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DEVELOPMENT OF DIETARY LC_{50} AND REPRODUCTION TEST PROTOCOLS USING MINK AND FERRETS AS REPRESENTATIVE MAMMALIAN CARNIVORES

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DEVELOPMENT OF DIETARY LC_{50} AND REPRODUCTION TEST PROTOCOLS USING MINK AND FERRETS AS REPRESENTATIVE MAMMALIAN CARNIVORES

by

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ABSTRACT

DEVELOPMENT OF DIETARY LC_{50} AND REPRODUCTION TEST PROTOCOLS USING MINK AND FERRETS AS REPRESENTATIVE MAMMALIAN CARNIVORES

by

Thomas C. Hornshaw

Representative mammalian wildlife species have not been designated as toxicological models for testing substances of environmental concern. The mink (Mustela vison) and the European ferret (M. putorius furo) have been suggested as representative mammalian species since they are among the most sensitive mammalian species to the toxic and reproductive effects of several substances. Also, as carnivores, these species are subject to the effects of bioaccumulation of lipophilic compounds. Therefore, dietary LC_{50} and reproduction tests were initiated with these species, using sodium monofluoroacetate (Compound 1080), *o*-cresol, tetramethylthiuram disulfide (thiram), and a polychlorinated biphenyl (Aroclor 1254) as test substances to develop protocols for these tests.

The results of the various tests demonstrate that mink and ferrets may be used in subacute and reproduction tests with a wide range of test substances, since the test substances used representated a wide range of solubilities, volatilities, acute toxicities, modes of action, and chemical classes. Since the tests were conducted indoors under controlled conditions, it is expected that the results should be reproducible in similarly equipped laboratories. Factors demonstrated in these tests

that may affect the determination of toxicity of a substance include the age of the animal at the beginning of a test and the carrier used to introduce the substance into the diet, while the diet's composition (as long as it meets the nutrient requirements of the test species) and the season of the year may have little or no effect on the results of the test. Dietary LC_{50} tests may be conducted with as few as 32 animals (3 test concentrations plus control, 8 animals per concentration) if an accurate acute oral LD_{50} is available, while reproduction tests may require 64 animals (3 test concentrations plus control, 16 animals per concentration) if unproven breeders are used.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

INTRODUCTION

Since the late 1960's-early 1970's, there has been an ever-increasing concern in this country about the effects of toxic compounds on man, his domestic animals, aquatic and terrestrial wildlife, and the environment. As this concern grew and as toxic compound-related incidents both large and small became more numerous, pressure mounted for a legal framework to better protect man and the ecosystem from the adverse effects of these compounds. As a result, several new laws were passed and some old laws were amended, and, in 1970, the Environmental Protection Agency (EPA) was created. This agency was charged with, among other things, the regulation of most toxic or hazardous chemicals. Toward this end, EPA has promulgated many rules and regulations, so that, today, most toxic or hazardous chemicals are regulated "from cradle to grave".

In the area of new compounds (or new uses of old compounds), the key to regulation of toxic or hazardous chemicals is toxicological testing of the compounds with an appropriate test species. Thus, on June 25, 1975, the Environmental Protection Agency published in the Federal Register (FR) proposed guidelines for registering pesticides in the United States, enumerating test procedures and data reporting requirements for evaluating the toxicity of compounds to man, domestic animals, and fish and wildlife. After review and modification, these guidelines were reproposed and published in the Federal Register

on July 10, 1978 and by the National Technical Information Service (EPA, 1983).

The species of choice for testing the toxicity of a compound to man and domestic animals, as specified in these guidelines, are common laboratory animals such as rats, mice, rabbits, guinea pigs, and hamsters. Fathead minnows, bluegill sunfish, and rainbow trout were suggested as representative fish species for toxicological testing, while the mallard duck and bobwhite quail were chosen as representative avian wildlife species. However, no representative mammalian wildlife species has been designated either under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) or under the Toxic Substances Control Act (TSCA).

In toxicological tests required on carnivorous species, the dog and cat have been the species of choice. However, paralleling the increasing concern about toxic compounds, there has been an increasing concern about using "pet" species in toxicological tests. And, for toxicological tests of all kinds, there has been an increasing awareness during this same time frame of the importance of using sensitive species as test animals.

Since no wildlife mammalian species has been designated under FIFRA or TSCA for toxicological testing, since dogs and cats have fallen into disfavor as carnivorous animal models in toxicological testing, and since the importance of using sensitive test species has been stressed, the mink (Mustela vison) has been suggested as a representative mammalian carnivore for

toxicological testing. In non-standard tests it has been shown to be among the most sensitive, if not the single most sensitive, mammalian species to polychlorinated biphenyl (PCB) toxicity. Research performed with mink was instrumental in setting U.S. water quality standards for PCBs (Aulerich and Bleavins, 1981). In subsequent studies, this species has been shown to be similarly sensitive to polybrominated biphenyls (PBBs) (Aulerich and Ringer, 1979), hexachlorobenzene (Bleavins et al., 1984), and sodium monofluoroacetate (Compound 1080) (present study), as well as to aflaxatoxins (Chou et al., 1976). Furthermore, since mink occupy a position high on the food chain, they may be subject to the effects of bioaccumulation of fat soluble compounds and thus, may be exposed to higher concentrations of a compound via the diet in the wild than species lower in the food chain.

This research was initiated to develop standard dietary LC_{50} and reproduction tests using the mink as a representative mammalian carnivore. Four test substances were chosen for use in developing the test protocols, sodium monofluoroacetate (Compound 1080), *o*-cresol, Aroclor 1254® (a PCB), and tetramethylthiuram disulfide (thiram), ranging from water soluble, to slightly water soluble, to lipid soluble, to practically insoluble, respectively. The test substances represented a wide range of acute oral LD_{50}s, ranging from highly toxic (Compound 1080) to practically non-toxic (Aroclor 1254), and also were representative of four different classes of chemical compounds. Where possible, the European ferret (Mustela

putorius furo), a closely related carnivore exhibiting high sensitivity to the same compounds as the mink, although generally not as sensitive as the mink, was tested and the results compared to those for mink using the same test procedures.

REVIEW OF LITERATURE

Legal Framework

In addition to FIFRA and TSCA, toxic or hazardous substances may be regulated under the Federal Food, Drug, and Cosmetic Act, the Federal Water Pollution Control Act, the Federal Hazardous Substances Act, the Occupational Safety and Health Act, and the Resource Conservation and Recovery Act of 1976. FIFRA provides for registration, re-registration, and classification of pesticides, while TSCA regulates most other toxic or hazardous substances not specifically covered by one of the other federal acts. This review will concentrate on the regulation of pesticides under FIFRA.

The Federal Insecticide, Fungicide, and Rodenticide Act (7U.S.C.136 et seq.) provides, under Section 3 of the Act, for the establishment of regulations for the registration, re-registration, and use classification of pesticides in the United States. The EPA is required to formulate Registration Guidelines for pesticides, which, after a suitable period of public comment, revision, further public comment, and final issuance, become a part of the Code of Federal Regulations (CFR). These Guidelines, which must specify the kinds of information to be required to support the registration of a

pesticide, were published for public comment in the Federal Register on June 25, 1975 and republished after revision on July 10, 1978 as Guidelines for Registering Pesticides in the United States, 40 CFR Parts 162 and 163.

The Act requires the EPA to register a pesticide if it can be determined that:

1. Its composition is such to warrant the proposed claims for it;
2. Its labelling complies with the requirements of the Act;
3. It will perform its intended function without unreasonable adverse effects on the environment; and
4. When used in accordance with widespread and commonly recognized practices, it will not cause unreasonable adverse effects on the environment.

Toward these ends, various sections of the Guidelines deal with product performance, label development, product chemistry, and hazard evaluation. Of particular interest is Section 162.8, dealing with hazard evaluation.

Section 162.8 addresses the toxicological information necessary to assess the hazard of a pesticide to man and domestic animals, and to fish and wildlife. The data required by this Section enables the EPA:

1. To determine whether or not to approve a registration for a pesticide, and if approved, whether it should be classified for general or restricted use;

2. To determine whether the proposed labelling contains adequate warnings to protect pesticide users, and exposed humans, domestic animals, and fish and wildlife;
3. To determine residue tolerances for the protection of consumers of exposed foods; and
4. To determine conditions under which farm workers may safely re-enter treated fields.

Required tests for all pesticide registrations include acute oral LD_{50}, acute dermal LD_{50}, primary dermal irritation, and primary eye irritation tests. For pesticides which may present a hazard to man and domestic animals, a series of conditional tests, depending on the physical and chemical nature of the pesticide, its proposed use, its expected residue on foods, and other "triggers", are enumerated. These tests range from acute inhalation LC_{50} to subacute oral and dermal to chronic feeding tests. For pesticides which may present a hazard to fish and wildlife (i.e., pesticides that will be used outdoors or that may contaminate water or other environmental resources), avian acute oral LD_{50} and subacute dietary LC_{50}, fish acute LC_{50}, and aquatic invertebrate acute LC_{50} tests are also required. These tests constitute the first level, or tier, of tests for all pesticides. A second tier of longer term tests are required for certain pesticides intended for outdoor use, depending on the toxicity found in the first level tests, the probable environmental exposure calculated from the environmental chemistry data and the

proposed use patterns, the species likely to be exposed to the pesticide, the probable routes of exposure of these species, and the persistence and expected degree of bioaccumulation of the pesticide and/or its metabolites. These second tier tests include subacute, reproduction, and/or chronic feeding studies. Finally, if the second level tests arouse suspicions that unreasonable adverse effects on fish or wildlife may occur, a third tier of simulated or actual field tests may be required.

As mentioned previously, required tests for the registration of a pesticide to be used outdoors include acute studies on rats, birds, fish, and aquatic invertebrates and a subacute dietary test on birds, while no tests so far are required on wildlife mammals. It was at first thought in the original issuance of the Registration Guidelines (FR, June 25, 1975) that the rat acute oral and, if performed, the subacute dietary and reproduction studies would be sufficient to assess the hazard of most pesticides to wildlife mammals, and that if unique conditions of proposed usage were identified as potential hazards to certain mammals, acute oral and subacute dietary studies could be required for these mammals. Among the approximately 80 sets of public comments received regarding this first issuance were several replies questioning the adequacy of protection provided to wildlife under the Guidelines. Many of the questions raised were addressed in the reproposal of three sections of the Guidelines (FR, July 10, 1978), although this document still contains no mention of

specific requirements for dietary LC_{50} or reproduction tests for wildlife mammals. Currently, if concerns exist about the effects of a proposed use of a pesticide on wildlife mammals, dietary LC_{50} and reproduction tests are required, following protocols established for these tests for man and domestic animals (i.e., using rats and one other non-rodent).

Test Protocols

Standard test protocols for rats have been in existence for two decades or more. Acceptable protocols for the study of acute oral (Hagan, 1965), subacute dietary (Fitzhugh and Schouboe, 1965), chronic (Barnes and Denz, 1954; Fitzhugh, 1965), and reproductive (Oser and Oser, 1956) toxicity are listed in the Registration Guidelines. The Guidelines also specify acceptable protocols for acute LC_{50} tests for fish and aquatic invertebrates (Stephan, 1975) and birds (Anonymous, 1968), and subacute dietary LC_{50} and reproduction tests for birds (FR, July 10, 1978).

The subacute dietary LC_{50} test for birds is designed to test the effects of a substance applied to plant matter in the field. It allows ingestion and degradation of the substance, as well as absorption and metabolism over the five days of dietary exposure. The test also allows exposure to the compound via inhalation from the daily diet, and dermal exposure from contact of the feet and face with the daily diet. A 3-day withdrawal period, during which the birds are given untreated feed, allows for observation of delayed mortality or recovery. The test specifies the use of one

aquatic species, preferably the mallard, and one upland species, preferably the bobwhite quail, and requires that hatchlings be used. Hatchlings are used to ensure that (1) the substance is tested at the most sensitive period of the animal's life, and (2) the substance will be tested via the diet, since very young birds cannot survive five days without eating. In addition to an estimate of the dietary concentration of the substance lethal to young birds, this test also may provide (1) an estimate of the maximum concentration of the substance tolerated in the daily diet, (2) signs of intoxication not found in the acute LD_{50} test, (3) indications of the target organ or organ system of the substance, and (4) indications of behavior changes, including rejection of the treated diet.

The avian reproduction test should be used when the substance or its metabolite(s) persists in the environment or when wildlife are subjected to repeated or continual exposure. Specifically, these studies are required when:

1. The pesticide or major metabolites or degradation products is persistent in the environment;
2. The pesticide or major metabolites or degradation products is stored or accumulated in plant or animal tissues;
3. The pesticide is intended for use under conditions in which birds may be subjected to repeated or continued exposure to it; or
4. Other test information indicates reproduction may be affected adversely.

The test also specifies the use of one aquatic and one upland species, as in the dietary LC_{50} test. It requires a period of exposure prior to egg-laying, and provides data on a longer term of exposure than the dietary LC_{50} as well as effects of the substance on the reproduction of adult birds and survival of the young.

A short-term dietary LC_{50} test has been proposed for small mammals (McCann et al., 1981), based on the avian dietary LC_{50} test. This test substitutes immature rats (90-120 g) for hatchling birds, using the same experimental protocol as that described for the avian test, with the exception that the withdrawal period for the rats was 9 days, and the housing was appropriate for rats. Dietary tests were conducted on 21 pesticides, and dietary LC_{50}s were calculated for 17 of them. Where duplicate tests were run on a pesticide, good reproducibility was usually obtained.

A protocol is specified by the Canadian Environmental Protection Service (1975) for testing the effects of chemicals on mink reproduction. This protocol describes normal reproduction in the mink, housing and lighting conditions adequate for conducting a reproduction test with mink, two diets suitable for mink and methods for incorporating a test chemical into the diet, and an experimental design utilizing 5 males and 15 females in each of 2 or 3 test concentrations plus a similarly-sized control group. Parameters measured in this test include adult mortality, weight of adults at the beginning and end of the test, feed consumption per week, number mated,

number whelped, length of gestation, number of males with motile sperm, number of implantation sites (which persist in mink well past parturition, and can be counted at 3 weeks, the suggested time of termination), number of resorbed embryos, number and weight of newborn (kits) born alive and dead, deformed kits, number and weight of kits alive at 1, 2, and 3 weeks post-partum, sex ratio of kits, organ weights of adults at 3 weeks post-partum, and pathology. The length of exposure to the test chemical is not specified in this protocol.

It is important to note that, when conducting a toxicological test with a wildlife species instead of standard, often highly inbred laboratory test species, certain inherent factors will contribute to a greater degree of variability in the test. Age, sex, species and strain, and factors associated with unnatural conditions, such as diet, social (or anti-social) interactions, and stress are all known to influence toxicological test results (Hurni, 1970). The age of the animal at the beginning of a test can be very important. Hill and Camardese (1981) noted an increase in the subacute dietary LC_{50} with increasing age of the test birds in 60 subacute tests with four different classes of pesticides. It has also been shown that carriers can affect the dietary toxicity of a substance in the avian LC_{50} test. Gile *et al.* (1983) noted carrier-related differences in the toxicities of some pesticides tested with distilled water, corn oil, propylene glycol, or carboxymethylcellulose as the carrier. Carrier-related differences in feed consumption and body weight gain were also noted in this study.

Test Substances

Sodium Monofluoroacetate (Compound 1080)

Compound 1080 (analytical reagent; Pfaltz & Bauer, Stamford, CT) is a white, odorless, nearly tasteless hygroscopic powder, soluble in water and nearly insoluble in organic solvents. It has no vapor pressure. It has a molecular weight of 100.03. Its molecular formula and structure are:

$C_2H_2O_2FNa$; $FCH_2C(=O)O^-Na^+$

Compound 1080 is a potent rodenticide and predacide approved for pest control in several countries and recently re-approved for coyote control in the United States. In the past in the U.S., it has been used for coyote, gopher, ground squirrel, prairie dog, and field mouse control. However, it's extreme toxicity to non-target species and the possibility of secondary poisoning led to its restriction to rodent control by licensed exterminators until its recent re-registration for coyote control. It was first synthesized by Swarts (1896). Its synonyms and trade names include: SMFA; Gifblaar poison; ten-eighty; "1080"; sodium fluoroacetate; and fratol.

During World War II, when the major sources of red squill, thallium, and strychnine were cut off, intense research efforts on both sides of the Atlantic were directed at finding an effective rodenticide to replace those in short supply. SMFA was among ten compounds selected for study by the Fish and Wildlife Service, and was given the laboratory acquisition number 1080 (hence the name Compound 1080). The results with

laboratory rats were encouraging, and extensive field trials further showed SMFA's potential for pest animal control. Independently of this wartime research, Marais (1944) identified monofluoroacetic acid (FCH_2COOH) as the toxic agent of the Gifblaar plant, Dichapetalum cymosum (Hook), of South Africa, which had long been associated with livestock poisonings in South Africa. Monofluoroacetic acid has since been identified as the toxic agent in certain plants of the genera Palicourea of South America and Gastrolobium, Oxylobium, and Acacia of Australia (Hall, 1972).

Monofluoroacetate is itself an innocuous compound. Peters (1952) showed that SMFA must first be converted to fluorocitrate (which he termed the "lethal synthesis") in mitochondria before a toxic response will be elicited. This conversion occurs in the Krebs cycle, similar to the synthesis of citrate from acetic acid. Monofluoroacetate is first converted to monofluoroacetic acid, which, in the presence of adenosine triphosphate (ATP), combines with coenzyme A (CoA) to form fluoroacetyl-CoA. Fluoroacetyl-CoA then combines with oxaloacetate and water in the presence of "condensing enzyme" to form fluorocitrate. Whereas citrate continues through the Krebs cycle, fluorocitrate does not, constituting a block in the cycle which leads to a buildup of citrate (Buffa et al., 1973). Fluorocitrate specifically inhibits aconitase (Morrison and Peters, 1954), which is responsible for catalyzing the conversion of citrate to cis-aconitate, and succinate dehydrogenase (Fanshier et al., 1964), which is responsible

for catalyzing the conversion of succinate to fumarate. The resulting accumulation of citrate leads to interference with cellular respiration, permeability barriers, and energy production, and ultimately cellular function. Citrate tends to accumulate in cells of metabolically active tissues, such as kidneys, liver, and testes (Buffa and Peters, 1950). For example, Buffa and Peters (1949) found levels of citrate up to 70 times normal in kidneys from rats treated with SMFA.

Eventually, the cellular disruptions caused by fluorocitrate cause organ or organ system disruptions. There is a latent period of from ½ to 2½ hours, during which vomiting usually occurs. This latent period is a product of the "lethal synthesis", since time is required for: (a) hydrolysis of SMFA to monofluoroacetic acid; (b) synthesis of fluorocitrate from monofluoroacetic acid; and (c) fluorocitrate to disrupt cellular functions enough to produce gross clinical signs. When clinical signs begin, the onset is acute and the course rapid.

The clinical signs of SMFA poisoning vary markedly among species. Man, monkeys, horses, and rabbits suffer cardiac arrhythmias and ventricular fibrilation, while canids suffer intermittent excitation and depression of the central nervous system (CNS), with death ultimately due to convulsions or subsequent respiratory paralysis. Animals that die from SMFA poisoning develop rigor mortis very rapidly, with the limbs usually rigidly extended (Edwards, 1977). In general, herbivores die as a result of cardiac disorders, carnivores as a

result of CNS disorders, and omnivores as a result of both types of disorders. Cold-blooded animals are less sensitive than warm-blooded ones to SMFA (Chenoweth, 1949).

The LD_{50} for SMFA also varies markedly among species, ranging from 0.05 mg/kg for the dog (Buch et al., 1976) to over 500 mg/kg for the South African clawed toad (Chenoweth, 1949). The LD_{50} does not appear to be markedly affected by age in the cow (Robinson, 1970), but young mallard ducks appear to be approximately one-half as sensitive as older ducks (Hudson et al., 1972). The estimated LD_{50} for man ranges from 0.7 mg/kg (Kaye, 1970) to 10 mg/kg (Harrison et al., 1952). The LD_{50} for mink has been reported to be approximately 1 mg/kg (Robinson, 1953), and 1.41 mg/kg for ferrets (Tucker and Crabtree, 1970).

SMFA may be absorbed through the gastrointestinal tract, respiratory tract, mucous membranes, and abraded skin, but not through intact skin (Edwards, 1977). Its toxicity is approximately the same regardless of the route of administration (Chenoweth and Gilman, 1946; Ward and Spencer, 1947), and the oral toxicity is also approximately the same whether the carrier is water, meat, grain, oil, gum acacia suspension, or gelatin capsule (Atzert, 1971).

There are no pathognomonic lesions associated with SMFA poisoning. There is a generalized cyanosis of mucous membranes and other tissues, and the liver and kidneys usually exhibit marked congestion. The heart may be flaccid and may show subepicardial hemorrhages. Hyperglycemia and elevated citrate

levels, especially in the kidneys, will usually be present (Edwards, 1977).

