the present feeding program. Lack of volatility of the compound favors containment but its stability and excretion in unmetabolized (but toxigenic) form makes decontamination of surfaces difficult and enhances accumulation. Dispersion of toxicants from food residual and as dust aerosols or spray particles (during cleaning of cages or pans), appears to be the main foci of hazard. Inhalation and ingestion of hazardous substances is the most likely method of potential intoxication.

C. Safety Recommendations

1. Animal Facility

Entry restricted to authorized personnel. Post hazard sign on door indicating toxicant used. Copy of safety protocol outline should also be affixed.

a. Ventilation - maintain present directional air flow i.e., Prep room at negative pressure to corridor, and animal quarters negative to prep room. Exhaust filter bags and blower assemblies in plenums servicing animals fed toxicant should be changed or adjusted with extreme care. Access to these areas should be identified with Biohazard signs. Only persons who are protectively garbed, using face respirators and who have been trained in handling hazardous materials should be permitted to work in these plenums. Upon disposal, filter bags should be double bagged in containers ("Garbax"), identified as to hazards and removed for incineration.

b. Animal Waste Disposal - If feasible, use disposable plastic liners in animal cage pans (Encl. 3), bag and discard in covered non-leakable "GI" can. All disposal operations should be done in animal room.

c. Cage Area Clean-up - Use two-bucket, wet mop method. Cleaner should contain an anionic detergent (1% triton X-100). HEPA-filtered vacuum cleaners, (wet/dry combination model) are satisfactory, provided the filter has been certified by DOP containment test (Encl. 4), but check with Biosafety Office before purchasing.

2. Personnel Practices

All persons connected with the operation or support of the project should receive proper instruction in the nature of the hazard, areas of potential contamination, precautions to be observed, garments to be worn and proper manner of clean-up and disposals.

Directions should be as explicit as possible, with few optional alternatives. When required, protective clothing,

gloving, properly adjusted face respirators with dust and organic vapor cartridges should be worn. Rigorous attention to personal hygiene following animal handling, cage or pan cleaning and any contact with residual hazardous material is essential.

a. Clothing

No uncovered street clothing should be worn in the facility. Type and sources of disposable headwear and clothing are given in the attached sheets (Encl. 5). A gloving-combination of nitrile inner and latex outer gloves is recommended. Outer gloves should be discarded immediatley after use and nitrile gloves cleansed in detergent, rinsed and dried each day for further use. Plastic booties should be worn over work shoes and should remain in (preferably) or near entrance to animal rooms. They should never be worn in the outer corridor. When cage pans are being dumped or transferred for a cleaning, plasti overaprons should be worn. Use of pan liners will decreas the frequency of scraping and washing of metal pans.

During those times when such cleansing is required, it will be necessary to wear the full complement of garments in the wash area. After completion of pan washing, disposable garments and gloves should be containerized for incineration and all other apparel stored in the wash area for cleansing before reuse.

Research employees should use the same protective outer clothing when entering contaminated animal quarters and remove and retain them in the prep room. Thorough hand washing should always be done before leaving the facility. Since no eating, smoking or drinking can be allowed in the facility, this should be done only in designated areas of the Biotron. No garments, gloves, booties, caps etc. contaminated with toxicants or by animals, should be permitted in the dining or rest areas.

D. Health Programs

At the initiation of a hazardous project in the Biotron, all personnel should receive a physical exam. In monkey experiments, a TB skin test or X-ray program is already in effect. Females of child-bearing age should be advised of possible additional risks if pregnancy occurs during project tenure. They should be encouraged to ask for pregnancy testing if they believe conception has occurred.

Also, TCDD has been reported to have an immunosuppressive effect in animals. This may result in activation of latent indigenous simian viruses, or other infections, some of which are extremely dangerous to humans. Care should be exercised at all times even when handling animals ordinarily docile. Changes in behavior patterns or presence, facial or oral lesions should be reported for veterinary evaluation. Bites, scratches or other animal

injuries from animal contact should be washed immediately (3 min. with soap and water) and disinfectant (1% Zephiran) applied to affected area. The incident should be promptly reported to the supervisor and/or Biotron physician for further treatment.

Any illness thought to be occupationally related should be brought to the attention of the Biotron medical director for evaluation and clinical laboratory workup if necessary. The latter should include a serum sample and serological assay of this specimen with a previous (base line) serum sample, if available.

E. Waste Disposal Considerations

From a safety point of view, it would be desirable to handle all wastes emanating from the entire project facility as hazardous. However, this would increase the work load and the economic burden involved. Because of the low levels of toxicant utilized and if reasonable precautions are taken to segregate toxic wastes from other wastes, there would be minimal opportunities for cross-contamination. All wastes which are derived from animals under toxic diets should be double bagged, put in leak-proof containers, identified with biohazard sticker tape and routed for terminal incineration.