Several secondary biochemical effects have been reported with SMFA poisoning. The accumulation of citrate has been shown to inhibit phosphofructokinase (PFK) in the heart both *in vitro* (Bowman, 1964) and *in vivo* (Williamson *et al.*, 1964). The inhibition of PFK causes a decrease in glucose metabolism, resulting in a further decrease in energy production (Bowman, 1964), and a concurrent increase in free glucose, resulting in hyperglycemia (Elliott and Phillips, 1954). Ketonemia has also been reported (Engel *et al.*, 1954). Roy (Shapira) *et al.* (1980) have found a significant decrease in ionized calcium in the blood of SMFA-poisoned cats (citrate is a potent chelator of calcium ions), which contributes to the pathogenesis of SMFA poisoning.

Repeated sublethal doses of monofluoroacetate can increase the tolerance to a subsequent challenging dose in some species. Golden eagles (Atzert, 1971), rats (Miller and Phillips, 1955), mice (Quin and Clark, 1947), and rhesus monkeys (Chenoweth, 1949) have been shown to acquire a tolerance for monofluoroacetate, although only for slightly increased challenging doses and for a limited period of time. Conversely, repeated sublethal doses of SMFA have accumulated in some species until they reached lethal levels. Dogs, guinea pigs (Foss, 1948), rabbits (Rowley, 1963), and mallards (Tucker and Crabtree, 1970) have exhibited this response.

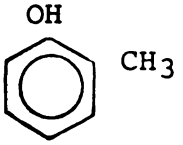
SMFA has been shown to cause testicular atrophy, decreased testicular ATP concentration, and seminiferous tubular atrophy in rats at doses of 6.6 and 20 ppm in their drinking water for 7 days, and altered appearance and decreased numbers of spermatids even at the lowest dose of 2.2 ppm (Sullivan et al., 1979).

Secondary poisoning of dogs eating the carcasses of animals poisoned by SMFA has been reported (Meldrum et al., 1957). Such instances are quite rare, since the poison has been diluted. Of greater concern is the ingestion of the vomitus of an animal poisoned by SMFA by another animal, in which case a toxic dose may be consumed.

O-cresol

O-cresol (analytical reagent; Pfaltz & Bauer, Stamford, CT) is a light to dark brown volatile solid or liquid (mp = 30°C). It is slightly soluble in water (2.9 mg/l at 46°) and is miscible with alcohols, chloroform, and ether. Other physical characteristics include: bp = 191-192°; d_4^{20} = 1.047; flash point = 81-83°; vp = 1 at 36.6°. It has a molecular weight of 108.14. Its molecular formula and structure are:

C_7H_8O;



O-cresol is used as a disinfectant, like phenol, often in a mixture with *m*-cresol and *p*-cresol known as cresyl, as a solvent, and as an intermediate in other chemical processes. A major use of *o*-cresol is in the manufacture of the herbicides

dinitro-*o*-cresol (DNOC) and 2-methyl-4-chlorophenoxyacetic acid (MCPA) (Klapproth, 1976). The cresols are derived from petroleum or coal tar acids (McNeil, 1965), and they are also components of the phenolic wastes of certain manufacturing processes, and as such pose a threat to aquatic species both directly as a toxicant and indirectly as a behavior modifier (Buikema et al., 1979). Synonyms of *o*-cresol include: 2-methylphenol; *o*-cresylic acid; 2-cresol; *o*-hydroxytoluene; and *o*-oxytoluene.

One of the earliest uses of *o*-cresol was in the disinfectant Lysol. Lysol originally contained phenol as its active ingredient when it was introduced in 1860, but a new formulation, introduced in 1872, contained the cresols (6-50%) in glycerin or saponified linseed oil. Cresol was removed from Lysol in the U.S. in 1951 and replaced by *o*-phenylphenol (U.S. Dept. of Health, Education, and Welfare, 1978). Attempts at suicide involving ingestion of 4-120 ml of the Lysol preparation with cresol have revealed the signs of acute intoxication in humans, which include abdominal pain and cramps, vomiting, burning sensation of the mouth, throat, esophagus, and epigastrium, cyanosis, unconsciousness, and respiratory failure (Isaacs, 1922).

In animals, clinical signs of *o*-cresol intoxication include twitching, pupil contraction followed by dilation, some salivation, and marked dyspnea upon original exposure in all species tested. In addition, rats showed continued twitching

and uncoordinated movements until death. Cats eventually became lethargic or comatose before death, while rabbits behaved similar to cats, but also exhibited asphyxial convulsions just before death. Signs and symptoms of *o*-cresol intoxication, in general, resemble those for phenol, but the convulsions are less severe while the signs of weakness and/or depth of coma are more severe (Deichmann and Witherup, 1944). The main effect of *o*-cresol exposure is CNS depression, and death often results from respiratory failure. The cresols are also corrosive and can cause chemical burns and dermatitis. Absorption through the skin is rapid (Sax, 1963). Exposure to cresol vapors can lead to inflammation of mucous membranes, nervous irritation, and kidney damage (Deichmann and Witherup, 1944). The cresols are readily metabolized, being conjugated in the body to form glucuronides and sulfates (Bakke and Scheline, 1970).

Rat oral LD_{50} estimates include values of 121 mg/kg for male white rats (Bio Fax Techniques, 1969) and 1350 mg/kg (Deichmann and Witherup, 1944) and 1470 mg/kg (Uzhdavini et al., 1974) for unspecified strains of rats. The mouse oral LD_{50} has been reported to be 344 mg/kg (Uzhdavini et al., 1974). *O*-cresol is as toxic by the dermal route as by the oral. Uzhdavini et al. (1974) have reported the rat dermal LD_{50} to be 620 mg/kg, while LD_{50} estimates for rabbits include values of 890 mg/kg (Vernot et al., 1977) and 1380 mg/kg (Bio Fax Techniques, 1969). The clinical signs reported by Bio Fax Techniques included skin irritation,

hyperemia, convulsions, and tremors. The inhalation LC_{50} for mice has been reported to be 0.179 mg/l (= 179 mg/m^3) (Uzhdavini et al., 1974), but the conditions reported in this study included a degree of saturation of the air such that dermal contact was also likely.

O-cresol is much more toxic to aquatic species. Pickering and Henderson (1966) reported 96-hr LC_{50}s of 20.78, 12.55-13.42, 23.25, and 18.85 mg/l for bluegills, fathead minnows, goldfish, and guppies, respectively. Concentrations much lower than these values may lead to avoidance behavior by fish and/or tainting of edible flesh (Buikema et al., 1979).

Very few longer-term studies have been conducted with *o*-cresol. Savolainen (1979) reported no effect on the body weights of rats receiving 0.3 g/l *o*-cresol in their drinking water for 20 weeks. The ingested cumulative dose exceeded the acute oral LD_{50} by the fourth week in this study. Biochemical effects of this exposure on the brain were inconspicuous. The cresols have been shown to be tumor promoters in mouse-skin assays, causing an increase in papillomas but not in carcinomas (Boutwell and Bosch, 1959). Reproduction tests with *o*-cresol are lacking in the literature (U.S. Dept. of Health, Education, and Welfare, 1978).

Workplace exposure to cresol has been regulated since 1952. The recommended Threshold Limit Value (TLV) was set at 5 ppm (American Conference of Governmental Industrial Hygienists, 1952). This value was expressed as a Time Weighted Average (TWA), being 22 mg/m^3 for an 8-hour workday and 40-hour work-week, in 1956 (American Conference of Governmental Industrial

Hygienists, 1956). A "Skin" notation was added in 1961, acknowledging the importance of dermal exposure in toxicity and the rapid absorption of cresol through the skin (American Conference of Governmental Industrial Hygienists, 1961). The U.S. Dept. of Health, Education, and Welfare (1978) has proposed lowering the TWA to 10 mg/m^3 for a 10-hour workday and 40-hour week, and has proposed labeling and precautionary measures for workers exposed to cresol.

The TSCA Interagency Testing Committee has recommended that cresol be a priority substance to be tested for carcinogenicity, mutagenicity, and teratogenicity (Federal Register, October 12, 1977).

Thiram

Thiram (analytical reagent; Pfaltz & Bauer, Stamford, CT) is a fine light green powder, insoluble in water, slightly soluble in alcohol (< 0.2%), ether (< 0.2%), acetone (1.2%), and benzene (2.5%), and more soluble in chloroform. It has a melting point of 70° and a density of 1.30. It has a molecular weight of 240.44. Its molecular formula and structure are:

$$C_6H_{12}N_2S_4;\quad (CH_3)_2N\text{-}\overset{\overset{S}{\|}}{C}\text{-}S\text{-}S\text{-}\overset{\overset{S}{\|}}{C}\text{-}N(CH_3)_2$$

Thiram, a dithiocarbamate, is used as a rubber accelerator and vulcanizer, as a seed disinfectant, as a fungicide, and as a bacteriostat in soap (it is the main ingredient of the antiseptic spray Nobecutan). It is also used, when suspended in

water, as a paint or spray on ornamental plants to prevent gnawing. It is prepared by the oxidation of sodium dimethyldithiocarbamate by hydrogen peroxide or iodine. Its synonyms and trade names include: tetramethylthioperoxydicarbonic diamide; bis (dimethylthiocarbamoyl) disulfide; bis (dimethylthiocarbamyl) disulfide; tetramethylthiuram disulfide; TMTD; Thiurad; Thiuram; Thiosan; Thylate; Tiuramyl; Thiuramyl; Puralin; Fernasan; Nomersan; Rezifilm; Pomarsol; Tersan; Tuads; Tulisan; Arasan; TTD; and disulfuram.

In acute human intoxication, thiram causes liver and kidney injury, and can cause brain damage (Sax, 1963). It is an irritant of the eye, nose, and throat, and can cause dermatitis. Sub-acute exposure can cause nausea, vomiting, diarrhea, hyperexcitability, weakness, or ataxia (Anon., 1975). The toxicity is greater in the presence of fats, oils, and fat solvents (Merck Index, 1976).

The mammalian acute oral LD_{50} ranges from 350 mg/kg for rabbits (Matthiaschk, 1973) to 4000 mg/kg for male rats and mice (Lee et al., 1978). Sex differences have been reported for rats, with an LD_{50} of 4000 mg/kg for male CD rats and 1900 mg/kg for females (Lee et al., 1978). Strain differences are also apparent for rats, since Gaines (1969) has reported an oral LD_{50} of 620-640 mg/kg for male and female Sherman rats. Manifestations of acute exposure in animals include severe effects on the nervous system, with ataxia, incoordination (especially of the lower extremities), and clonic convulsions being reported (Fishbein, 1976).

In subacute exposure, whether by diet or by mouth, thiram has repeatedly been shown to significantly decrease feed consumption and body weight gain. Rats (Lee et al., 1978; Lowy et al., 1980), chickens (Waibel et al., 1957; Rasul and Howell, 1974), turkeys, and geese (Waibel et al., 1957) have been reported to reduce feed consumption upon exposure to ⩾ 225 mg/kg (in diet), 178 mg/kg (po), ⩾ 400 mg/kg (in diet), and ⩾ 150 mg/kg (in diet), respectively. Further manifestations of toxicity in these studies included: tubular degeneration of the testes with atypical spermatids in the epididymis of rats fed 2500 ppm for 13 weeks (Lee et al., 1978); decreased weight of kidneys, epididymal and perirenal fat pads, testes, and seminal vesicles, and increased weight of livers of rats fed ⩾ 225 ppm for 29 days (Lowy et al., 1979; 1980); abnormal gait in four of seven chicks due to enlarged epiphyses of the long bones, and degenerative changes in the epithelium of the seminiferous tubules, at a dose of 178 mg/kg po for 18 weeks, and mortality of five of five adults within 3 weeks exposure to the same dose (Rasul and Howell, 1974); and leg weakness and enlargement of the hock joint in chicks fed ⩾ 150 ppm, poults fed 400 or 800 ppm, and goslings fed 400 or 800 ppm for 3 weeks (Waibel et al., 1957).

Chronic and/or reproduction studies have revealed other adverse effects of exposure to thiram. Lee and Peters (1976) found further evidence of neurotoxicity in rats. They have reported demyelination and degeneration of peripheral nerves, with secondary degeneration of muscle, and behavioral

deficiencies in gait and jump tests, and hyperactivity in an 80-week chronic study with rats fed 100, 400, or 1000 ppm thiram in the diet. They also found decreased weight gain in males at all doses and females at 400 and 1000 ppm, ataxia in 8 of 24 females fed 1000 ppm, alopecia in some animals of both sexes at 1000 ppm, and increased weights of thyroid and testes in high-dose males and liver, spleen, kidney, thyroid, ovary, and brain in high-dose females. The spleen weights of females fed the 400 ppm diet were also increased. No changes in blood chemistry were reported in this study.

Adverse effects on several reproductive indices have been reported following exposure to thiram. Robens (1969) noted increased resorption of hamster fetuses at oral doses of 125 and 250 mg/kg, given from days 5-15 of gestation, and low birth weight and viability of surviving fetuses. Short et al. (1976) found, in addition to the tubular degeneration of testes of male rats already noted (Lee et al., 1978), that female rats fed 400 ppm thiram in the diet for 14 days prior to mating experienced a significant decrease in the number of implants and the number of pups per female. They also found that, at a dose of 2000 ppm, only 1 of 20 females mated and 5 of 20 had died by 4½ weeks. The cause of the failure of these females to mate was determined to be a prolonging of the diestrus phase of the estrus cycle. Extension of the estrus cycle at the expense of the resting cycle has been reported for albino rats exposed to 3.8 mg thiram/m^3 air for 5 hr/day, 5 days/wk, for 4.5 months (Davydova, 1973), and inhibition of

ovulation has been reported in bobwhite quail at doses as low as 8.8 mg/kg/day (Wedig et al., 1968).

In further studies, Short et al. (1976) reported only 33% survival of female rats given 200 mg/kg/day of thiram po during days 6-15 of gestation, decreased number of implants and 100% resorption of fetuses at 164 and 200 mg/kg, decreased number of pups/female at 136, 164, and 200 mg/kg, and decreased birth weight for pups in all treatment groups (40, 90, 136, 164, and 200 mg/kg). In a similar study with mice given 100 or 300 mg/kg/day po during days 6-14 of gestation, these authors reported 78% survival of females at the high dose, and no changes in weight gains of adults, litter sizes, incidence of resorption, or birth weight. In peri- and postnatal studies with rats fed 300 or 1000 ppm thiram in the diet from day 16 of gestation through day 21 of lactation, they found no effect at 300 ppm and decreases in viability and growth at 1000 ppm. All offspring of dams fed 1000 ppm were dead by day 21 of lactation. A cross-fostering study conducted simultaneously determined that thiram is most toxic to the pups during the nursing period, suggesting that it can be transferred via the milk.

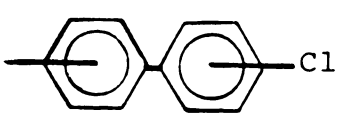
Thiram has been shown to be teratogenic in hamsters (Robens, 1969), rats, and mice (Short et al., 1976). Major anomalies noted in hamsters were cranial abnormalities, fused ribs, and limb and tail abnormalities. Occasional heart anomalies and missing kidneys were also noted. The major anomaly found in rats and mice was hydrocephalus, and cardiovascular defects were also noted in mice. The tumor incidence

in rats fed 100, 400, or 1000 ppm thiram in the diet for 80 weeks was found not to be different from controls (Lee et al., 1978).

A dietary no-effect level of 48 ppm has been reported for thiram in the rat (WHO, 1965). This value has been confirmed by Lowy et al. (1979; 1980), who used a log-probit model to extrapolate a no-effect level of 38 ppm from a 29-day feeding study. They determined that the most sensitive parameters, in the rat, for measuring toxicity were the weights of the epididymal and perirenal fat pads, while the least sensitive parameter was kidney weight.

Aroclor 1254

Aroclor 1254 (electrical grade, lot #KB-05-612; Monsanto Co., St. Louis, MO) is a clear to yellowish viscous, oily liquid, nearly insoluble in water and soluble in organic solvents. It is a mixture of over 100 individual chlorobiphenyls, made by reacting biphenyl with chlorine to produce a product that is 54% chlorine by weight. By virtue of its high chlorine content, Aroclor 1254 is virtually non-flammable. Aroclor mixtures have variable molecular weights, since they are mixtures of many congeners, and are usually designated by the percent chlorine in the mixture (i.e. Aroclor 1254 contains 54% chlorine). Its molecular formula and structure are:

$C_{12}H_{10-n}Cl_n$; 

Currently, Aroclor 1254 is restricted to use in existing electrical transformers as an insulating fluid, and is being phased out. Its manufacture is prohibited in the U.S. Due to its excellent heat-resistance, it has been used widely in the past for many applications requiring non-flammable liquids, including electrical transformers and capacitors, as a heat exchange medium, as plasticizers in paints, as hydraulic fluids, and for many other uses. Due to its widespread use over a long period of time (over 40 years), the PCBs have become nearly ubiquitous in the environment (Peakall, 1975). Its synonyms and trade names include: PCBs, polychlorobiphenyls; Kanechlor, Clophen, and Phenochlor.

There are numerous theories of the mode of action of PCB toxicity. It has been shown to cause liver enlargement and induction of microsomal enzymes in almost all species tested. Decreased feed consumption and severe loss of body weight (sometimes called the "Wasting Syndrome") has also been associated with acute or subacute PCB exposure in most species. The nature of the toxicity of PCBs is still an area of intense investigation. Clinical signs of PCB intoxication are often species-specific, and can include inanition, weight loss, liver enlargement, edema, hydropericardium and/or ascites, chloracne, hyperkeratinization, gastric ulcers, and lethargy or unconsciousness. The PCBs have been the subject of numerous reviews, monographs, and symposia, and the reader is referred to Hutzinger et al. (1974) and to Kimbrough (1974; 1980), Nicholson and Moore (1979), and Poland and Knutson (1982)

for comprehensive reviews of the chemistry and biology, respectively, of these compounds. This review will focus mainly on the effects of PCBs on mink and ferrets.

Mink and ferrets appear to be among the most sensitive species to PCB toxicity. The first indication of this sensitivity to PCBs came in the mid-1960s, when mink ranchers began to note reproductive problems with mink fed diets containing Great Lakes fish (Hartsough, 1965). Research by Ringer, Aulerich, and associates in the early 1970s ultimately isolated PCBs as the causative factor in these reproductive problems (Aulerich et al., 1970; Aulerich et al., 1971; Ringer et al., 1972; Aulerich et al., 1973). Further research by this laboratory showed that consumption of 2 or more ppm of Aroclor 1254 in the daily diet for 8 months (Aulerich and Ringer, 1977) or 5 or more ppm of Aroclor 1242 for 9 months (Bleavins et al., 1980) resulted in severe reproductive problems in mink. Recent research (Hornshaw et al., 1983) has shown that PCBs which have been "metabolized" by another animal before being fed to mink are even more detrimental to mink reproduction, a response also noted by Platonow and Karstad (1973).

The PCBs are only slightly toxic to mink on an acute oral basis. The acute oral LD_{50}s for Aroclors 1221, 1242, and 1254 are approximately 1, 3, and 4 g/kg (Aulerich and Ringer, 1977), and are slightly less than those reported for the rat (4-10 g/kg: Kimbrough et al., 1978). On a subacute or chronic basis, however, the PCBs are highly toxic to mink. Concentrations of Aroclors 1242 and 1254 only slightly higher than those which caused reproductive problems resulted in outright mortality in mink during the 8-9 months of these

studies, leading to dietary LC_{50} estimates of 8.60 and 6.65 ppm for these two Aroclors (Ringer et al., 1981). Clinical signs of toxicity noted were: enlarged livers, as found in most species following exposure to PCBs, inanition, reduced growth rate, enlarged kidneys, and hemorrhagic gastric ulcers, which have also been observed in monkeys (Allen and Norback, 1973) and swine (Hansen et al., 1975). A further indication of the extreme sensitivity of mink to certain components of the Aroclor mixtures is the result of a recent study in which 50% of the female mink fed a diet containing 50 ppb of the PCB congener 3,4,5,3',4',5'-hexachlorobiphenyl died during 6 month's exposure (unpublished research, Michigan State University).

The ferret is also highly sensitive to PCB toxicity, although not as sensitive as the mink. Studies in which the two species have been tested at comparable concentrations of PCB mixtures have shown the ferret to be 2-4 times less sensitive than the mink. Ferrets fed up to 20 ppm of Aroclor 1242 in the diet survived 9 month's exposure, whereas 15 of 15 mink fed this same diet died during 9 month's exposure. Mink mortality was noted at concentrations as low as 5 ppm in this study (Bleavins et al., 1980). Similarly, ferrets fed 30% Great Lakes fish in the diet experienced no reproductive problems, whereas mink suffered total reproductive failure at this level (Ringer, 1983).

Several subacute or chronic dietary studies with PCBs with other species further illustrate the sensitivity of mink and ferrets to PCBs. 1000 ppm of Aroclor 1254 in the diet of

rats resulted in 50% mortality in an 8-month study (Kimbrough et al., 1972) and 100% mortality by 53 days in another study (Tucker and Crabtree, 1970). One of six rhesus monkeys fed 25 ppm of Aroclor 1248 for 2 months died after a 2-month withdrawal period, with a total PCB intake of approximately 400 mg (Allen, 1975). Five of five mice fed 10 ppm of 3,4,5,-3',4',5'-hexachlorobiphenyl died after 36-47 days of exposure (Kimbrough et al., 1978), a dose 200 times greater than the concentration that caused 50% mortality in mink.

MATERIALS AND METHODS

At the beginning of each test a log book was kept, in which the name(s), reason(s) for entry, and time(s) of entry and departure of all personnel entering the test rooms were recorded. Also, the following were posted at the entrance to each test room: name(s), address(es), and telephone number(s) of person(s) to contact in case of emergency; compound(s) and concentrations being tested; and appropriate warning labels. The following materials and methods were used in four experiments (I. Compound 1080; II. *O*-cresol; III. Thiram; and IV. Aroclor 1254), unless specifically noted in the text.

Test Animals

The species used were the ranch mink (Mustela vison) and the European ferret (M. putorius furo). The mink were the standard dark color variety, and the ferrets were the agouti variety. Both species were from stock raised at the Michigan

State University Experimental Fur Farm. All animals were immunized (at approximately 8 weeks of age) against botulism, virus enteritis, and canine distemper. Each animal was assigned a unique identification number. Only alert, active, injury-free animals were used in the tests.

Housing

Test animals were housed indoors (see Figure 1) in two adjoining 14.0 x 6.7 m rooms, with a maximum of 64 animals housed per room. Each animal was kept in a 61 (W) x 76 (L) x 46 (H) cm cage (see Figure 2) constructed of 2.54 x 2.54 cm galvanized steel welded wire on the front, back, and top, with the bottom wire being vinyl coated. Solid fiberglass dividers separated adjacent cages, to prevent cross-contamination and incidental contact between the animals. The cages were suspended 61 cm above the floor. Each cage was provided with a 15.2 x 22.9 cm feed grid on the top of the cage and an aluminum water cup attached to the front. For reproduction tests, all cages of female animals were equipped with 1.27 x 1.27 cm mesh vinyl-coated false bottoms prior to whelping, to prevent the kits from falling through the bottom of the cages. The wire fabric used in the front and back of the cages contained a "Kit Guard", which consists of a 1.27 x 2.54 cm mesh on the lower 10.2 cm of the fabric, to prevent the newborn from falling out of the cage. All cages of female animals were also equipped prior to whelping with 38.1 (L) x 27.9 (W) x 26.7 (H) cm wooden nest boxes. The nest boxes were partially filled with a sugar cane product used as nesting

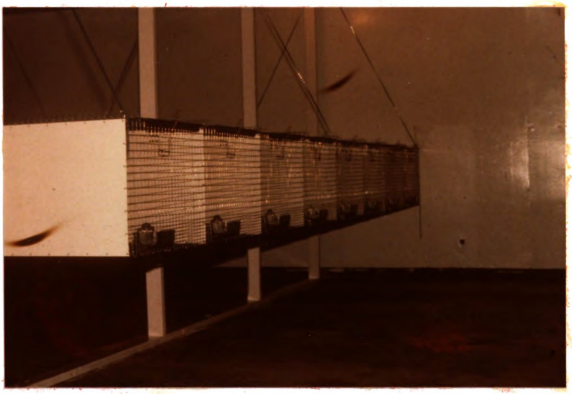


FIGURE 1. Housing for test animals.

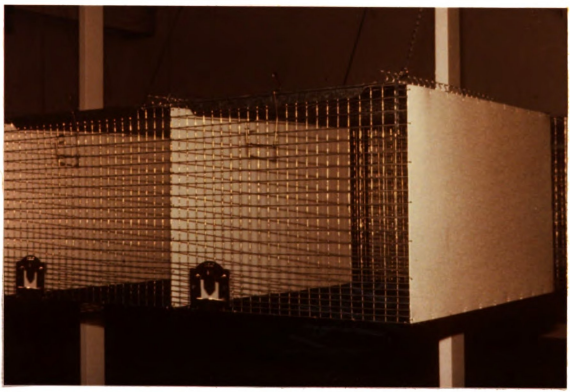


FIGURE 2. Typical cage for test animals.

material. Wood shavings were spread below the cages before the start of all trials and were changed monthly. The walls and ceilings of the test rooms were painted with an epoxy-based primer coat and an oil-based final coat, which provided an easily washable surface. The concrete floors were untreated.

Each room was equipped with a thermostatically controlled 60 AMP, 250 VAC, 15 h.p. heater-blower system to maintain room temperature above freezing to facilitate estimation of feed consumption. During warm months, the temperature in the rooms was allowed to approximate ambient air temperature, and ventilation was provided by an exhaust fan and ceiling vents. Lighting was provided by four 200 watt incandescent bulbs per room, and was controlled by a timer to approximate ambient light/dark schedules. Fifteen minutes of light were added to the time of local sunrise and sunset to approximate twilight. The light/dark settings were changed weekly during reproduction tests and were held constant at the schedule in effect during the last week of the acclimation period for the LC_{50} test. All cages, nest boxes, walls, and floors were thoroughly cleaned with a high pressure sprayer after each test.

Diet

The basal diet (Table 1) was formulated to meet the nutrient requirements of the mink (National Research Council, 1982) and was comparable to diets used commercially. Studies have shown that this diet also supports adequate growth and reproduction of the ferret (Bleavins et al., 1980). Feed ingredients were obtained locally, with the exception of the ocean fish

Table 1. Composition and nutrient analysis of basal diet.

Ingredient	Percentage
Commercial mink cereal[1]	16.7
Whole chicken	20.0
Ocean fish scrap mix[2]	12.5
Beef tripe	6.7
Beef liver	3.3
Beef lungs	3.3
Beef trimmings	3.3
Cooked eggs	3.3
Added water	30.9
	100.0
Nutrient Analysis[3]	
Moisture	63.40
Protein	14.30
Fat	7.91
Ash	2.54
	93.15

[1] XK-40, XK Mink Foods, Thiensville, WI.

[2] Consists of cod, haddock, and flounder; National Fur Foods, New Holstein, WI.

[3] Analyzed by Rosner/Runyon Laboratories, Inc., Chicago, IL.

scraps and cereal. The feed ingredients were ground in a commercial meat grinder (except cereal), mixed in a commercial paddle-type mixer, and stored at -18°C in metal containers. Feed and water were provided ad libitum. Any remaining feed was removed and discarded prior to feeding each day.

Prior to the whelping period (April 15) and until the newborn were weaned (June 15), corn oil (~1% W:W) and supplemental vitamin E (18.3 IU/kg) were added to the diets to promote lactation and kit survival, as is standard practice in the mink industry.

Reproduction

Mating attempts were begun at the beginning of March for mink and the end of April for ferrets. In breeding the mink, each female was presented to a male and, if receptive, was allowed to mate. If not receptive, the female was removed and presented to a male 4 days later. Once a successful mating occurred (as verified by the presence of normal-appearing, motile spermatozoa in a vaginal smear taken just after copulation) the female was given the opportunity to mate a second time, either 8 days after the initial mating or the next day (if the first mating occurred late in the breeding season). In breeding the ferrets, females were presented to males when they were judged to be in estrus (determined by the extent of vulvar swelling) and left overnight. Vaginal smears were not taken from female ferrets, nor were they given the opportunity for an additional mating(s).

Mated females of both species were checked daily for newborn (kits) during the parturition (whelping) period (April 20-May 15 for mink, approximately 42 days after mating for ferrets). All kits (alive and dead) were counted and weighed on the day of birth and at 3 and 6 weeks of age. During the reproductive period undue handling and stress were avoided, including cessation of routine weighing and measurement of feed consumption, to maximize reproductive potential.

Weights and Measurements

During the definitive test, adult animals were weighed weekly (LC_{50} tests) or bi-weekly (every 2 weeks; reproduction tests) on a digital balance (Fisher Scientific Co., Model EWO-4010) to the nearest gram. Kits were weighed on a top-loading balance (Mettler Model P-1210) to the nearest tenth of a gram.

In estimating feed consumption by mink or ferrets, several precautions were necessary. Since feed consumption can be adversely affected on a short-term basis by temperature, weather, and other factors, estimates were based on at least 2 consecutive days' consumption. These days were also days in which the animals were not handled (e.g. during weighing, moving, etc.), since handling can produce a temporary reduction in feed consumption. Since ranch-kept mink, and to a lesser extent ferrets, are known to attempt to store away or hide food, and to examine or "play" with objects (i.e. feed containers) in their cages, certain measures were taken to ensure an accurate measurement of feed consumed. In these trials,

each cage was equipped with a "holder" for the cups in which the feed was presented. This holder consisted of a 7.5 cm by 33 cm length of cage material which was bent into a circle and fastened to the side and floor of the cage. Also, when feed consumption was measured, all cages (and nest boxes, during reproduction trials) and the wood shavings beneath them were examined for hidden, stored, or spilled feed. Where possible (i.e., if feed was stored inside a nest box or spilled in a discrete pile where it could be picked up without shavings) stored or spilled feed was returned to the feed cup. If this was not possible, the animal's consumption was not included in the estimate for that group.

To measure feed consumption, uniform feed cups were important. In these trials, #2 and #3 tuna cans (6½ and 12 oz.) were used for females and males, respectively. A cup was filled with a portion of feed in excess of the amount normally consumed, weighed, and recorded to the nearest gram, and placed in the holder. At approximately the same time the next day, the cup was removed, weighed, and recorded to the nearest gram, emptied, and then the procedure was repeated. Average feed consumption was estimated on the basis of 2 consecutive days' consumption. Feed consumption was measured weekly for LC_{50} tests and bi-weekly for reproduction tests.

PRE-TEST PROCEDURE

Acclimation

Prior to the start of an LC_{50} test, the cages were divided randomly throughout the test room into groups of two or four

cages, to hold the 10 animals (5 male, 5 female) per test group in sub-groups of 4, 4, and 2 animals (equal numbers of each sex). For the reproduction tests, the cages were divided into groups of 4, to accommodate the 16 animals (4 male, 12 female) per test group in sub-groups of 4 animals (1 male, 3 female).

The animals used in a test were weighed, then randomly allocated to treatment groups (with the exception that littermates were not assigned to the same group), and were acclimated to the test facilities for a minimum of 7 days, with most tests having an acclimation period of 14 days. Each animal was observed daily and weighed at weekly intervals until the beginning of the test. Feed consumption was measured at least once, and usually twice, during the acclimation period. Any animal that died or exhibited sickness or reduced feed consumption was replaced.

Dietary Concentrations

For the LC_{50} tests, if a search of the literature failed to provide sufficient toxicological data to set dietary concentrations for the test substance, a range-finding trial was conducted in which the compound was administered by gavage to animals at several concentrations. The test compound was either dissolved or suspended in an innocuous vehicle (e.g. distilled water or corn oil) and serial dilutions of a stock solution were made so that the volume administered was equal for all animals.

The range-finding procedure varied slightly, depending on whether LD_{50} estimates were available from the literature. If LD_{50} estimates were found for other species, a dose (mg/kg of body weight) approximating the LD_{50} became the highest of a series of five concentrations, each one-half the previous concentration, administered to two animals (1 male, 1 female). If LD_{50} values were not found, the test substance was first administered to one animal at doses of 1, 10, 100, or 1000 mg/kg of body weight to find a lethal concentration. If a lethal concentration was found, it became the median dose of a series of five concentrations, two concentrations being one-half and one-fourth of the median and two concentrations being two and four times greater than the median. These doses were administered, as above, to two animals per dose. After dosing, all animals were observed for 2-3 hours for signs of intoxication and for vomiting. For either range-finding procedure, the approximate LD_{50} was the dose at which one or two animals died after an appropriate observation period (usually one week).

Based on estimates of the LD_{50} found in the literature or determined by range-finding techniques, the dietary concentrations for the test substance were determined as follows: using the estimated LD_{50} and the estimate(s) of feed consumption during the acclimation period, the highest dietary concentration (in mg/kg of feed) was calculated such that an animal would consume a lethal dose in one day's feed, and four more concentrations were calculated, each being 1.8 times smaller than the previous concentration. If an estimate of

the LD_{50} was not determined, or if the animals vomited the oral dose during range-finding procedures, the highest dietary concentration was set at 5000 mg/kg (since concentrations higher than this are considered to be non-toxic), and four more concentrations were calculated as above.

Finally, it was necessary to determine whether the animals would consume the feed dosed at the highest concentration. One kg of the standard diet (enough to feed an animal for 3-5 days) was dosed with an appropriate weight of the compound or volume of the stock solution to yield a concentration equal to the highest test concentration to be used. Two animals were then fed this diet to determine if they would reject or avoid the feed. (Due to time constraints, this procedure was not carried out in some of the trials, leading to problems in two of the trials). If it was determined that they would not eat the feed at this concentration, several lower concentrations were fed to different animals until a concentration was found which the animals would eat for 3-5 days. This then became the new highest concentration, and the four lower concentrations were calculated as before.

The dietary concentrations of the test substance to be used in the reproduction tests were based on the results of the LC_{50} test performed previously with the test substance. This involved the determination of the maximum tolerated dose (MTD), which was the highest concentration of the substance found not to produce mortality or gross abnormalities in the LC_{50} trial. Since blood parameters and organ weights were

analyzed statistically, these factors were also examined to determine the MTD. The MTD then approximated the highest concentration for the reproduction test. Three concentrations plus a control were used, with each being one-fourth the previous concentration.

After the dietary concentrations were set for the LC_{50} or reproduction test, enough feed to last each of the test groups approximately 30 days, based on feed consumption data from the acclimation period, was mixed. An appropriate amount of the test substance for the amount of feed mixed was either dissolved in an innocuous solvent (e.g. distilled water or corn oil), or dissolved in a volatile solvent (e.g. acetone, hexane), mixed with ground mink cereal, and evaporated to dryness. The resultant premix was then added to the basal diet and mixed thoroughly (the solvent alone was likewise added in the same manner as the compound to the control diet). A sample of each diet was frozen for subsequent residue analysis. The diets were placed in metal containers large enough to hold 1-2 days' feed and frozen at -18°C. In the case of volatile test substances, the containers were lined with sealable plastic bags. For reproduction tests, feed was mixed and frozen as needed throughout the trial.

DEFINITIVE TEST

LC_{50} Tests

The prescribed length of the LC_{50} test was 28 days, with an optional withdrawal period (not more than 14 days) to be used if animals were still dying or exhibiting signs of

intoxication. For the LC_{50} test, the animals used were approximately 6 months old ($\pm$ 14 days) unless the test was designed specifically for younger animals. The use of 6-month old animals guarantees that the animals have attained the adult body size and that any body weight changes still occurring are due to fat deposition. Each group of 10 animals (5 males, 5 females) was randomly assigned to either the control group or one of the five dietary concentrations.

Body weight and feed consumption were recorded, as described above. Each animal was observed at least once daily for signs of intoxication, abnormal behavior, or mortality. Mortalities were recorded, and gross examination of organs was performed as soon after death as possible. Weights of whole body, brain, heart, lungs (5 lobes only), liver, spleen, and kidneys (minus capsules) were recorded for all mortalities. Samples of these organs, as well as muscle and fat, were frozen for subsequent residue analysis.

Upon termination of the test, a suitable volume of blood was taken from all survivors (2-3 ml, via toe clip) for analysis of blood parameters (HCT, RBC, WBC, Hb). The animals were then weighed and euthanized with CO_2 gas. CO_2 gas was used for terminal kills because, unlike decapitation or cervical dislocation, bleeding does not occur, thus allowing blood to pool in organs. This allows comparison of organ weights of terminally-killed animals with mortalities during the test. Gross examination of organs was performed, and weights of brain, heart, lungs (5 lobes only), liver, spleen, and kidneys

(minus capsules) were recorded. Samples of these organs, as well as muscle and fat, were frozen for residue analysis and preserved in 10% formalin for histopathological examination.

The LC_{50} for the test substance, the slope of the dose-response curve, and the confidence limits for these values were determined according to the methods of Litchfield and Wilcoxon (1949). Blood parameters were analyzed via 1-factor analysis of variance. Organ weights were transformed to percentages of body and brain weights, and the body and transformed organ weights of male and female animals were then analyzed via 2-factor analysis of variance. Organ weights were transformed to percentage of brain weight to account for the possibility of a decrease in body weight not giving a corresponding decrease in organ weight. Since brain weight remains relatively constant regardless of changes in body weight, it was used as a benchmark against which to measure organ weight changes, relative to the organ weight: brain weight ratios of controls. Significant differences revealed by analysis of variance were then analyzed by Dunnett's method for comparison with a control (Gill, 1978).

Reproduction Tests

The prescribed length of the reproduction test was approximately 23 weeks, divided into the following segments during which the animals were exposed to the test substance.

1. Pre-breeding period of 8 weeks, starting the beginning of January for mink and the middle of February for ferrets.

2. Breeding and whelping period of 9-12 weeks (depending on species), starting the beginning of March and lasting through mid-May for mink and starting the end of April and lasting through the end of June for ferrets.
3. Post-whelping period of 3 or 6 weeks.

Animals used in the reproduction test were approximately 9 months old (± 14 days) at the beginning of the trial. Each group of 16 animals (4 males, 12 females) was randomly assigned to either the control group or one of the three dietary concentrations. Body weight and feed consumption were recorded according to the methods described previously. All mating attempts were made within dietary groups, and no extraordinary methods (e.g. sedation, use of overaggressive males from ranch stock) were employed to breed uncooperative females. Use of first-year females may result in a limited number of females which will not breed under ordinary circumstances, or within the normal time range of the breeding season.

If mortalities occurred, the animal was weighed and necropsy performed as soon as possible after death, with the necropsy report recorded. Upon termination of the test at 3 weeks post-partum or at weaning (6 weeks post-partum), the four males and four randomly chosen females were weighed, sampled for analysis of blood parameters, and euthanized with CO_2 gas. Gross examination of organs was performed and weights of brain, heart, lungs (5 lobes only), liver, spleen, and kidneys (minus capsules) were recorded. Samples of organs, as well

as muscle and fat, were collected for residue and histopathological analysis.

Reproductive parameters were calculated for the females' performance (see Table 2 for some representative average values). The parameters measured and the statistical procedures used were:

1. Percent whelped and not whelped[a]
2. Live kits/female whelped[b]
3. Total kits/female whelped[b]
4. Length of gestation[b]
5. Average birth weight[b]
6. Average litter weight[b]
7. Percent kit survival to 3 weeks[a]
8. Average 3 week weight[b]

[a] Analyzed by contingency table. Significant differences were then tested by Bonferroni's Chi-square test (Gill, 1978).

[b] Analyzed by 1-factor analysis of variance. Significant differences were then tested by Dunnett's method for comparison with a control (Gill, 1978).

When subtle reproductive effects were observed (i.e., when statistical analysis yielded levels of significance of $0.1 < \alpha < 0.2$ for two or more reproductive parameters), it was sometimes advisable to combine these parameters according to the method of Brown (1975) to give a more accurate indication of the effect of a compound on reproduction. Any kit

Table 2. Reproductive parameters of control groups from reproductive trials involving mink fed several compounds.

Compound	Females mated/ total	% whelped	Live kits/♀ whelped	Average birth wgt. (g)	Average litter wgt. (g)	% kit survival to 3 wks	Average kit wgt. 3 wk. (g)	Reference
1080[1]	12/12	75.0	5.89	9.40	57.6	94.3	98.6	This study
o-cresol	9/12	88.9	4.50	8.31	42.9	75.0	91.0	This study
Thiram[2]	12/12	83.3	3.90	9.71	37.8	85.0	96.5	This study
HCB-1[3]	10/10	100.0	4.90	9.10	44.6	91.8	97.3	Bleavins, 1983
HCB-2[4]	20/20	90.0	6.10	9.04	54.8	96.7	99.6	Bleavins, 1983
Larvadex[5]	12/12	100.0	5.30	9.24	49.0	74.6	105.2	Aulerich et al., 1983
Rabon[5]	11/11	81.8	5.11	9.00	45.9	70.0	103.0	Aulerich et al., 1982
$NaOCl$[6]	11/11	72.7	6.00	8.90	53.4	93.7	102.9	Napolitano and Aulerich, 1982

[1] Sodium monofluoroacetate

[2] Tetramethylthiuram disulfide

[3] Hexachlorobenzene, reproduction study

[4] Hexachlorobenzene, cross-fostering study

[5] Trade name for experimental oral fly larvicide

[6] Sodium hypochlorite

abnormalities were recorded. Blood parameters were analyzed via 1-factor analysis of variance. Organ weights were transformed to percentages of body and brain weights, and the body and transformed organ weights of male and female animals were then analyzed via 2-factor analysis of variance. Significant differences revealed by analysis of variance were then analyzed by Dunnett's method for comparison with a control (Gill, 1978).