Under present circumstances, installation of a high temperature incinerator (2,000 - 2,200°F) solely for use connected with this project, does not appear warranted. Should more investigators wish to pursue hazardous projects in the Biotron, which require high temperature disposal, then consideration should be given to purchasing an incinerator. Also, it might be advisable to give some thought to constructing an addition to the Biotron, expressly for the conduct of hazardous research.

COMMENTS

The above recommendations and comments are critical of the Biotron's animal spaces and configuration only in light of hindsight and contemporary design. Present concepts favor "clean-dirty" access areas contiguous with animal care facilities where hazardous work is performed. Since such safety conveniences are not presently available, it limits the type of hazardous experiments which can be allowed in the Biotron. Even with such facilities, the level of safety is largely dependent upon the attitude and training of the personnel involved.

I fully realize that some of the suggestions made above may require some adaptation or modification to make them practical. If we can assist in any way to achieve this, we shall be glad to do so.

If further clarification is needed on any of the points raised, please let me know.

II. MATERIALS AND METHODS

A. Chemical

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) -- CAS No. 1746-01-6--was synthesized in the Chemistry Division of IITRI, Chicago, Illinois, by the condensation of potassium 2,4,5-trichlorophenate in the presence of the Ullman copper catalyst as described in Appendix E. IITRI reported the purity to be 99.4%, based on results of gas chromatographic analysis of the chemical. Samples analyzed at Dow Chemical Company, Midland, Michigan, were found to contain less than 1% of two impurities, tentatively identified as a trichlorodibenzo-p-dioxin and a pentachlorodibenzo-p-dioxin. The presence of 0.1% to 0.2% hexachlorodibenzo-p-dioxin was detected by gas chromatography and mass spectrometry (Stehl, 1974).

The TCDD was stored at room temperature in brown glass vials in an unlighted glove-box hood and was exposed to light only at 3-month intervals, when samples were removed for preparation of stock suspensions in acetone.

B. Dosage Preparation

TCDD is insoluble in corn oil and in most other solvents but is partially soluble in acetone. Therefore, TCDD was administered as a suspension in a 9:1 corn oil-acetone solution. Fresh stock suspensions in acetone (Mallinkrodt, Inc., St. Louis, MO) containing 10.0 μg/ml were prepared every 3 months, and working suspensions were prepared every 2 weeks from the stock suspensions. The stock suspensions in acetone were shaken well, and suitable aliquots were added to corn oil (Tek-Lad Laboratories, Madison, WI) and to additional acetone to give the desired concentrations of the test chemical in 9:1 corn oil-acetone. The working suspensions were administered to rats at a volume of 0.05 ml/100 g body weight and 0.05 ml/10 g body weight to mice.

The suspensions of TCDD in either acetone alone or in the corn oil-acetone vehicle were kept in brown glass bottles with Teflon-lined caps. The bottles were sealed with tape, triple-bagged in plastic, and stored at

Animals were maintained in sealed, individually ventilated cages to ensure containment of the test chemical. Rats were housed 3 per cage and mice 10 per cage in polystyrene cages (Table 1) covered with a special tight-fitting polystyrene lid adapted to hold two metal filter housings and a water bottle. The filter housings contained FG 50 filters, one of which was left open to the room atmosphere while the other was attached to a hose that led to a pipe running the length of the shelf on the rack. Pipes on each of four shelves of the rack led to a large vertical pipe at the end of the rack. The large pipe was connected by flexible hose to the HEPA filtered exhaust system. This arrangement of individually vented cages provided a constant flow of air that was filtered as it entered and as it left the cages to prevent the release of the test chemical from the cages into the room.

The cages (including lids) housing the groups of animals dosed with TCDD were discarded after 1 week of use. The polystyrene lids were changed once a month. The used cages and lids were triple-sealed in plastic bags and incinerated, as was all waste material from the animal rooms and the hoods. The glass water bottles and stainless steel sipper tubes from the used cages were rinsed in the same rooms, using the organic solvent chlorothene, N.U.® (Table 1) to dilute out any dioxin present, and were then sanitized at 82°C in an automatic washer. The polycarbonate cages in the room housing the vehicle-control groups of animals were recycled 3 times and incinerated. The corresponding water bottles and sipper tubes used in these rooms were not rinsed in chlorothene before washing.

Disposable clothing was worn by all personnel and, after use, was incinerated by the procedure used for cages and other waste material. Respirators were worn in the animal rooms. All dosing of animals was carried out in hoods.

Animals were provided with fresh hardwood chip bedding once a week (Table 1). They were fed Wayne® Lab Blox (Table 1) in pellet form and were provided with fresh food when their cages were changed. Tap water was provided ad libitum. Clean water bottles were provided once a week, and the bottles were refilled once a week.