RESULTS AND DISCUSSION

The following tests were conducted with the test substances:

Experiment I. Compound 1080 - LC_{50} tests with mink and young and old ferrets; reproduction test with mink;

Experiment II. *O*-cresol - LC_{50} tests with mink and ferrets; reproduction test with mink;

Experiment III. Thiram - LC_{50} tests with mink and ferrets; reproduction tests with mink and ferrets;

Experiment IV. Aroclor 1254 - LC_{50} tests with young and old mink.

All tests followed the procedures outlined above, unless specifically noted. The results of each individual experiment will be discussed separately. A discussion of the protocols developed from these experiments will follow.

Experiment I - Results

Mink LC_{50} Test:

Since a literature search yielded only an approximate LD_{50} for mink of around 1 mg/kg (Robinson, 1953), it was decided to perform a range-finding study. From the results, the LD_{50} was estimated to be 0.25 mg/kg of body weight (Table 3). Based on the results, dietary concentrations of 0, 0.50, 0.90, 1.62, 2.90, and 5.25 ppm were chosen. Distilled water was the carrier used. The nominal dietary concentrations and those determined analytically were:

0 ppm	- 0 ppm
0.50 ppm	- 0.51 ppm
0.90 ppm	- 0.64 ppm
1.62 ppm	- 1.31 ppm
2.90 ppm	- 2.10 ppm
5.25 ppm	- 3.93 ppm

(Analysis performed by Howard H. Casper, Ph.D., Dept. of Veterinary Science, North Dakota State University, Fargo, ND 58105). It should be noted that the feed samples saved for analysis were thawed and refrozen several times during the development of the analytical procedure and this may have resulted in the loss of some of the 1080 residues in the feed.

A 13-day acclimation period was begun on 27 October, 1981. The 28-day LC_{50} trial began on 9 November, 1981 and ended on 7 December, 1981. Signs of intoxication were first noted on day 5, when 2 animals fed the 5.25 ppm diet exhibited difficulty

Table 3. Results of range-finding study with mink exposed to sodium monofluoroacetate (Compound 1080) by gavage.

Dose (mg/kg)	Died/ total	Comments
10.0	2/2	
1.00	2/2	
0.50	2/2	
0.25	1/2	3 days to death
0.10	0/2	No effects observed

Table 4. Average body weight changes and feed[a] and compound[b] consumed by mink fed various concentrations of sodium monofluoroacetate (Compound 1080) for 28 days.

Concentration (ppm)	Parameter measured	Acclimation	Week 1	Week 2	Week 3	Week 4	4 weeks Cumulative
0	Weight change (g)	+29.4	---	-33.9	+14.9	+96.0	+243.2(10)[c]
	Feed consumption (g/d)	279	252	218	265	296	---
	Compound consumption (mg/d)	0	0	0	0	0	---
	Compound consumption (mg/wk)	0	0	0	0	0	---
0.50	Weight change (g)	+10.5	---	-36.9	+59.7	+33.5	+203.8(10)[c]
	Feed consumption (g/d)	311	276	205	282	249	---
	Compound consumption (mg/d)	0	0.14	0.10	0.14	0.12	---
	Compound consumption (mg/wk)	0	0.97	0.72	0.99	0.88	3.56
0.90	Weight change (g)	+43.7	---	-65.4	+20.7	-20.2	- 39.8(10)[c]
	Feed consumption (g/d)	283	181	170	215	208	---
	Compound consumption (mg/d)	0	0.16	0.15	0.19	0.19	---
	Compound consumption (mg/wk)	0	1.14	1.07	1.35	1.31	4.87
1.62	Weight change (g)	+27.9	---	-98.4	-39.4	- 6.3	-160.2(10)[c]
	Feed consumption (g/d)	310	118	152	196	199	---
	Compound consumption (mg/d)	0	0.19	0.25	0.32	0.32	---
	Compound consumption (mg/wk)	0	1.34	1.73	2.23	2.25	7.55
2.90	Weight change (g)	+41.0	---	-143.4	-110.5	-88.6	-439.1(6)[c]
	Feed consumption (g/d)	274	46	81	104	96	---
	Compound consumption (mg/d)	0	0.13	0.23	0.30	0.28	---
	Compound consumption (mg/wk)	0	0.94	1.64	2.11	1.95	6.64
5.25	Weight change (g)	+24.1	---	-195.2	-164.6	-148	-789(1)[c,d]
	Feed consumption (g/d)	279	34	40	69	45	---
	Compound consumption (mg/d)	0	0.18	0.21	0.36	0.24	---
	Compound consumption (mg/wk)	0	1.25	1.47	2.55	1.64	6.91

[a] Feed consumption based on the average of two consecutive day's consumption.

[b] Daily compound consumption calculated from average feed consumption x dietary concentration; weekly compound consumption calculated from daily consumption x 7.

[c] Week 1 weights not recorded; therefore, cumulative weight change is measured from the acclimation period.

[d] Body weight change based on the number of live animals shown in parentheses.

Table 5. Initial and final body weights of male (M) and female (F) mink fed various concentrations of sodium monofluoroacetate (Compound 1080) for 28 days.

Concentration (ppm)	Sex	n	Initial wgt. (g)	Final Wgt. (g)	Change (g)
0	M	5	1617.4	1899.8	+282.4
	F	5	904.4	1108.4	+204.0
0.50	M	5	1540.4	1894.4	+354.0
	F	5	882.2	935.8	+ 53.6
0.90	M	5	1452.2	1487.8**	+ 35.6
	F	5	1011.4	896.2	-115.2*
1.62	M	5	1490.4	1285.8**	-204.6**
	F	5	956.6	840.8	-115.8*
2.90	M	5	1448.8	835.0**	-613.8**
	F	5	1006.6	641.6**	-365.0**
5.25	M	5	1474.4	929.6**	-544.8**
	F	5	938.8	494.0**	-444.8**

* Significantly different from control ($P \leq .05$).

** Significantly different from control ($P \leq .01$).

Table 6. Mortality pattern of mink fed sodium monofluoroacetate (Compound 1080) during a 28-day LC_{50} test.

Concentration (ppm)	No. of animals dying/day of test																											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
0																												
0.05																												
0.90																												
1.62																												
2.90																							1	2			1	
5.25					1			1			1				1				1	1	1					1	1	

Table 7. Blood parameters of mink fed various concentrations of sodium monofluoroacetate (Compound 1080) for 28 days.

Concentration (ppm)	n	RBC ($x10^6$)	WBC ($x10^3$)	Hb (g/dl)	Hct (%)
0	10	9.89[a] ± 0.189	19.9 ± 5.56	23.2 ± 0.90	53.0 ± 1.94
0.50	10	9.55 ± 0.554	23.3 ± 11.21	22.3 ± 0.97	54.3 ± 2.99
0.90	10	9.61 ± 0.633	13.4 ± 10.51	22.7 ± 1.12	53.7 ± 2.72
1.62	10	9.63 ± 0.554	22.3 ± 14.90	21.8* ± 1.13	52.8 ± 2.61
2.90	6	9.26 ± 0.737	20.8 ± 10.73	21.1** ± 1.04	51.0 ± 3.18
5.25	1	9.62 ± 0	6.6 ± 0	21.3 ± 0	50.5 ± 0

[a] Mean ± S.D.

* Significantly different from control ($P \leq .05$).

** Significantly different from control ($P \leq .01$).

Table 8. Body and organ weights of male (M) and female (F) mink fed various concentrations of sodium monofluoroacetate (Compound 1080) for 28 days.

Concentration (ppm)		Body wgt (g)	Brain (% body)	Liver (% body)	Spleen (% body)	Kidney (% body)	Lung (% body)	Heart (% body)	Testes (% body)
0	M	1899.8	0.51	4.28	0.24	0.53	0.57	0.54	0.04
	F	1108.4	0.71	5.05	0.36	0.61	0.68	0.52	--
	C[a]	---	--	--	--	--	--	--	--
0.50	M	1894.4	0.55	3.55	0.26	0.56	0.55	0.50	0.03
	F	935.8	0.97	4.22	0.36	0.62	0.75	0.57	--
	C	---	--	--	--	--	--	--	--
0.90	M	1487.8**	0.68	3.99	0.28	0.60	0.72	0.55	0.04
	F	896.2	0.90	3.99	0.30	0.62	0.82	0.57	--
	C	---	--	--	--	--	--	--	--
1.62	M	1285.8**	0.83**	4.06	0.22	0.55	0.85*	0.62	0.03
	F	840.8	1.01*	4.02	0.33	0.66	0.75	0.66**	--
	C	---	--	--	--	--	--	--	--
2.90	M	835.0**	1.21**	3.77	0.18	0.76**	1.01**	0.72**	0.05
	F	641.6**	1.27**	4.58	0.32	0.76**	1.17**	0.72**	--
	C	---	--	--	--	--	--	--	--
5.25	M	929.6**	1.17**	3.66	0.20	0.72**	1.24**	0.70**	0.04
	F	494.0**	1.64**	4.23	0.20**	0.87**	1.51**	0.82**	--
	C	---	--	--	--	--	--	--	--
		(Organs expressed as a percent of brain weight)							
0	M			839.8	48.0	104.4	112.4	106.7	9.0
	F			714.7	50.1	86.0	96.5	74.4	--
	C			---	49.0	--	--	--	--
0.50	M			650.8*	47.9	103.2	101.1	91.8	4.9
	F			486.9**	41.7	71.2	86.1	65.4	--
	C			---	44.8	--	--	--	--
0.90	M			602.8**	42.0	89.7	107.4	81.8**	6.7
	F			448.1**	33.0	69.8*	91.9	63.6	--
	C			---	37.5*	--	--	--	--
1.62	M			497.1**	27.2	67.5**	103.8	76.6**	3.8
	F			399.2**	33.5	65.8**	74.0	65.5	--
	C			---	30.3**	--	--	--	--
2.90	M			321.3**	15.5	64.0**	84.0	59.9**	4.2
	F			367.6**	26.8	60.6**	92.3	62.4	--
	C			---	21.1**	--	--	--	--
5.25	M			336.9**	19.3	63.9**	109.8	62.7**	3.6
	F			263.1**	12.5	53.3**	92.9	50.4**	--
	C			---	15.9**	--	--	--	--

[a] Sexes are combined where no significant difference ($P \leq .05$) was found between them.

* Significantly different from control ($P \leq .05$).

** Significantly different from control ($P \leq .01$).

in moving their hind legs. (Feed consumption was affected earlier, since most of the animals fed the 5.25 ppm diet exhibited reduced feed consumption by day 3). Clinical signs included reduced feed consumption, incoordination or paralysis (especially of hindquarters), and unconsciousness. Body weight changes and feed and compound consumption are summarized in Tables 4 and 5. Initial body weights were not recorded, therefore, body weight changes have been calculated from the last week of the acclimation period.

At the conclusion of the 28-day exposure period, 4 of 10 animals had died on the 2.90 ppm diet and 9 of 10 died on the 5.25 ppm diet. The mortality pattern is described in Table 6. The data yielded a LC_{50} of 3.2 ppm, with a 95% confidence interval of 2.4 to 4.5 ppm, and a slope of 1.46, with a 95% confidence interval of 1.24 to 1.71.

No gross lesions were noted at necropsy of mortalities during the test or at the terminal kill (8 December, 1981). The hematologic parameters, summarized in Table 7, showed significant decreases in hemoglobin content at 1.62 and 2.90 ppm. The transformed organ weights, summarized in Table 8, revealed significant effects on the liver, spleen, kidney, lungs, and heart.

Ferret LC_{50} Tests:

Based on a published LD_{50} value of 1.41 mg/kg of body weight for yearling domestic ferrets (Tucker and Crabtree, 1970), dietary concentrations of 0, 1.08, 1.94, and 3.50 mg/kg of feed were chosen for young, rapidly growing ferrets (based on their

average body weights during the last week of the acclimation period), and 0, 4.76, 8.56, and 15.40 mg/kg of feed for adult ferrets. Distilled water was the carrier used. Actual concentrations of Compound 1080 in the test diets have not been determined.

A 7-day acclimation period was begun on 26 July, 1983. The 28-day LC_{50} trial began on 2 August, 1983 and ended on 30 August, 1983. The animals used were approximately 2½ months old and > 1 year old for the tests with growing and mature animals, respectively, since one of the objectives of the trial was to compare growing and mature animals' responses in an LC_{50} test. One adult ferret on the 4.76 ppm diet died the fourth day of the test from enteritis (not related to effects of the compound) and was not included among the results of the trial. Also, one adult ferret in the control group showed signs of the same illness, losing over 100 g over the last week of the trial and was thus excluded from the results.

Body weight changes and feed and compound consumption are summarized in Tables 9 and 10. Signs of intoxication were first noted on day 2 among the adult animals, when 2 animals fed the 15.40 ppm diet were observed to have lost coordination of their hindquarters. Reduced feed consumption was noted for some animals on all 3 treatment groups by day 2, also. Among the rapidly growing animals sign of intoxication were first noted on day 5, when a slight loss of coordination of the hindquarters was noted in 2 animals fed the 3.50 ppm diet. This group also showed a reduction in feed consumption

Table 9. Average body weight changes and feed[a] and compound[b] consumed by young or old ferrets fed various concentrations of sodium monofluoroacetate (Compound 1080) for 28 days.

Concentration (ppm)	Parameter measured	Acclimation	Week 1	Week 2	Week 3	Week 4	4 weeks Culumative
Young							
0	Weight change (g)	+121.2	+117.4	+91.4	+126.0	+80.8	+415.5(8)[c]
	Feed consumption (g/d)	188	240	272	237	242	---
	Compound consumption (mg/d)	0	0	0	0	0	---
	Compound consumption (mg/wk)	0	0	0	0	0	---
1.08	Weight change (g)	+134.4	+ 99.9	+71.1	+ 89.6	+55.6	+316.2(8)[c]
	Feed consumption (g/d)	178	228	220	239	261	---
	Compound consumption (mg/d)	---	0.25	0.24	0.26	0.28	---
	Compound consumption (mg/wk)	---	1.73	1.66	1.81	1.97	7.17
1.94	Weight change (g)	+135.9	+ 71.9	+58.9	+ 81.6	+48.2	+260.6(8)[c]
	Feed consumption (g/d)	188	158	230	193	230	---
	Compound consumption (mg/d)	---	0.31	0.45	0.37	0.45	---
	Compound consumption (mg/wk)	---	2.15	3.12	2.62	3.12	11.01
3.50	Weight change (g)	+120.0	+ 36.2	+51.8	+ 70.0	+71.8	+229.8(8)[c]
	Feed consumption (g/d)	173	111	181	167	223	---
	Compound consumption (mg/d)	---	0.39	0.63	0.59	0.78	---
	Compound consumption (mg/wk)	---	2.71	4.43	4.10	5.46	16.70

(con't.)

Table 9.(con't.)

Concentration (ppm)	Parameter measured	Acclimation	Week 1	Week 2	Week 3	Week 4	4 weeks Cumulative
Old							
0	Weight change (g)	- 13.9	+ 21.8	+ 3.9	+ 12.5	+22.3	+ 61.9(7)[c,d]
	Feed consumption (g/d)	82	94	160	102	148	---
	Compound consumption (mg/d)	---	0	0	0	0	---
	Compound consumption (mg/wk)	---	0	0	0	0	---
4.76	Weight change (g)	+ 11.3	- 81.9	-40.7	+ 2.8	+15.2	-115.7(6)[c,d]
	Feed consumption (g/d)	121	60	113	157	224	---
	Compound consumption (mg/d)	---	0.29	0.54	0.75	1.07	---
	Compound consumption (mg/wk)	---	2.01	3.75	5.22	7.46	18.44
8.56	Weight change (g)	+ 15.1	-116.6	-83.8	- 6.6	+ 7.3	-207.4(7)[c]
	Feed consumption (g/d)	113	44	84	97	167	---
	Compound consumption (mg/d)	---	0.38	0.72	0.83	1.43	---
	Compound consumption (mg/wk)	---	2.65	5.04	5.83	10.00	23.52
15.40	Weight change (g)	+ 25.1	-160.6	-132.0	- 80.7	-84.0	-640 (1)[c]
	Feed consumption (g/d)	141	37	50	52	67	---
	Compound consumption (mg/d)	---	0.58	0.77	0.80	1.03	---
	Compound consumption (mg/wk)	---	4.03	5.41	5.59	7.24	22.27

[a] Feed consumption based on the average of two consecutive day's consumption.

[b] Daily compound consumption calculated from average feed consumption x dietary concentration; weekly compound consumption calculated from daily consumption x 7.

[c] Cumulative weight change based on the number of live animals shown in parentheses.

[d] One animal afflicted with enteritis, not counted in totals.

Table 10. Initial and final body weights of male (M) and female (F) ferrets fed various concentrations of sodium monofluoroacetate (Compound 1080) for 28 days.

Concentration (ppm)	Sex	n	Initial wgt. (g)	Final wgt. (g)	Change (g)
Young					
0	M	4	692.2	1288.2	+596.0
	F	4	511.5	746.5	+235.0
1.08	M	4	729.5	1175.0**	+445.5**
	F	4	550.0	737.0	+187.0
1.94	M	4	756.0	1127.2**	+371.2**
	F	4	545.2	695.2	+150.0**
3.50	M	4	730.8	1072.5**	+341.7**
	F	4	561.5	679.2	+117.7**
Old					
0	M	4	1300.2	1387.2	+ 87.0
	F	3[a]	634.5	677.0	+ 42.5
4.76	M	4	1390.5	1280.0	-110.5*
	F	3[a]	670.0	597.0	- 73.0
8.56	M	4	1299.5	1080.0	-219.5**
	F	4	711.5	531.5**	-180.0**
15.40	M	4	1353.5	890.8**	-462.7**
	F	4	680.5	428.2**	-252.3**

[a] One animal died from causes not related to effects of the compound and was excluded.

* Significantly different from control ($P \leq .05$).

** Significantly different from control ($P \leq .01$).

Table 11. Mortality pattern of ferrets fed sodium monofluoroacetate (Compound 1080) during a 28-day LC_{50} test.

Concentration (ppm)	No. of animals dying/day of test																											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Young																												
0																												
1.08																												
1.94																												
3.50																												
Old																												
0																												
4.76										1																		
8.56														1														
15.40										2											1	1	1			1	1	

Table 12. Blood parameters of growing (young) and fully grown (old) ferrets fed various concentrations of sodium monofluoroacetate (Compound 1080) for 28 days.

Concentration (ppm)	n	RBC ($x10^6$)	WBC ($x10^3$)	Hb (g/dl)	Hct (%)
Young					
0	8	11.26[a] ± 1.020	12.0 ± 5.18	20.1 ± 1.82	46.7 ± 1.94
1.08	8	9.43** ± 0.719	6.9** ± 0.84	20.0 ± 1.53	45.4 ± 2.12
1.94	8	9.06** ± 0.320	6.9** ± 2.70	18.3 ± 1.81	45.1 ± 1.64
3.50	8	9.36** ± 1.170	5.0** ± 1.18	17.0** ± 2.54	43.0** ± 1.69
Old					
0	8	11.16 ± 2.029	6.4 ± 4.63	19.8 ± 2.43	51.7 ± 5.54
4.76	6	10.15 ± 1.971	4.3 ± 0.98	20.3 ± 3.08	47.7 ± 7.36
8.56	7	10.71 ± 2.017	4.4 ± 2.54	19.1 ± 4.19	41.4* ± 8.96
15.40	1	11.15 ± 0	3.0 ± 0	21.6 ± 0	54.8 ± 0

[a] Mean ± S.D.

* Significantly different from control ($P \leq .05$).

** Significantly different from control ($P \leq .01$).

Table 13. Body and organ weights of growing male (M) and female (F) ferrets fed various concentrations of sodium monofluoroacetate (Compound 1080) for 28 days.

Concentration (ppm)		Body wgt. (g)	Brain (% body)	Liver (% body)	Spleen (% body)	Kidney (% body)	Lung (% body)	Heart (% body)	Thymus (% body)	Testes (% body)
0	M	1288.2	0.68	3.99	0.58	0.63	0.75	0.41	0.78	0.07
	F	746.5	1.08	3.87	0.58	0.61	0.82	0.44	0.79	--
	C[a]	---	--	3.93	--	0.62	--	0.43	0.79	--
1.08	M	1175.0**	0.80*	3.79	0.57	0.61	0.81	0.46	0.78	0.07
	F	737.0	1.03	3.67	0.59	0.63	0.89	0.49	0.88	--
	C	---	--	3.73	--	0.62	--	0.47	0.83	--
1.94	M	1127.2**	0.82*	3.92	0.69	0.68	0.88*	0.48	0.74	0.06
	F	695.2	1.09	3.59	0.59	0.63	0.91	0.48	0.68	--
	C	---	--	3.76	--	0.66	--	0.48*	0.71	--
3.50	M	1072.5**	0.80**	3.92	0.66	0.63	0.88*	0.46	0.63	0.07
	F	679.2	1.13	3.31	0.55	0.63	0.92*	0.48	0.65	--
	C	---	--	3.62	--	0.63	--	0.47	0.64	--
		Organs expressed as a percent of brain weight								
0	M			594.4	86.9	93.5	110.5	60.4	117.2	10.2
	F			359.8	53.8	56.5	76.3	41.1	73.2	--
	C			---	--	--	---	--	---	--
1.08	M			475.3	71.3	77.0*	101.8	57.4	97.3	9.0
	F			355.3	56.6	61.4	85.7	47.4	84.7	--
	C			---	--	--	---	--	---	--
1.94	M			477.0	84.3	82.5	107.3	58.6	91.1	7.9*
	F			330.9	54.1	57.6	84.1	44.3	63.1	--
	C			---	--	--	---	--	---	--
3.50	M			447.6*	75.7	72.2**	100.9	52.2	71.7*	7.7*
	F			295.2	49.1	55.8	82.7	43.1	57.7	--
	C			---	--	--	---	--	---	--

[a] Sexes are combined where no significant difference ($P < .05$) was found between them.

* Significantly different from control ($P \leq .05$).

** Significantly different from control ($P \leq .01$).

Table 14. Body and organ weights of fully grown male (M) and female (F) ferrets fed various concentrations of sodium monofluoroacetate (Compound 1080) for 28 days.

Concentration (ppm)		Body wgt. (g)	Brain (% body)	Liver (% body)	Spleen (% body)	Kidney (% body)	Lung (% body)	Heart (% body)	Testes (% body)
0	M	1387.2	0.55	3.92	0.75	0.61	0.60	0.51	0.29
	F	677.0	0.85	2.74	0.57	0.53	0.68	0.60	--
	C	---	--	--	--	0.57	0.64	--	--
4.76	M	1200.0	0.64	4.03	0.79	0.68	0.64	0.56	0.25
	F	597.0	1.08	3.52	0.60	0.67	0.81	0.64	--
	C	---	--	--	--	0.68	0.71	--	--
8.56	M	1080.0	0.71	3.91	0.71	0.75	0.76	0.64	0.28
	F	531.5**	1.12	3.61	0.55	0.76	0.80	0.71	--
	C	---	--	--	--	0.76*	0.78	--	--
15.40	M	890.8**	0.90**	4.25	0.52	0.82	1.10	0.76*	0.41
	F	428.2**	1.47**	5.37**	0.98	0.99	1.16	0.81**	--
	C	---	--	--	--	0.91**	1.12**	--	--
		Organs expressed as a percent of brain weight							
0	M			716.6	137.8	112.1	108.9	93.6	53.0
	F			327.5	70.4	63.3	74.8	66.0	--
	C			---	---	---	---	--	--
4.76	M			629.0	122.7	106.8	100.3	86.6	38.0
	F			328.2	56.6	62.5	74.3	65.1	--
	C			---	---	---	---	--	--
8.56	M			552.6	99.2	106.0	106.4	90.7	39.5
	F			328.7	48.9	68.3	72.0	63.8	--
	C			---	---	---	---	--	--
15.40	M			495.0*	60.5**	93.1	121.9	87.1	46.7
	F			357.2	63.4	68.6	75.8	56.6	--
	C			---	---	---	---	--	--

[a] Sexes are combined where no significant difference ($P < .05$) was found between them.

* Significantly different from control ($P < .05$).

** Significantly different from control ($P < .01$).

by day 2. No signs of intoxication were noted in the groups fed the 1.08 or 1.94 ppm diets. Clinical signs among the adult animals included reduced feed consumption, incoordination or paralysis (especially of hindquarters), and unconsciousness, while among the younger animals the only signs noted were reduced feed consumption and occasional loss of coordination of the hindquarters.

At the conclusion of the 28-day exposure period, 1 of 7 animals had died on the 4.76 ppm diet, 1 of 8 had died on the 8.56 ppm diet, and 7 of 8 had died on the 15.40 ppm diet among the adult ferrets. No animals died among the young ferrets. The mortality pattern of the adult ferrets is described in Table 11. The data yielded an LC_{50} of 9.4 ppm, with a 95% confidence interval of 6.1 to 14.5 ppm, and a slope of 1.56, with a 95% confidence interval of 1.01 to 2.42.

No gross lesions were noted at necropsy of mortalities during the test or at the terminal kill (30 August, 1983). The blood parameters, summarized in Table 12, show significant effects on all measurements for younger animals and a decrease in hematocrit for the older animals. The transformed organ weights, summarized in Tables 13 and 14 for young and old ferrets, respectively, reveal effects on the liver, kidneys, thymus, and testes of the young animals and liver and spleen of the older animals.

Mink Reproduction Test:

Based on the results of the LC_{50} trial, where adverse effects were noted at concentrations $\geq$ 0.90 ppm, dietary

concentrations of 0, 0.05, 0.20, and 0.80 ppm were chosen. Distilled water was the carrier used. Actual concentrations of Compound 1080 in the test diets have not been determined.

The reproduction test was initiated on 7 January, 1982 and terminated on 29 June, 1982. Weight changes were recorded bi-weekly until 4 April, 1982, and feed consumption was measured only for weeks 6 and 8. These data are summarized in Tables 15 and 16. No signs of intoxication or mortalities were noted, nor were there any obvious birth defects found. The reproductive indices are presented in Tables 17 and 18.

No gross lesions were noted at necropsy at the terminal kill (29 June, 1982). The hematologic parameters, summarized in Table 19, reveal a decreased WBC count for the 0.80 ppm group. The transformed organ weights, listed in Table 20, show effects on the heart weights of males fed the 0.20 and 0.80 ppm diets.

DISCUSSION

Several noteworthy results can be obtained from an inspection of the LC_{50}, feed consumption, and body weight data. First, it is apparent from the cumulative weight changes that a dose-dependent response was elicited upon exposure to Compound 1080 in both mink and ferrets. Second, from the cumulative compound consumption data, it would appear that an upper limit may exist for the ingestion of Compound 1080 over 28 days, being approximately 7.5 mg for mink and 23.5 mg for ferrets. Similarly, from the daily compound consumption data, it would

Table 15. Average body weight changes and feed[a] and compound[b] consumed by mink fed various concentrations of sodium monofluoroacetate (Compound 1080) during a reproduction test.

Concentration (ppm)	Parameter measured	Acclimation	Weeks 1-2	Weeks 3-4	Weeks 5-6	Weeks 7-8	8 weeks Cumulative
0	Weight change (g)	+44.7	-33.3	- 7.9	+66.4	-17.4	+ 7.8[c]
	Feed consumption (g/d)	---	---	---	195.4	220.0	---
	Compound consumption (mg/d)	---	---	---	0	0	---
	Compound consumption (mg/2 wk)	---	---	---	0	0	0
0.05	Weight change (g)	+18.6	-41.1	-35.8	+75.3	+16.1	+14.5[c]
	Feed consumption (g/d)	---	---	---	246.5	267.3	---
	Compound consumption (mg/d)	---	---	---	0.012	0.013	---
	Compound consumption (mg/2 wk)	---	---	---	0.17	0.19	0.9[d]
0.20	Weight change (g)	+34.4	-56.8	-13.8	+87.8	-27.9	-10.6[c]
	Feed consumption (g/d)	---	---	---	250.4	226.5	---
	Compound consumption (mg/d)	---	---	---	0.050	0.045	---
	Compound consumption (mg/2 wk)	---	---	---	0.70	0.63	3.3[d]
0.80	Weight change (g)	+37.2	-100.8	-55.1	+44.1	+29.6	-82.2[c]
	Feed consumption (g/d)	---	---	---	220.0	189.0	---
	Compound consumption (mg/d)	---	---	---	0.176	0.151	---
	Compound consumption (mg/2 wk)	---	---	---	2.46	2.12	11.4[d]

[a] Feed consumption based on the average of two consecutive day's consumption.

[b] Daily compound consumption calculated from average feed consumption times dietary concentration; 2 week compound consumption calculated from daily consumption times 14.

[c] Body weight change based on values for 16 animals (4 males, 12 females).

[d] Values estimated, based on average of weeks5-8.

Table 16. Initial and final body weights of male (M) and female (F) mink fed various concentrations of sodium monofluoroacetate (Compound 1080) for 8 weeks prior to breeding.

Concentration (ppm)	Sex	n	Initial wgt. (g)	Final wgt. (g)	Change (g)
0	M	4	1786.8	1844.0	+57.2
	F	12	981.8	973.2	- 8.7
0.05	M	4	1788.2	1792.5	+ 4.2
	F	12	956.9	974.8	+17.9
0.20	M	4	1616.2	1645.2	+29.2
	F	12	924.8	900.9	-23.9
0.80	M	4	1899.5	1822.8	-76.8
	F	12	955.2	871.2*	-90.2**

* Significantly different from control ($P \leq .05$).

** Significantly different from control ($P \leq .01$).

Table 17. Reproductive performance of female mink fed various concentrations of sodium monofluoroacetate (Compound 1080) for 23 weeks.

Concentration (ppm)	♀'s bred/ total	♀'s whelped/ ♀'s bred	Gestation (days)	Live kits/ ♀ whelped	Total kits/ ♀ whelped
0	12/12	9/12	46.3 ± 4.00[a]	5.9 ± 1.96	6.1 ± 1.96
0.05	11/12	9/11	47.2 ± 1.72	6.0 ± 1.58	6.4 ± 1.59
0.20	10/12	7/10	45.4 ± 2.76	4.0 ± 2.31	4.0 ± 2.31
0.80	2[b]/12	1/ 2	43 ± 0	5 ± 0	6 ± 0

[a] Mean ± S.D.

[b] 9 other females accepted males but no live spermatozoa were observed in vaginal smears.

Table 18. Average kit body and litter weights and kit survival for dams fed various concentrations of sodium monofluoroacetate (Compound 1080) for 23 weeks.

Concentration (ppm)	Average kit body weight (g)			Average litter weight (g)			% survival	
	Birth	3 week	6 week	Birth	3 week	6 week	3 week	6 week
0	9.4 ± 1.56[a]	89.6 ± 20.10	235.3 ± 64.34	57.6 ± 17.31	497.6 ± 167.34	1353 ± 324.2	94.3	86.8
0.05	8.9 ± 1.40	93.6 ± 18.12	247.3 ± 48.69	53.1 ± 11.75	478.4 ± 211.06	1201 ± 400.9	85.2	75.6[b]
0.20	9.8 ± 1.51	108.9** ± 21.03	292.2** ± 79.77	39.2 ± 20.38	363.0 ± 196.57	1052 ± 413.4	95.2[b]	85.7[b]
0.80	8.8 ± 1.01	---	---	43.8 ± 0	---	---	0	0

[a] Mean ± S.D.

[b] Does not include kits killed by dam.

* Significantly different from control ($P \leq .05$).

** Significantly different from control ($P \leq .01$).

Table 19. Blood parameters of mink fed various concentrations of sodium monofluoroacetate (Compound 1080) for 6 months.

Concentration (ppm)	n	RBC ($x10^6$)	WBC ($x10^3$)	Hb (g/dl)	Hct (%)
0	8	9.27[a] ± 0.739	28.0 ± 13.77	22.4 ± 0.85	53.4 ± 3.06
0.05	8	8.50 ± 0.756	19.3 ± 6.97	22.1 ± 1.76	53.1 ± 3.07
0.20	8	9.44 ± 0.579	45.3 ± 24.94	22.7 ± 1.17	54.6 ± 2.66
0.80	8	9.10 ± 0.560	56.0* ± 28.34	23.3 ± 0.79	55.5 ± 2.57

[a] Mean ± S.D.

* Significantly different from control ($P \leq .05$).

Table 20. Body and organ weights of male (M) and female (F) mink fed various concentrations of sodium monofluoroacetate (Compound 1080) for 6 months.

Concentration (ppm)		Body wgt. (g)	Brain (% body)	Liver (% body)	Spleen (% body)	Kidney (% body)	Lung (% body)	Heart (% body)
0	M	1456.0	0.62	3.99	0.29	0.59	0.93	0.78
	F	899.0	0.84	4.28	0.45	0.65	0.86	0.64
	C[a]	----	--	4.14	--	--	0.90	0.71
0.05	M	1570.2	0.59	4.25	0.26	0.49	0.84	0.62
	F	871.2	0.91	4.45	0.42	0.64	0.95	0.71
	C	----	--	4.35	--	--	0.89	0.66
0.20	M	1437.0	0.66	4.11	0.31	0.58	0.95	0.65
	F	881.8	0.87	4.58	0.48	0.63	0.96	0.66
	C	----	--	4.35	--	--	0.95	0.66
0.80	M	1447.0	0.66	4.22	0.25	0.61	0.81	0.62
	F	843.8	0.95	4.50	0.41	0.66	0.95	0.64
	C	----	--	4.36	--	--	0.88	0.63
		(Organs expressed as a percent of brain weight)						
0	M			659.9	50.0	97.0	150.2	128.0
	F			518.0	53.4	79.5	103.5	76.9
	C			---	51.7	--	---	---
0.05	M			608.2	38.8	83.8	120.3	106.6
	F			494.3	47.5	70.8	104.9	78.3
	C			---	43.1	--	---	---
0.20	M			620.1	46.6	87.2	142.8	98.5*
	F			527.2	56.3	72.9	109.7	76.0
	C			---	51.5	--	---	---
0.80	M			642.1	38.5	92.2	121.4	92.7**
	F			480.8	43.9	71.6	100.5	68.1
	C			---	41.2	--	---	---

[a] Sexes are combined where no significant difference ($P \leq .05$) was found between them.

* Significantly different from control ($P \leq .05$).

** Significantly different from control ($P \leq .01$).

appear that the upper limit for consuming 1080 in one day would be approximately 0.35 mg for mink and 1.4 mg for ferrets (approximately equal to the oral LD_{50}s). Finally, from the feed consumption and weight change data, it would appear that tolerance to the compound has occurred by the third week in the mink, since feed consumption was considerably above the first 2 week's levels (except in the 5.25 ppm diet), and after 2-3 weeks exposure in the older ferrets. The same phenomenon may have occurred with the younger ferrets, especially those fed the 3.50 ppm diet. Tolerance to Compound 1080 has been reported by several authors (Quin and Clark, 1947; Chenoweth, 1949; Miller and Phillips, 1955; Atzert, 1971), although the protection provided was only slight.

Inspection of the data for the 1.08 ppm diet for the young ferrets reveals another interesting possibility. Since the feed consumption for all four weeks was nearly equal, yet the cumulative weight gain showed a difference of nearly 100 g and a highly significant difference for males, it may be possible that Compound 1080 had an effect on weight gain by growing animals unrelated to feed consumption (perhaps related to nutrient uptake, feed conversion, etc.).

Examination of the blood parameters from the two LC_{50} tests (Tables 7 and 12) revealed a significant decrease in the hemoglobin content for mink fed the three highest concentrations of 1080 (including the 5.25 ppm group, even though a statistical analysis was not performed since there is only one value), indicating a possible anemic condition in the mink.

The older ferrets fed corresponding dietary concentrations, however, did not show this decreased hemoglobin content, suggesting that the mink may be more sensitive to Compound 1080 than the ferret. For the younger ferrets, significant decreases were noted in all hematologic parameters at 3.50 ppm 1080 and in RBC and WBC counts at the other two dietary concentrations. This may be an indication of an effect on the hematopoietic system in the young ferrets, although further tests would be necessary to confirm this result.

The organ weight data from the mink and ferret LC_{50} tests (Tables 8 and 13) also revealed interesting information about the two species' responses to 1080 exposure. The most notable observations from Table 8 were: (1) a significant decrease in the liver weight when expressed as a percentage of brain weight for all treatment groups (although these groups do not show a decrease when the liver weight was expressed as a percentage of body weight, since the body weights declined with increasing concentration of 1080); (2) significant decreases of spleen, kidney, and heart weights (as a percentage of brain weight) for all dietary concentrations except 0.50 ppm for at least one sex (although the kidneys were significantly increased as a percentage of body weight at 2.90 and 5.25 ppm and the heart was increased at 1.62, 2.90, and 5.25 ppm); (3) a significant increase in the lung weight expressed as a percentage of body weight for at least one sex at 1.62, 2.90, and 5.25 ppm coupled with no significant difference in lung weight as a percentage of brain weight, which may be an

indication that the lungs (and heart) are the last organs to decline in size as the body loses weight; and (4) no significant decrease in testicular weight. Previous research with rats exposed to Compound 1080 has shown significant decreases in testicular weight during subacute exposure (Sullivan et al., 1979). However, it must be noted that the animals used in this test were not in active spermatogenesis at the time of necropsy.

In contrast with the mink, the adult ferrets did not experience widespread decreases in organ weights upon dietary exposure to Compound 1080. The only significant findings were decreases in liver and spleen weights as percentages of brain weight for males fed 15.40 ppm. This may be a further indication of the greater sensitivity of mink to 1080.

In the LC_{50} test with young ferrets, significant decreases were noted for liver, kidney, thymus, and testes of males fed 3.50 ppm and for testes at 1.94 ppm as well. The decrease in thymus weight may be correlated with the reduction in WBCs noted above. The decrease in testis weight of the young ferrets fed 1.94 and 3.50 ppm of 1080 may be indicative of a preferential effect on this organ, although it must be noted that the testis is relatively undeveloped in ferrets at this age.

Taken in toto, the results from these LC_{50} tests indicate a systemic deleterious effect on adult mink and ferrets upon exposure to dietary concentrations of Compound 1080 of $\geqslant$ 0.90 ppm and $\geqslant$ 8.56 ppm, respectively. This is consistent

with the mode of action of Compound 1080, in which fluoroacetate, when converted to fluorocitrate, constitutes a block in the Krebs cycle, and produces an accumulation of citrate. This citrate accumulation leads to interference with energy production and cellular function and is likely involved in the decrease in body and organ weights noted above, and probably also in the anemia seen at the higher dietary concentrations in mink.

In comparing the responses of the young and old ferrets, Compound 1080 at first glance appears to affect the mature animals more severely, since all three dietary groups exhibited reduced feed consumption almost immediately, whereas only the 3.50 ppm group showed this response among the young, rapidly growing animals, and mortalities were only recorded among the adult ferrets. However, due to this rapid growth the young ferrets were able to "outgrow" the dose (which was calculated on the basis of an approximate body weight of 500 grams), so that at the end of the 28 days of the trial the animals on the 3.50 ppm diet were actually receiving a dose of approximately 0.9 mg/kg of body weight instead of 1.4 mg/kg. Since young, rapidly growing ferrets appear capable of "outgrowing" a lethal dose within a 28-day LC_{50} trial, it would appear that this age group is not suitable for use in a trial of this length. If it is desirable to conduct LC_{50} trials using young, rapidly growing animals, either the length of the trial should be shortened or the dietary concentration of the compound should be changed weekly to account for changes in body weight.

The younger ferrets do appear to be more sensitive to hematological derangements upon exposure to Compound 1080. There were significant decreases noted for all parameters at the highest concentration fed to the young ferrets and decreased RBC and WBC counts even at the lowest dose, whereas the only decrease noted among the older ferrets was the hematocrits of the 8.56 ppm group.

Comparison of the LC_{50} values obtained from the two tests (3.2 ppm for mink, 9.4 ppm for ferrets) indicates that the mink is approximately 3 times more sensitive to Compound 1080 than the ferret. This is also reflected in the apparent upper limits for daily 1080 consumption (0.35 mg/d vs. 1.4 mg/d), and the apparently greater effect on the blood parameters and organ weights noted above.

In the reproduction test with Compound 1080, adverse effects on body weight were found for female mink fed 0.80 ppm, although there was only a slight decrease in feed consumption noted. This dietary concentration proved to have an adverse effect on the reproductive performance of the mink, although this effect was only seen in males in this test. The impaired reproduction in this group is presumed to be due to oligo- or aspermia, since motile spermatozoa were only seen twice in vaginal aspirations taken after copulation, even though the males were repeatedly accepted by the females in this group. Testes degeneration and altered spermatogenesis have been reported in rats exposed to sodium monofluoroacetate (Sullivan et al., 1979). Further tests are necessary to

evaluate the reproductive effects of 0.80 ppm of Compound 1080 in the diet on females.

No significant differences were noted in reproductive performance between controls and dietary concentrations of 0.05 and 0.20 ppm, although the decrease in the number of live kits per female and average litter weight for the 0.20 ppm group indicate that there may be subtle effects on reproduction at this dietary concentration. (The significant increase in average kit body weight at 3 and 6 weeks was a product of decreased litter size, which allowed individual kits to attain relatively larger sizes. Note the decrease in the corresponding litter weights at 3 and 6 weeks).

Examination of Tables 19 and 20 reveals that the maximum tolerated dose estimated by the LC_{50} test was quite accurate, since the only parameters significantly different from the controls were the WBC counts of the 0.80 ppm group and the heart weights as a percentage of brain weight of the males of the 0.20 and 0.80 ppm groups.

CONCLUSIONS

Based on the results of tests conducted with mink and ferrets, which were fed sodium monofluoroacetate (Compound 1080) in the diet, the following conclusions may be drawn:

1) The dietary LC_{50} for mink and ferrets is 3.2 ppm and 9.4 ppm, respectively.
2) Dose-dependent decreases in body weight and feed consumption are elicited in both species upon dietary exposure to Compound 1080.

3) Mink are more severely affected by dietary exposure to Compound 1080 than ferrets.
4) Young, rapidly growing animals may be unsuitable test animals for LC_{50} tests, since they may be able to "outgrow" a lethal concentration.
5) Red and white blood cell counts are negatively affected by dietary concentrations of Compound 1080 as low as 1.08 ppm fed to young, rapidly growing ferrets.
6) 0.80 ppm of Compound 1080 in the diet causes reproductive failure in mink. The presumed cause of this failure is oligo- or aspermia.

Experiment II - Results

Mink LC_{50} Test:

Since a literature search yielded acute oral LD_{50} values ranging from 121 mg/kg (Bio Fax Techniques, 1969) to 1470 mg/kg (Uzhdavini et al., 1974) for rats, range-finding tests were performed for mink and ferrets. From the results (Table 21), LD_{50}s were estimated to be between 100 and 500 mg/kg for mink and between 300 and 500 mg/kg for ferrets. Based on these results, dietary concentrations of 0, 240, 432, 778, 1400, and 2520 ppm were chosen for mink. Corn oil was the carrier used. Actual dietary concentrations of *o*-cresol, analyzed by a modification of an analytical technique published by Supelco Co. (1975), were:

Table 21. Results of range-finding studies with mink and ferrets exposed to *o*-cresol by gavage.

Dose (mg/kg)	Died/total	Comments
Mink		
1000	1/1	
500	1/1	
300	0/2	Both unconscious after dosing
200	1/2	4 days to death
100	0/2	Both lost coordination after dosing
50	0/1	No effects observed
Ferrets		
500	1/1	
400	1/1	1 day to death
300	0/2	1 unconscious, 1 lost coordination after dosing
200	0/1	Lost coordination after dosing

0 ppm	- 0 ppm
240 ppm	- 213.5 ppm
432 ppm	- 473.1 ppm
778 ppm	- 862.3 ppm
1400 ppm	- 1533.7 ppm
2520 ppm	- 3680.3 ppm

A 13-day acclimation period was begun on 27 October, 1981. The 28-day trial began on 9 November, 1981 and ended on 7 December, 1981. No overt signs of toxicity were observed during the test. No animals died during the test and there were no gross lesions noted at the terminal kill (8 December, 1981). Body weight changes and feed and *o*-cresol consumption are summarized in Tables 22 and 23. Initial body weights were not recorded for the mink, therefore, body weight changes have been calculated from the last week of the acclimation period.

Feed consumption was negatively affected at first at 2520 ppm, but returned to normal levels shortly afterward. Weight gain was affected at this concentration also, with levels of significance of $\alpha = .05$ for the males and $\alpha = .10$ for the females.

The hematologic parameters, summarized in Table 24, showed significant decreases in RBC counts at 1400 and 2520 ppm and a decrease in hemoglobin at 2520 ppm. The organ weight data, summarized in Table 25, revealed an increase in liver weight (as a percentage of body weight) at all concentrations except 240 ppm and an increase in heart weight at 2520 ppm. However, no significant changes were seen in the organ weights when expressed as percentages of brain weight.

Table 22. Average body weight changes and feed[a] and compound[b] consumed by mink fed various concentrations of o-cresol for 28 days.

Concentration (ppm)	Parameter measured	Acclimation	Week 1	Week 2	Week 3	Week 4	4 weeks Cumulative
0	Weight change (g)	+103.9	--	+10.5	+21.2	+53.4	+204.9(10)[c,d]
	Feed consumption (g/d)	261	219	220	245	235	---
	Compound consumption (mg/d)	0	0	0	0	0	---
	Compound consumption (mg/wk)	0	0	0	0	0	0
240	Weight change (g)	+41.2	--	+ 3.6	+32.0	+ 7.8	+137.1(10)[c,d]
	Feed consumption (g/d)	279	256	214	226	208	---
	Compound consumption (mg/d)	0	61.4	51.4	54.2	50.0	---
	Compound consumption (mg/wk)	0	429.7	360.0	379.5	350.1	1519.0
432	Weight change (g)	+118.3	--	-12.0	+51.3	+50.9	+243.1(10)[c,d]
	Feed consumption (g/d)	308	290	243	291	255	---
	Compound consumption (mg/d)	0	125.4	105.1	125.8	110.3	---
	Compound consumption (mg/wk)	0	877.6	735.7	880.6	772.3	3266.2
778	Weight change (g)	+91.4	--	-11.1	+39.7	+47.8	+130.7(10)[c,d]
	Feed consumption (g/d)	284	222	211	260	268	---
	Compound consumption (mg/d)	0	173.0	163.8	202.2	208.8	---
	Compound consumption (mg/wk)	0	1211.2	1146.9	1414.3	1461.4	5233.2
1400	Weight change (g)	+61.3	--	- 3.6	-12.8	- 0.4	+106.7(10)[c,d]
	Feed consumption (g/d)	293	217	212	195	229	---
	Compound consumption (mg/d)	0	303.5	296.2	272.4	320.3	---
	Compound consumption (mg/wk)	0	2124.6	2073.7	1907.1	2242.2	8346.8
2520	Weight change (g)	+111.9	--	+28.0	- 7.7	+24.7	- 20.4(10)[c,d]
	Feed consumption (g/d)	271	121	217	209	212	---
	Compound consumption (mg/d)	0	303.9	547.8	527.7	533.8	---
	Compound consumption (mg/wk)	0	2127.4	3835.0	3693.8	3736.6	13392.4

[a] Feed consumption based on the average of two consecutive days consumption.

[b] Daily compound consumption calculated from average feed consumption times dietary concentration; weekly compound consumption from daily consumption times 7.

[c] Body weight change based on the number of live animals shown in parentheses.

[d] Week 1 weights not recorded; cumulative weight change is measured from the acclimation period.

Table 23. Initial and final body weights of male (M) and female (F) mink fed various concentrations of o-cresol for 28 days.

Concentration (ppm)	Sex	n	Initial wgt. (g)	Final wgt. (g)	Change (g)
0	M	5	1537.0	1836.2	+299.2
	F	5	969.8	1080.4	+110.6
240	M	5	1515.2	1763.4	+248.2
	F	5	980.6	1006.6	+ 26.0
432	M	5	1544.4	1966.8	+422.4
	F	5	941.8	1005.6	+ 63.8
778	M	5	1528.2	1767.0	+238.8
	F	5	1030.4	1053.0	+ 22.6
1400	M	5	1592.2	1806.3	+214.6
	F	5	1013.2	1012.0	- 1.2
2520	M	5	1582.6	1592.6	+ 10.0*
	F	5	938.2	887.4	- 50.8

* Significantly different from control ($P \leq .05$).

Table 24. Blood parameters of mink fed various concentrations of *o*-cresol for 28 days.

Concentration (ppm)	n	RBC ($x10^6$)	WBC ($x10^3$)	Hb (g/dl)	Hct (%)
0	10	11.51[a] ± 0.700	-[b]	24.4 ± 1.43	53.4 ± 3.01
240	10	11.21 ± 0.449	-	24.5 ± 1.97	54.8 ± 1.95
432	10	11.14 ± 0.544	-	25.9 ± 1.38	55.7 ± 2.19
778	10	10.92 ± 0.926	-	25.4 ± 1.72	53.7 ± 2.63
1400	10	9.53** ± 0.398	-	23.1 ± 1.12	54.9 ± 2.72
2520	10	9.62** ± 0.707	-	22.5* ± 0.80	54.3 ± 2.04

[a] Mean ± S.D.

[b] Not analyzed due to problems with lysing agent.

* Significantly different from control ($P \leq .05$).

** Significantly different from control ($P \leq .01$).

Table 25. Body and organ weights of male (M) and female (F) mink fed various concentrations of o-cresol for 28 days.

Concentration (ppm)		Body wgt (g)	Brain (% body)	Liver (% body)	Spleen (% body)	Kidney (% body)	Lung (% body)	Heart (% body)	Testes (% body)
0	M	1836.2	0.54	3.60	0.25	0.49	0.55	0.45	0.04
	F	1080.4	0.81	3.83	0.32	0.56	0.70	0.49	--
	C[a]	----	--	--	0.29	--	--	--	--
240	M	1763.4	0.56	3.98	0.25	0.54	0.63	0.50	0.06
	F	1006.6	0.82	4.79	0.32	0.60	0.66	0.55	--
	C	----	--	--	0.28	--	--	--	--
432	M	1966.8	0.53	4.48	0.28	0.53	0.67	0.46	0.05
	F	1005.6	0.85	5.03*	0.28	0.59	0.75	0.52	--
	C	----	--	--	0.28	--	--	--	--
778	M	1767.0	0.59	4.32	0.28	0.53	0.58	0.50	0.04
	F	1053.0	0.79	5.03*	0.32	0.60	0.77	0.55	--
	C	----	--	--	0.30	--	--	--	--
1400	M	1806.8	0.59	4.55	0.32	0.53	0.65	0.48	0.06
	F	1012.0	0.81	5.19*	0.26	0.61	0.79	0.55	--
	C	----	--	--	0.29	--	--	--	--
2520	M	1592.6	0.64	5.31**	0.29	0.53	0.66	0.54*	0.06
	F	887.4	0.94*	5.95**	0.38	0.67	0.75	0.61**	--
	C	----	--	--	0.33	--	--	--	--
		(Organs expressed as a percent of brain weight)							
0	M			671.4	47.2	91.8	102.0	84.3	8.2
	F			475.9	39.9	69.9	86.6	61.9	--
	C			---	--	--	---	--	--
240	M			722.2	44.7	97.2	116.1	89.7	10.4
	F			594.3	38.7	73.3	91.1	67.5	--
	C			---	--	--	---	--	--
432	M			849.5	52.3	101.7	127.9	87.2	9.5
	F			592.5	32.6	69.7	88.8	61.6	--
	C			---	--	--	---	--	--
778	M			741.2	47.9	93.6	101.4	86.9	6.2
	F			648.5	39.9	76.6	99.0	69.3	--
	C			---	--	--	---	--	--
1400	M			768.9	44.7	87.1	109.7	80.8	9.8
	F			650.1	39.6	76.0	93.3	67.8	--
	C			---	--	--	---	--	--
2520	M			845.0	44.9	84.5	104.4	86.7	9.8
	F			639.3	40.3	71.5	80.2	65.9	--
	C			---	--	--	---	--	--

[a] Sexes are combined where no significant difference ($P \leq .05$) was found between them.

* Significantly different from control ($P \leq .05$).

** Significantly different from control ($P \leq .01$).

Ferret LC_{50} Tests:

Based on the estimated LD_{50} (Table 21), dietary concentrations of 0, 432, 778, 1400, 2520, and 4536 ppm were chosen, providing overlap of four dietary concentrations with the mink LC_{50} test. The overlapping diets were taken from the same batch of feed prepared for the mink. Analytically determined dietary concentrations were:

0 ppm -	0 ppm
432 ppm -	473.1 ppm
778 ppm -	862.3 ppm
1400 ppm -	1533.7 ppm
2520 ppm -	3680.3 ppm
4536 ppm -	5188.7 ppm

A 13-day acclimation period was begun on 27 October, 1981. The 28-day trial began on 9 November, 1981 and ended on 7 December, 1981. No overt signs of toxicity were observed during the test. No animals died during the test and there were no gross lesions noted at the terminal kill (9 December, 1981). Body weight changes and *o*-cresol consumption are summarized in Tables 26 and 27, which show a slight reduction in feed consumption at 4536 ppm but no effects on body weight.

The hematologic parameters, summarized in Table 28, show a reduced RBC count at 4536 ppm, while the organ weight data (Table 29) reveals increases in liver and kidney weights and a decrease in the lung weight (as a percentage of body weight) at some dietary concentrations. When the organs were expressed as a percentage of brain weight, however, the only changes were increases in livers at concentration $\geq$ 1400 ppm, and an increase in the females' kidney weight at 4536 ppm.

Table 26. Average body weight changes and feed[a] and compound[b] consumed by ferrets fed various concentrations of o-cresol for 28 days.

Concentration (ppm)	Parameter measured	Acclimation	Week 1	Week 2	Week 3	Week 4	4 weeks cumulative
0	Weight change (g)	+70.1	+30.4	+24.2	+18.4	- 0.9	+ 72.1(10)[c]
	Feed consumption (g/d)	205	199	169	168	169	---
	Compound consumption (mg/d)	0	0	0	0	0	---
	Compound consumption (mg/wk)	0	0	0	0	0	0
432	Weight change (g)	+60.1	+33.3	+26.5	+ 4.9	+ 3.7	+ 68.4(10)[c]
	Feed consumption (g/d)	174	193	171	169	159	---
	Compound consumption (mg/d)	0	83.5	73.9	73.2	68.6	---
	Compound consumption (mg/wk)	0	584.5	517.1	512.3	480.5	2094.4
778	Weight change (g)	+64.8	+31.6	+17.4	+19.0	+ 2.4	+ 70.4(10)[c]
	Feed consumption (g/d)	193	204	169	180	190	---
	Compound consumption (mg/d)	0	159.0	131.3	140.2	148.0	---
	Compound consumption (mg/wk)	0	1113.2	919.3	981.4	1036.2	4050.1
1400	Weight change (g)	+63.2	+12.4	+13.5	+11.3	+12.2	+ 49.4(10)[c]
	Feed consumption (g/d)	173	177	161	158	163	---
	Compound consumption (mg/d)	0	248.1	225.8	220.9	228.4	---
	Compound consumption (mg/wk)	0	1736.6	1580.7	1546.4	1598.9	6462.6
2520	Weight change (g)	+64.0	+23.4	+46.9	+30.1	+11.9	+112.3(10)[c]
	Feed consumption (g/d)	207	217	178	212	186	---
	Compound consumption (mg/d)	0	546.3	449.8	543.2	470.0	---
	Compound consumption (mg/wk)	0	3924.4	3148.7	3739.7	3290.3	14003.1
4536	Weight change (g)	+52.4	+ 9.4	+15.1	+28.6	+ 8.3	+ 61.4(10)[c]
	Feed consumption (g/d)	208	166	140	145	154	---
	Compound consumption (mg/d)	0	754.3	635.0	659.5	698.3	---
	Compound consumption (mg/wk)	0	5280.4	4445.3	4616.7	4888.0	19230.4

[a]Feed consumption based on the average of two consecutive days consumption.

[b]Daily compound consumption calculated from average feed consumption times dietary concentration; weekly compound consumption from daily consumption times 7.

[c]Body weight change based on the number of live animals shown in parentheses.

Table 27. Initial and final body weights of male (M) and female (F) ferrets fed various concentrations of o-cresol for 28 days.

Concentration (ppm)	Sex	n	Initial wgt. (g)	Final wgt. (g)	Change (g)
0	M	5	1780.6	1913.2	+132.6
	F	5	950.6	962.2	+ 11.6
432	M	5	1637.0	1781.0	+144.0
	F	5	952.4	945.2	- 7.2
778	M	5	1708.4	1816.8	+108.4
	F	5	972.0	1004.4	+ 32.4
1400	M	5	1709.6	1795.2	+ 85.6
	F	5	960.2	933.4	- 26.8
2520	M	5	1799.4	1998.4	+199.0
	F	5	1000.4	1026.0	+ 25.6
4536	M	5	1689.8	1803.2	+113.4
	F	5	905.4	914.8	+ 9.4

Table 28. Blood parameters of ferrets fed various concentrations of *o*-cresol for 28 days.

Concentration (ppm)	n	RBC ($x10^6$)	WBC ($x10^3$)	Hb (g/dl)	Hct (%)
0	10	10.46[a] ± 0.671	27.5 ± 9.03	20.9 ± 1.96	50.4 ± 2.95
432	10	10.64 ± 0.975	25.2 ± 8.22	22.2 ± 1.76	51.8 ± 2.59
778	10	10.66 ± 0.762	24.9 ± 7.95	21.2 ± 1.90	50.8 ± 2.77
1400	10	10.52 ± 0.510	30.2 ± 10.34	21.5 ± 1.29	49.9 ± 2.04
2520	10	10.59 ± 0.806	36.2 ± 10.60	21.4 ± 1.31	52.4 ± 3.51
4536	10	9.52* ± 0.380	28.3 ± 15.09	19.7 ± 1.04	49.1 ± 2.83

[a] Mean ± S.D.

* Significantly different from control ($P \leq .05$).

Table 29. Body and organ weights of male (M) and female (F) ferrets fed various concentrations of p-cresol for 28 days.

Concentration (ppm)		Body wgt. (g)	Brain (% body)	Liver (% body)	Spleen (% body)	Kidney (% body)	Lung (% body)	Heart (% body)	Testes (% body)
0	M	1913.2	0.42	3.74	0.65	0.49	0.64	0.33	0.16
	F	962.2	0.64	4.85	0.61	0.54	0.70	0.42	--
	C[a]	----	--	--	0.63	--	--	--	--
432	M	1781.0	0.41	4.00	0.67	0.58	0.53	0.36	0.14
	F	945.2	0.66	5.29	0.60	0.60	0.67	0.49**	--
	C	----	--	--	0.64	--	--	--	--
778	M	1816.8	0.42	4.12	0.59	0.55	0.54	0.37	0.14
	F	1004.4	0.64	5.72	0.65	0.62	0.64	0.46	--
	C	----	--	--	0.62	--	--	--	--
1400	M	1795.2	0.43	4.93*	0.69	0.58	0.54	0.39*	0.15
	F	933.4	0.65	6.12*	0.69	0.66*	0.61	0.45	--
	C	----	--	--	0.69	--	--	--	--
2520	M	1998.4	0.40	4.51	0.56	0.55	0.44	0.35	0.19
	F	1026.0	0.59	6.26*	0.73	0.59	0.56*	0.43	--
	C	----	--	--	0.64	--	--	--	--
4536	M	1803.2	0.45	5.19**	0.75	0.58	0.49	0.38	0.19
	F	914.8	0.69	7.41**	0.68	0.77**	0.68	0.50**	--
	C	----	--	--	0.71	--	--	--	--
		(Organs expressed as a percent of brain weight)							
0	M			904.5	156.5	119.9	130.8	81.2	38.8
	F			760.5	91.3	84.8	110.2	66.2	--
	C			----	---	---	---	--	--
432	M			986.7	165.0	141.6	129.5	89.6	33.5
	F			814.4	92.7	91.8	102.5	73.8	--
	C			----	---	---	---	--	--
778	M			991.9	141.7	135.6	129.6	89.3	33.8
	F			895.4	102.8	98.7	99.6	72.4	--
	C			----	---	---	---	--	--
1400	M			1156.7*	163.8	137.0	126.1	90.8	35.8
	F			936.3	104.8	100.7	92.9	68.2	--
	C			----	---	---	---	--	--
2520	M			1135.5	142.2	140.5	111.8	89.1	47.8
	F			1089.7**	126.3	103.6	96.9	74.8	--
	C			----	---	---	---	--	--
4536	M			1168.7*	168.4	131.7	110.3	95.4	43.1
	F			1077.8**	98.3	112.4*	98.9	72.4	--
	C			----	---	---	---	--	--

[a] Sexes are combined where no significant difference ($P \leq .05$) was found between them.

* Significantly different from control ($P \leq .05$).

** Significantly different from control ($P \leq .01$).

Mink Reproduction Test:

Based on the results of the LC_{50} trial, where adverse effects were noted at 2520 ppm, dietary concentrations of 0, 100, 400, and 1600 ppm were chosen. Corn oil was the carrier used. Actual dietary concentrations of *o*-cresol have not been analyzed.

A 14-day acclimation period was begun on 23 December, 1981. The reproduction test was initiated on 7 January, 1982 and terminated on 29 June, 1982. Weight changes were recorded bi-weekly until 4 April, 1982, and feed consumption was measured only for weeks 6 and 8. One female assigned to the 1600 ppm diet was later discovered to be a male during weighing for week 2 and was replaced from farm stock, and one female on this diet died from virus enteritis, not related to effects of *o*-cresol exposure.

Weight changes and feed and *o*-cresol consumption are summarized in Tables 30 and 31. No effect on feed consumption was observed, although the males fed 1600 ppm differed in weight gain from control at the .10 level of significance. No signs of intoxication were observed during the test.

The reproductive indices, summarized in Tables 32 and 33, show the average birth weight of kits from the 100 ppm diet to be increased over control kits' birth weights. No obvious birth defects were noted in this study.

The hematologic parameters, summarized in Table 34, reveal a significant increase in RBC count for the 1600 ppm group. The organ weight data, presented in Table 35, show an increase in females' liver weight at 1600 ppm.

Table 30. Average body weight changes and feed[a] and compound[b] consumed by mink fed various concentrations of o-cresol during a reproduction test.

Concentration (ppm)	Parameter measured	Acclimation	Weeks 1-2	Weeks 3-4	Weeks 5-6	Weeks 7-8	8 weeks cumulative
0	Weight change (g)	+ 6.3	-23.9	+16.6	+82.9	-18.7	+56.9[c]
	Feed consumption (g/d)	--	--	--	222	193	--
	Compound consumption (mg/d)	0	0	0	0	0	0
	Compound consumption (mg/2 wk)	0	0	0	0	0	0
100	Weight change (g)	+41.9	-11.8	-13.8	+60.8	-22.4	+12.8[c]
	Feed consumption (g/d)	--	--	--	184	197	--
	Compound consumption (mg/d)	0	--	--	18.4	19.7	--
	Compound consumption (mg/2 wk)	0	--	--	257.6	275.2	1332[d]
400	Weight change (g)	+35.4	-14.5	-20.5	+58.4	-23.9	- 0.6[c]
	Feed consumption (g/d)	--	--	--	194	189	--
	Compound consumption (mg/d)	0	--	--	77.8	75.6	--
	Compound consumption (mg/2 wk)	0	--	--	1089.0	1058.2	5368[d]
1600	Weight change (g)	+ 5.1	-79.4	- 0.3	+80.3	- 1.0	-15.9[e]
	Feed consumption (g/d)	--	--	--	228	225	--
	Compound consumption (mg/d)	0	--	--	364.9	359.4	--
	Compound consumption (mg/2 wk)	0	--	--	5108.3	5031.2	25349[d]

[a] Feed consumption based on the average of two consecutive days consumption.

[b] Daily compound consumption calculated from average feed consumption times dietary concentration; bi-weekly compound consumption from daily consumption times 14.

[c] Body weight changes based on values for 16 animals (4 males, 12 females).

[d] Values estimated, based on average of weeks 5-8.

[e] One female died from causes not related to effects of the test substance and one female was discovered to be a male; body weight change based on 14 animals (4 males, 10 females).

Table 31. Initial and final body weights of male (M) and female (F) mink fed various concentrations of o-cresol for 8 weeks prior to breeding.

Concentration (ppm)	Sex	n	Initial wgt. (g)	Final wgt. (g)	Change (g)
0	M	4	1748.5	1822.5	+ 74.0
	F	12	958.2	1009.5	+ 42.8
100	M	4	1713.5	1796.0	+ 82.5
	F	12	997.2	986.8	- 10.4
400	M	4	1697.5	1648.0	- 49.5
	F	12	955.5	971.2	+ 15.8
1600	M	4	1752.8	1606.8	-146.0
	F	10[a]	946.5	982.6	+ 36.1

[a] One female died from causes not related to effects of the test substance, one female found to be male, both not included in totals.

Table 32. Reproductive performance of female mink fed various concentrations of o-cresol for 23 weeks.

Concentration (ppm)	♀'s bred/ total	♀'s whelped/ ♀'s bred	Gestation (days)	Live kits/ ♀ whelped	Total kits/ ♀ whelped
0	9/12	8/9	46.8 ± 2.71[a]	4.50 ± 2.88	5.12 ± 2.70
100	11/12	9/11	47.9 ± 6.17	4.77 ± 1.92	5.33 ± 2.00
400	12/12	9/12	47.6 ± 6.11	4.89 ± 1.90	5.22 ± 2.17
1600	9/11	8/ 9	47.9 ± 2.42	4.25 ± 2.38	5.12 ± 1.96

[a] Mean ± S.D.

Table 33. Average kit body and litter weights and kit survival for dams fed various concentrations of o-cresol for 23 weeks.

Concentration (ppm)	Avg. kit body wgt. (g)			Avg. litter wgt. (g)			% survival	
	Birth	3 week	6 week	Birth	3 week	6 week	3 week	6 week
0	8.3 ± 1.42[a]	91.0 ± 15.01	250.8 ± 47.46	42.9 ± 18.19	491.6 ± 292.80	1630 ± 319.4	75.0	72.2
100	9.4* ± 1.94	99.0 ± 18.02	240.8 ± 70.34	46.7 ± 20.47	461.9 ± 228.63	1144 ± 560.8	97.7	88.4
400	8.7 ± 1.98	97.2 ± 30.92	274.9 ± 55.80	42.4 ± 19.40	399.7 ± 236.95	1031 ± 641.0	84.1	68.2
1600	8.0 ± 2.23	96.6 ± 11.19	242.6 ± 38.84	33.8 ± 21.48	425.0 ± 285.62	1455 ± 469.4	64.7	52.9

[a] Mean ± S.D.

* Significantly different from control ($P \leq .05$).

Table 34. Blood parameters of mink fed various concentrations of *o*-cresol for 6 months.

Concentration (ppm)	n	RBC ($x10^6$)	WBC ($x10^3$)	Hb (g/dl)	Hct (%)
0	8	9.37[a] ± 0.525	-[b]	23.0 ± 1.47	54.1 ± 2.43
100	8	9.13 ± 0.612	-	22.8 ± 1.32	53.0 ± 3.07
400	8	9.52 ± 0.532	-	24.5 ± 1.73	55.8 ± 2.41
1600	8	10.42** ± 0.642	-	23.6 ± 1.27	55.0 ± 2.87

[a] Mean ± S.D.

[b] Not analyzed due to problems with lysing agent.

** Significantly different from control ($P \leq .01$).

Table 35. Body and organ weights of male (M) and female (F) mink fed various concentrations of *o*-cresol for 6 months.

Concentration (ppm)		Body wgt. (g)	Brain (% body)	Liver (% body)	Spleen (% body)	Kidney (% body)	Lung (% body)	Heart (% body)
0	M	1822.0	0.56	3.59	0.29	0.55	0.66	0.52
	F	1009.5	0.93	4.01	0.32	0.61	0.73	0.58
	C[a]	----	--	--	--	0.58	--	0.55
100	M	1796.0	0.60	3.97	0.28	0.60	0.75	0.66
	F	968.8	0.88	4.43	0.30	0.64	0.77	0.66
	C	----	--	--	--	0.62	--	0.66*
400	M	1648.0	0.61	4.33	0.32	0.59	0.67	0.56
	F	971.2	0.84	4.81	0.48*	0.65	0.79	0.60
	C	----	--	--	--	0.62	--	0.58
1600	M	1606.8	0.64	4.52	0.31	0.59	0.76	0.59
	F	982.6	0.96	5.62**	0.44	0.73	0.88	0.66
	C	----	--	--	--	0.66	--	0.63
		(Organs expressed as a percent of brain weight)						
0	M			641.2	50.8	99.4	118.9	92.7
	F			445.6	35.7	67.0	80.0	63.3
	C			---	43.2	---	---	---
100	M			670.6	47.3	102.6	125.3	109.9
	F			508.5	33.9	74.0	88.4	75.5
	C			---	40.6	---	---	---
400	M			729.6	53.4	98.9	109.8	94.1
	F			579.3	57.1	77.7	94.7	72.7
	C			---	55.3	---	---	---
1600	M			712.9	48.7	94.7	120.7	94.3
	F			596.4	46.1	78.0	92.3	69.6
	C			---	47.4	---	---	---

[a] Sexes are combined where no significant difference ($P \leq .05$) was found between them.

* Significantly different from control ($P \leq .05$).

** Significantly different from control ($P \leq .01$).

DISCUSSION

Examination of the body weight and feed consumption data revealed that, with the exception of weight change for male mink fed 2520 ppm, no significant results were observed in the mink and ferret LC_{50} tests. Since the cresols are known to be easily excreted as conjugated glucuronides and sulfates (Bakke and Scheline, 1970), it is assumed that the animals were able to excrete enough ingested *o*-cresol from any one feeding during the day that a toxic dose was not reached, even though the mink consumed in excess of the estimated LD_{50} in an average day's feed consumption on the 2520 ppm diet, and possibly on the 778 and 1400 ppm diets. Similarly the ferrets consumed in excess of the estimated LD_{50} on the 2520 and 4536 ppm diets. Similar results have been reported for rats exposed to 0.3 g/l of *o*-cresol in their drinking water. Savolainen (1979) found no effect on body weight at this concentration, even though the rats had consumed a cumulative dose in excess of the acute oral LD_{50} by the fourth week of this study.

In comparing results from diets that overlapped on the two tests, it appears that mink are more sensitive than ferrets to *o*-cresol. At a dietary concentration of 2520 ppm, both male and female ferret weight changes were in excess of control values, while the male mink weight change was significantly less than controls at the .05 level of significance, and females' weight change was also well below that of controls. Feed consumption was negatively affected in the first week for mink fed 2520 ppm, while it was slightly above control levels for ferrets in the first week at this dietary concentration.

This sensitivity is also reflected in the hematologic parameters. Significant decreases in RBC count were found in the mink fed 1400 and 2520 ppm and in the hemoglobin concentration of mink fed 2520 ppm, while no significant changes were noted in ferrets fed the same concentrations. It must be noted, however, that the RBC counts of the mink fed 1400 and 2520 ppm were well within the normal range for mink at this time of year. In fact, the control and 240 ppm groups' RBC counts were actually quite high for this time of year (Fletch and Karstad, 1972). Another puzzling result from the blood data was the decreased RBC count with increasing dietary concentration of *o*-cresol in the mink LC_{50} test, whereas the RBC count increased with concentration in the reproduction test.

In regard to the reproduction test, no biologically significant results were obtained. No significant negative results were observed in the weight change data or in the reproductive indices, although male weight change on the 1600 ppm diet was significantly different from control at the .10 level of significance. Average birth weight of kits born on the 100 ppm diet was significantly greater than control, although the average of 9.4 g is within the range of normal birth weights. In fact, the 8.0 g average birth weight for kits on the 1600 ppm diet is near the range of abnormally low birth weights, but is not significantly different from the 8.3 g average of the control group (see Table 2).

CONCLUSIONS

Based on the results of tests conducted with mink and ferrets, which were fed *o*-cresol in the diet, the following conclusions may be drawn:

1) The dietary LC_{50} for mink is > 2520 ppm, and for ferrets is > 4536 ppm.
2) Since *o*-cresol is easily metabolized and conjugated, mink and ferrets appear able to excrete enough of the compound ingested during a meal to avoid consuming a lethal dose during a whole day's feed consumption.
3) Mink are more sensitive to *o*-cresol than ferrets, based on results from diets of equal concentration fed to both species.
4) Dietary concentrations of *o*-cresol up to 1600 ppm have no significant effect on reproduction in mink.

Experiment III - Results

Mink LC_{50} Test:

A literature search yielded acute oral LD_{50} values ranging from 350 ppm for rabbits (Matthiaschk, 1973) to 4000 ppm for male rats and mice (Lee et al., 1978), so a range-finding study was performed with mink and ferrets (Table 36). In the mink range-finding study, thiram was administered first at concentrations of 10, 100, and 500 mg/kg suspended in water (since this was the intended carrier for mixing thiram in the test diets), with no observable effect. Since the toxicity of thiram has been reported to be greater in the presence of fats and oils (Merck Index, 1976), it was then decided to

Table 36. Results of range-finding studies with mink and ferrets exposed to thiram by gavage.

Dose (mg/kg)	Died/ total	Comments
Mink		
2000	0/2	Corn oil carrier; both vomited dose within 20 min.
1000	0/2	Corn oil carrier; both vomited dose within 20 min.
1000	0/2	Water carrier; no effects observed
500	0/2	Corn oil carrier; both vomited dose within 20 min.
500	0/2	Water carrier; no effects observed
100	0/2	Water carrier; no effects observed
10	0/2	Water carrier; no effects observed
Ferrets		
200	0/2	Water carrier; both vomited dose within 20 min.
100	0/2	Water carrier; both vomited dose within 20 min.
50	0/2	Water carrier; 1 animal vomited dose after 14 min.

administer the compound suspended in corn oil. Doses of 500, 1000, and 2000 ppm were administered, and were vomited by all animals.

Since a lethal dose could not be found, 5000 ppm was selected as the highest concentration to be fed to the mink in the LC_{50} test. It was decided to perform a palatability test, since one of the uses of thiram is as a protective coating for ornamentals to prevent gnawing. A small portion of the basal diet was supplemented with enough of the 2000 ppm suspension of thiram in corn oil used in the range-finding trial to yield 5000 ppm of thiram in the diet, and was fed to three mink which had been starved overnight. Since the mink ate all or nearly all of the ration provided, 5000 ppm was selected as the highest dietary concentration, with the other dietary concentrations being 1.8 times smaller than the previous concentration, or 2778, 1543, 857, and 476 ppm plus the control. Unfortunately, the thiram was added to the diets suspended in water, and, as became apparent later, thiram in water behaves very differently from thiram in oil. At the beginning of the 28-day exposure period, the 5000 and 2778 ppm diets were rejected outright by the mink, while consumption of the 1543, 857, and 476 ppm diets was severely reduced within the first week. The animals on the 5000 and 2778 ppm diets were removed from the trial after 3 days, fed the basal diet for 4 days, and were then re-assigned to two new dietary concentrations, again 1.8 times smaller than the previous lowest concentration, or 265 and 147 ppm. Eventually, the animals on the 1543, 857, 476, 265, and 147 ppm

diets were all removed from the trial to avoid starving them to death, since they all eventually exhibited severely reduced feed consumption within 2 weeks of the start of a 28-day trial. Finally, in an effort to determine a dietary concentration mink would consume for 28 days so as to be able to set dietary concentrations for the reproduction test to follow, two groups of four animals (which had been placed in the four extra cages in both test rooms along with the test animals at the start of the acclimation periods, to provide replacements in case of injury or sickness) were fed dietary concentrations 1.8 times smaller than the previous lowest concentration, or 82 and 45 ppm, for 28 days. At the same time, the eight remaining animals originally fed the 1543 ppm diet, which had been fed the basal diet since being removed from the trial (1 week), were fed a diet which contained 1543 ppm of thiram added in corn oil for 25 days, to observe the effects of thiram in this carrier. Actual dietary concentrations of thiram have not been determined.

An 18-day acclimation period was begun on 7 October, 1982 for mink. The 28-day trial for the original groups began on 25 October, 1982. The 5000 and 2778 ppm groups were removed from the trial on 28 October, 1982, fed the basal diet until 31 October, 1928, and reassigned to the 265 and 147 ppm diets, respectively, on 1 November, 1982. They were subsequently removed from the trial on 15 November, 1982. The 1543 ppm group was removed from the trial on 3 November, 1982, fed the basal diet until 10 November, 1982, and was then fed a diet containing 1543 ppm of thiram added in corn oil from 11 November,

1982 until 6 December, 1982. The 857 and 476 ppm groups (the last of the original dietary concentrations) were removed from the trial on 8 November, 1982. The 28-day exposure period for the 82 and 45 ppm groups began on 8 November, 1982 and ended on 6 December, 1982.

For the 28-day LC_{50} trial, signs of intoxication were seldom noted. In the 45 and 82 ppm groups (fed thiram added in water for 28 days) the only signs of intoxication were reduced feed consumption and subsequent tarry stools in the 82 ppm group. In the group fed 1543 ppm added in corn oil (fed 25 days) no signs of intoxication were noted. Of the remaining dietary concentrations (fed thiram added in water for 3-14 days), seven mink died during the exposure to thiram (two each on the 857 and 1543 ppm diets and three on the 476 ppm diet), and all groups exhibited either complete initial rejection of the feed or near avoidance of the feed within 2 weeks of the start of the test, and concurrent weight loss. Body weight changes and feed and compound consumption are summarized in Tables 37 and 38 for mink. Statistical analyses were not performed on these data due to the many problems and small numbers involved.

Clinical signs observed among the groups terminated, besides severe weight loss, included occasional listlessness, tarry stools, and on several occasions violent convulsions accompanied by intense vocalizations. On two occasions, the convulsions were preceded by low carriage, and on several occasions they were followed by a peculiar "corkscrewing" of the body. The convulsions were always followed by unconsciousness. Death came ½ to 1 hour after the convulsions, although

Table 37. Average body weight changes and feed[a] and compound[b] consumed by mink fed various concentrations of thiram (added in water) for 28 days.

Concentration (ppm)	Parameter measured	Acclimation	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	4 week cumulative
0	Weight change (g)	+67.8	+73.4	+ 1.0	+ 33.8	+69.6	-27.3	-13.2	+ 62.9(10)[c]
	Feed consumption (g/d)	362	339	358	328	273	255	234	---
	Compound consumption (mg/d)	0	0	0	0	0	0	0	---
	Compound consumption (mg/wk)	0	0	0	0	0	0	0	0
45	Weight change (g)				+ 60.8	+68.5	-10.8	-27.0	+ 91.5(4)[c]
	Feed consumption (g/d)				329	283	256	170	---
	Compound consumption (mg/d)				14.80	12.72	11.51	7.67	---
	Compound consumption (mg/wk)				103.60	89.04	80.57	53.69	326.90
82	Weight change (g)				- 99.0	+25.2	+ 8.2	-98.0	-163.5(4)[c]
	Feed consumption (g/d)				229	134	230	121	---
	Compound consumption (mg/d)				18.79	11.01	18.83	9.93	---
	Compound consumption (mg/wk)				131.53	77.07	131.81	69.51	409.92
147	Weight change (g)			-137.1	-138.4	---	(off trial)		---
	Feed consumption (g/d)			314	94	57			---
	Compound consumption (mg/d)			46.13	13.83	8.33			---
	Compound consumption (mg/wk)			322.91	96.81	58.31			---
265	Weight change (g)			-229.5	-167.9	(off trial)			---
	Feed consumption (g/d)			160	58				---
	Compound consumption (mg/d)			42.53	15.45				---
	Compound consumption (mg/wk)			297.71	108.15				---

(Con't.)

Table 37. (Con't.)

Concentration (ppm)	Parameter measured	Acclimation	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	4 week cumulative
476	Weight change (g)	+35.4	-303.4	-233.1	(off trial)				---
	Feed consumption (g/d)	340	73	39					---
	Compound consumption (mg/d)	0	34.72	18.69					---
	Compound Consumption (mg/wk)	0	243.04	130.83					---
857	Weight change (g)	+40.6	-300.8	---	(off trial)				---
	Feed consumption (g/d)	305	31	44					---
	Compound consumption (mg/d)	0	26.52	37.46					---
	Compound consumption (mg/wk)	0	185.64	262.22					---
1543[d]	Weight change (g)	+66.6	-358.1	+ 33.5[e]	+79.0[f]	+187.5	+103.9	-26.8	+343.6(8)[c]
	Feed consumption (g/d)	359	25	---	260	251	321	289	---
	Compound consumption (mg/d)	0	38.34	---	400.99	435.30	495.59	445.83	---
	Compound consumption (mg/wk)	0	268.40	---	2806.9	3047.2	3469.1	3120.8	12444.0

[a] Feed consumption based on the average of two consecutive days consumption.

[b] Daily compound consumption calculated from average feed consumption times dietary concentration; weekly compound consumption from daily consumption times 7.

[c] Body weight changes based on number of live animals shown in parentheses.

[d] Concentrations of 2778 and 5000 mg/kg were rejected immediately, and were removed from the trial.

[e] Remaining animals fed basal diet during week 2.

[f] Remaining animals fed diet with thiram added in corn oil for duration of trial.

Table 38. Initial and final body weights of male (M) and female (F) mink fed various concentrations of thiram for 28 days.

Concentration (ppm)	Sex	n	Initial weight (g)	Final weight (g)	Change (g)
0	M	5	1752.8	1867.0	+114.2
	F	5	962.0	973.6	+ 11.6
45	M	2	1453.0	1626.0	+173.0
	F	2	1200.0	1248.5	+ 48.5
82	M	2	1677.0	1468.5	-208.5
	F	2	1053.0	934.5	-118.5
147	M	5	1536.0	1213.0	-323.0
	F	5	1135.2	907.2	-228.0
265	M	4	1576.5	1071.5	-505.0
	F	4	920.0	636.8	-283.2
1543 (oil)	M	4	1231.5	1628.2	+396.7
	F	4	713.2	1003.8	+290.6

Table 39. Organ weights of mink fed various concentratios of thiram for 28 days.

Concentration (ppm)	n	Brain (% body)	Liver (% body)	Spleen (% body)	Kidney (% body)	Lung (% body)	Heart (% body)
0	10	0.66	4.72	0.27	0.53	0.71	0.59
45	4	0.68	4.36	0.42	0.59	0.60	0.52
82	4	0.77	4.59	0.42	0.55	0.76	0.66
1543 (oil vehicle)	8	0.72	4.54	0.45*	0.58	0.70	0.61
		(Organs expressed as a percent of brain weight)					
0	10		739.5	40.9	83.6	110.7	92.4
45	4		645.9	59.6	86.4	88.2	77.2
82	4		604.9	55.3	72.7	99.5	88.1
1543 (oil vehicle)	8		645.1	62.2**	82.1	98.5	87.1

* Significantly different from control ($P \leq .05$).

** Significantly different from control ($P \leq .01$).

not every episode resulted in death. Curiously, two mink in groups that were removed from the trial were observed to have these convulsions after several days on clean feed. No gross lesions were noted at necropsy of mortalities during the trial or at the terminal kill (7 December, 1982).

The only hematologic parameter analyzed in the 28-day test was hematocrit. Averages obtained for the 0, 45, and 82 ppm groups were 57.6, 51.2, and 48.5, respectively, with the two treated groups being significantly different from control ($P \leq .01$). The hematocrit for the 1543 ppm group. 51.9, was also significantly different from control ($P \leq .01$).

The organ weight data, summarized in Table 39, was not analyzed by sex as in other tests, due to the small numbers involved. Significant increases were found in the spleen weight of the 1543 ppm group, however.

Ferret LC_{50} Test:

Since the ferret LC_{50} test was conducted after the mink test, the previously mentioned problems were avoided. Doses of 50, 100, and 200 ppm, suspended in water, were administered, and 5 of 6 animals likewise vomited the dose. Again, no lethal concentration was found in the range-finding test, so a palatability test was conducted. Concentrations of 100, 500, and 1000 ppm of thiram were added to the basal diet in water and fed to previously-starved ferrets, and only the 100 ppm diet was consumed in normal amounts. Therefore, dietary concentrations of 0, 8, 20, 50, 125, and 312 ppm were chosen (each concentration being 2.5 times the previous concentration, since it was

decided that a wider range of dietary concentrations would be necessary to ensure finding a concentration that would be eaten for 28 days.

A 21-day acclimation period was begun on 16 December, 1982 for the ferrets. The 28-day test began on 6 January, 1983 and ended on 2 February, 1983. Signs of intoxication were first noted on day 4, when two animals fed the 312 ppm diet were found to have tarry stools. Reduced feed consumption was noted in this group, and a transient reduction in feed consumption was also noted for some animals in the 50 and 125 ppm groups in the first week. Body weight changes and feed and compound consumption are summarized in Tables 40 and 41. Clinical signs were only noted in the 312 ppm group, and included inanition, tarry stools, listlessness, uncoordination, and the same type of convulsions described for the mink trial. All animals on the 312 ppm diet had died by day 16, while no deaths were noted on any other dietary concentration. The mortality pattern is described in Table 42. No gross lesions were noted at necropsy of mortalities during the trial or at the terminal kill (3 February, 1983). The data obtained from the LC_{50} test was unsuitable for analysis. The LC_{50} for thiram for ferrets was between 125 and 312 ppm.

The hematologic parameters, summarized in Table 43, showed decreases in RBC count and hemoglobin concentration at 50 and 125 ppm. A significant decrease in hematocrit was seen in the 50 ppm group, but not the 125 ppm group. The organ weight data, summarized in Table 44, revealed significant increases in

Table 40. Average body weight changes and feed[a] and compound[b] consumed by ferrets fed various concentrations of thiram for 28 days.

Concentration (ppm)	Parameter measured	Acclimation	Week 1	Week 2	Week 3	Week 4	Cumulative
0	Weight change (g)	+29.3	+ 45.1	+ 6.4	+40.9	- 7.3	+ 85.1(10)[c]
	Feed consumption (g/d)	194	214	264	235	219	---
	Compound consumption (mg/d)	0	0	0	0	0	---
	Compound consumption (mg/wk)	0	0	0	0	0	0
8	Weight change (g)	+30.4	+ 50.9	+ 28.0	+24.5	-12.4	+ 91.0(10)[c]
	Feed consumption (g/d)	207	191	252	211	201	---
	Compound consumption (mg/d)	0	1.53	2.02	1.69	1.61	---
	Compound consumption (mg/wk)	0	10.71	14.14	11.83	11.27	47.95
20	Weight change (g)	+50.0	+ 19.3	- 14.3	+15.8	-11.2	+ 9.6(10)[c]
	Feed consumption (g/d)	211	161	246	237	216	---
	Compound consumption (mg/d)	0	3.21	4.92	4.74	4.33	---
	Compound consumption (mg/wk)	0	22.47	34.44	33.18	30.31	120.40
50	Weight change (g)	+31.2	- 1.9	+ 7.7	+24.1	-17.8	+ 12.1(10)[c]
	Feed consumption (g/d)	207	138	206	194	177	---
	Compound consumption (mg/d)	0	6.91	10.28	9.68	8.87	---
	Compound consumption (mg/wk)	0	48.37	71.96	67.76	62.09	250.18
125	Weight change (g)	+35.8	- 67.9	- 24.5	+ 5.3	-21.2	-108.3(10)[c]
	Feed consumption (g/d)	209	136	241	205	181	---
	Compound consumption (mg/d)	0	17.06	30.15	25.62	22.65	---
	Compound consumption (mg/wk)	0	119.42	211.05	179.34	158.55	668.36
312	Weight change (g)	+23.9	-176.2	-105.5	---	---	---
	Feed consumption (g/d)	184	141	113	---	---	---
	Compound consumption (mg/d)	0	44.97	35.14	---	---	---
	Compound consumption (mg/wk)	0	314.79	245.98	---	---	560.77

[a] Feed consumption based on the average of two consecutive days consumption.

[b] Daily compound consumption calculated from average feed consumption times dietary concentration; weekly compound consumption from daily consumption times 7.

[c] Body weight change based on the number of live animals shown in parentheses.

Table 41. Initial and final body weights of male (M) and female (F) ferrets fed various concentrations of thiram for 28 days.

Concentration (ppm)	Sex	n	Initial wgt. (g)	Final wgt. (g)	Change (g)
0	M	5	1923.6	2081.8	+153.2
	F	5	933.4	950.4	+ 17.0
8	M	5	1899.6	2074.4	+174.8
	F	5	795.6	802.8	+ 7.2
20	M	5	2033.2	2097.4	+ 64.2*
	F	5	906.8	861.8	- 45.0
50	M	5	1906.6	1957.2	+ 50.6*
	F	5	860.8	834.4	- 26.4
125	M	5	1993.2	1875.8*	-122.4**
	F	5	901.6	807.4	- 94.2**
312	M	5	1951.6	1623.0**	-323.6**
	F	5	949.2	763.0	-186.2**

* Significantly different from control ($P \leq .05$).

** Significantly different from control ($P \leq .01$).

Table 42. Mortality pattern of ferrets fed thiram during a 28-day LC50 test.

Concentration (ppm)	No. of animals dying/day of test																										
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
0																												
8																												
20																												
50																												
125																												
312											2	2	2	2	1	1												

Table 43. Blood parameters of ferrets fed various concentrations of thiram for 28 days.

Concentration (ppm)	n	RBC ($x10^6$)	WBC ($x10^3$)	Hb (g/dl)	Hct (%)
0	10	11.29[a] ± 0.682	10.0 ± 3.55	20.9 ± 1.31	48.8 ± 3.37
8	10	10.57 ± 0.875	8.6 ± 2.14	20.1 ± 1.76	47.6 ± 5.37
20	10	10.24 ± 0.791	18.5 ±11.22	18.7 ± 2.01	48.4 ± 2.31
50	10	8.19** ± 1.549	22.4 ±34.08	16.6** ± 3.02	42.3* ± 6.92
125	10	8.57** ± 1.151	16.8 ±11.57	17.1** ± 2.17	46.0 ± 4.24
312	0	---	---	---	---

[a] Mean ± S.D.

* Significantly different from control ($P \leq .05$).

** Significantly different from control ($P \leq .01$).

Table 44. Body and organ weights of male (M) and female (F) ferrets fed various concentrations of thiram for 28 days.

Concentration (ppm)		Body wgt. (g)	Brain (% body)	Liver (% body)	Spleen (% body)	Kidney (% body)	Lung (% body)	Heart (% body)
0	M	2081.8	.386	3.359	.561	.480	.432	.348
	F	950.5	.657	3.846	.393	.484	.574	.435
	C[a]	---	--	---	.477	--	--	--
8	M	2074.4	.375	3.524	.572	.475	.413	.314
	F	802.8	.773*	3.859	.481	.506	.621	.489
	C	---	--	---	.527	--	--	--
20	M	2097.4	.405	3.960	.682	.497	.434	.389
	F	861.8	.764*	3.913	.551	.539	.592	.519*
	C	---	--	---	.617	--	--	--
50	M	1957.2	.411	3.744	.704	.520	.481	.406
	F	834.4	.716	4.160	.911	.536	.612	.517*
	C	---	--	---	.807*	--	--	--
125	M	1875.8*	.436	3.928	.796	.516	.495	.418
	F	807.4	.813**	4.168	1.003	.571*	.678	.526**
	C	---	--	---	.900**	--	--	--
312	M	1628.0**	.501*	4.091*	.668	.525	.655**	.460**
	F	763.0	.892**	4.455	.825	.642**	.877**	.596**
	C	---	--	---	.746*	--	--	--
		Organs expressed as a percent of brain weight						
0	M			869.3	145.4	124.7	112.0	90.1
	F			592.6	60.4	74.2	87.4	56.5
	C			---	---	---	---	--
8	M			949.4	153.4	127.5	110.8	85.1
	F			500.5	62.0	65.5	80.3	63.4
	C			---	---	---	---	--
20	M			985.1	170.3	123.0	107.0	96.7
	F			520.4	73.7	71.1	77.7	68.4
	C			---	---	---	---	--
50	M			913.6	169.9	126.7	117.1	99.3
	F			577.7	122.0	74.4	84.9	71.8
	C			---	---	---	---	--
125	M			901.9	184.0	118.5	113.9	96.2
	F			517.7	125.3*	70.4	83.0	65.1
	C			---	---	---	---	--
312	M			824.1	133.5	106.0	131.3*	92.4
	F			501.3	92.5	72.1	98.8	67.0
	C			---	---	---	---	--

[a] Sexes are combined where no significant difference ($P \leq .05$) was found between them.

* Significantly different from control ($P \leq .05$).

** Significantly different from control ($P \leq .01$).

all organs as a percent of body weight at 312 ppm, but only in the lungs of males when expressed as a percent of brain weight. An increase in spleen weight was also seen at 125 ppm, both as a percentage of body and of brain weight, and the kidneys and hearts of females, expressed as a percentage of body weight, were increased at 125 ppm.

Mink Reproduction Test:

Based on the results of the LC_{50} trial, where adverse effects were noted in mink at 82 ppm, dietary concentrations of 0, 2.5, 10.0, and 40.0 ppm were selected. Distilled water was the carrier used.

A 21-day acclimation period was begun on 16 December, 1982. The reproduction test was initiated on 6 January, 1983 and terminated on 22 June, 1983. Weight changes were recorded bi-weekly until 3 March, 1983, and feed consumption was measured during weeks 1, 3, 5, and 7.

Body weight changes and feed and compound consumption are summarized in Tables 45 and 46. No signs of intoxication or mortalities due to effects of the compound were noted (one female mink died of virus enteritis), but male mink fed 40 ppm gained significantly less weight than did controls over the 8 weeks of the pre-breeding period. No obvious birth defects were found, and no gross lesions were noted at necropsy at the terminal kill (22 June, 1983).

The reproductive indices for mink (Tables 47 and 48) revealed no significant effects of thiram on the reproductive performance of the females except decreased birth weight at

Table 45. Average body weight changes and feed[a] and compound[b] consumed by mink fed various concentrations of thiram during a reproduction test.

Concentration I (ppm)	Parameter measured	Acclimation	Weeks 1-2	Weeks 3-4	Weeks 5-6	Weeks 7-8	8 weeks cumulative
0	Weight change (g)	+57.0	+26.6	+ 4.5	+ 6.1	-34.1	+ 3.0[c]
	Feed consumption (g/d)	213	220	261	213	194	---
	Compound consumption (mg/d)	0	0	0	0	0	0
	Compound consumption (mg/2 wk)	0	0	0	0	0	0
2.5	Weight change (g)	+33.8	+ 6.2	+18.0	+14.3	-44.2	- 5.8[c]
	Feed consumption (g/d)	150	222	220	192	220	---
	Compound consumption (mg/d)	0	0.56	0.55	0.48	0.55	---
	Compound consumption (mg/2 wk)	0	7.84	7.70	6.72	7.70	29.96
10.0	Weight change (g)	+20.6	- 2.4	+ 7.1	+22.9	-55.5	-27.9[d]
	Feed consumption (g/d)	169	207	216	226	219	---
	Compound consumption (mg/d)	0	2.07	2.16	2.26	2.19	---
	Compound consumption (mg/2 wk)	0	28.98	30.24	31.64	30.62	121.48
40.0	Weight change (g)	+48.0	-44.1	+ 6.6	-14.6	-30.6	-82.8[c]
	Feed consumption (g/d)	189	189	207	173	182	---
	Compound consumption (mg/d)	0	7.56	8.30	6.93	7.26	---
	Compound consumption (mg/2 wk)	0	105.84	116.20	97.02	101.64	420.70

[a] Feed consumption based on the average of two consecutive days consumption.

[b] Daily compound consumption calculated from average feed consumption times dietary concentration; bi-weekly compound consumption from daily consumption times 14.

[c] Body weight changes based on values for 16 animals (4 males, 12 females).

[d] One female died from causes not related to effects of the test substance; body weight changes based on 15 animals (4 males, 11 females).

Table 46. Initial and final body weights of male (M) and female(F) mink fed various concentrations of thiram for 8 weeks prior to breeding.

Concentration (ppm)	Sex	n	Initial wgt. (g)	Final wgt. (g)	Change (g)
0	M	4	2095.2	2222.0	+126.8
	F	12	1127.8	1089.6	- 38.2
2.5	M	4	1790.2	1849.2	+ 59.0
	F	12	1060.8	1033.5	- 27.3
10.0	M	4	1831.5	1840.0	+ 8.5
	F	11[a]	1142.3	1101.2	- 41.1
40.0	M	4	2079.5	1940.2	-139.2*
	F	12	1108.6	1044.7	- 63.9

[a] One female died from causes not related to effects of the test substance.

* Significantly different from control ($P \leq .05$).

Table 47. Reproductive performance of female mink fed various concentrations of thiram for 23 weeks.

Concentration (ppm)	♀'s bred/ total	♀'s whelped/ ♀'s bred	Gestation (days)	Live kits/ ♀ whelped	Total kits/ ♀ whelped
0	12/12	10/12	48.9 ± 5.86[a]	3.9 ± 2.08	4.1 ± 1.79
2.5	9/12	6[b]/ 9	48.5 ± 6.16	5.0 ± 2.45	5.3 ± 1.97
10.0	11/11	11/11	47.9 ± 4.78	4.8 ± 2.44	5.3 ± 2.41
40.0	12/12	7/12	47.1 ± 2.67	3.7 ± 2.81	4.7 ± 1.80

[a] Means ± S.D.

[b] One female died from problems associated with parturition.

Table 48. Average kit body and litter weights and kit survival for dams fed various concentrations of thiram for 23 weeks.

Concentration (ppm)	Average kit body weight (g)			Average litter weight (g)			% survival	
	Birth	3 weeks	6 weeks	Birth	3 weeks	6 weeks	3 weeks	6 weeks
0	9.7 ± 1.69[a]	96.5 ± 16.14	254.2 ± 66.30	37.9 ± 20.31	410.1 ± 151.89	1126 ± 341.7	85.0	85.0[b]
2.5	9.8 ± 1.88	93.7 ± 22.91	260.2 ± 72.12	48.9 ± 22.85	393.4 ± 236.52	1041 ± 661.9	84.0	80.0
10.0	8.9 ± 1.99	114.2** ± 10.35	318.0** ± 46.04	43.1 ± 21.44	445.2 ± 233.70	1240 ± 615.6	73.6	73.6
40.0	7.9** ± 1.34	87.4 ± 25.38	258.1 ± 28.0	29.4 ± 21.63	349.5 ± 175.43	1118 ± 284.4	88.9[c]	72.2[c]

[a] Means ± S.D.

[b] Does not include litter killed by dam.

[c] Does not include litter lost from death of dam due to causes unrelated to effects of test substance.

** Significantly different from control ($P \leq .01$).

Table 49. Blood parameters of mink fed various concentrations of thiram for 6 months.

Concentration (ppm)	n	RBC ($x10^6$)	WBC ($x10^3$)	Hb (g/dl)	Hct (%)
0	8	11.05[a] ± 0.606	12.7 ± 2.68	23.1 ± 0.83	55.2 ± 2.74
2.5	8	10.38 ± 1.073	12.0 ± 5.16	21.7 ± 1.93	54.2 ± 1.86
10.0	8	10.05 ± 1.172	18.8 ± 5.70	22.3 ± 1.41	54.8 ± 3.18
40.0	7	8.79** ± 0.761	17.8 ± 7.69	20.7** ± 1.17	51.2* ± 3.11

[a] Mean ± S.D.

* Significantly different from control ($P \leq .05$).

** Significantly different from control ($P \leq .01$).

Table 50. Body and organ weights of male (M) and female (F) mink fed various concentrations of thiram for 6 months.

Concentration (ppm)		Body wgt. (g)	Brain (% body)	Liver (% body)	Spleen (% body)	Kidney (% body)	Lung (% body)	Heart (% body)
0	M	1833.0	.573	3.527	.338	.566	.692	.494
	F	912.5	.849	3.971	.406	.600	.739	.594
2.5	M	1654.8	.578	3.449	.312	.604	.681	.568
	F	970.8	.850	3.698	.269	.573	.822	.585
10.0	M	1597.5	.614	3.719	.437	.594	.599	.552
	F	1002.5	.788	4.130	.468	.601	.699	.659
40.0	M	1614.2	.595	4.034	.558	.594	.846	.613
	F	980.0	.776	4.212	.690	.586	.675	.516
		Organs expressed as a percent of brain weight						
0	M			620.3	59.8	99.3	119.4	87.0
	F			470.6	48.4	70.7	86.7	70.0
2.5	M			608.9	54.9	105.5	118.3	99.0
	F			439.1	31.8	68.3	96.7	69.1
10.0	M			609.6	72.2	97.9	98.2	90.5
	F			524.0	59.2	76.1	88.7	83.6
40.0	M			677.2	94.0**	101.1	141.2	103.4
	F			545.4	88.3**	75.1	87.2	66.8

** Significantly different from control ($P \leq .01$).

40 ppm. In fact, significant increases in kit body weight at 3 and 6 weeks were seen for the 10 ppm group.

The hematologic parameters (Table 49) showed decreases in RBC count, hemoglobin concentration, and hematocrit at 40 ppm. The organ weight data (Table 50) revealed a significant increase in the spleen (as a percentage of brain weight) for both sexes at 40 ppm.

Ferret Reproduction Test:

Based on the results of the LC_{50} test, where adverse effects were noted in ferrets at 20 ppm, dietary concentrations of 0, 4, 16, and 64 ppm were selected. Distilled water was the carrier used.

A 9-day acclimation period was begun on 7 February, 1983. The reproduction test was initiated on 17 February, 1983 and terminated on 7 July, 1983. Weight changes were recorded bi-weekly until 14 April, 1983, and feed consumption was measured during weeks 1, 3, 5, and 7.

Body weight changes and feed and thiram consumption are summarized in Tables 51 and 52. Female ferrets fed 64 ppm lost less weight than did controls during the 8 weeks of the pre-breeding period. No signs of intoxication or mortalities due to effects of the compound were noted during the test, but necropsy at the terminal kill revealed white nodules ranging in size from 0.5-3 mm on the lungs of some ferrets on all dietary concentrations, including controls, and may be indicative of pneumonia in the ferret strain.

Table 51. Average body weight changes and feed[a] and compound[b] consumed by ferrets fed various concentrations of thiram during a reproduction test.

Concentration (ppm)	Parameter measured	Acclimation	Week 1	Week 2	Week 3	Week 4	Cumulative
0	Weight change (g)	+54.7	+62.0	+ 5.1	-95.8	-99.6	-128.3[c]
	Feed consumption (g/d)	215	198	176	166	124	---
	Compound consumption (mg/d)	0	0	0	0	0	---
	Compound consumption (mg/2 wk)	0	0	0	0	0	0
4.0	Weight change (g)	+10.4	+58.9	- 0.8	-94.3	-90.1	-123.1[c]
	Feed consumption (g/d)	201	188	169	139	112	---
	Compound consumption (mg/d)	0	0.75	0.68	0.56	0.57	---
	Compound consumption (mg/2 wk)	0	10.50	9.52	7.84	7.98	35.84
16.0	Weight change (g)	+ 8.9	+56.8	-11.1	-52.3	-80.4	- 87.0[c]
	Feed consumption (g/d)	214	170	151	152	138	---
	Compound consumption (mg/d)	0	2.72	2.41	2.43	2.20	---
	Compound consumption (mg/2 wk)	0	38.08	33.74	34.02	30.80	136.64
64.0	Weight change (g)	+ 5.8	+19.7	- 6.9	-86.5	-46.4	- 80.1[c]
	Feed consumption (g/d)	205	162	154	133	124	---
	Compound consumption (mg/d)	0	10.34	9.88	8.52	7.96	---
	Compound consumption (mg/2 wk)	0	144.76	138.32	119.28	111.44	513.80

[a] Feed consumption based on the average of two consecutive days consumption.

[b] Daily compound consumption calculated from average feed consumption times dietary concentration; bi-weekly compound consumption from daily consumption times 14.

[c] Body weight changes based on values for 16 animals (4 males, 12 females).

Table 52. Initial and final body weights of male (M) and female (F) ferrets fed various concentrations of thiram for 8 weeks prior to breeding.

Concentration (ppm)	Sex	n	Initial wgt. (g)	Final wgt. (g)	Change (g)
0	M	4	1980.2	1831.8	- 98.5
	F	12	925.0	787.2	-137.8
4.0	M	4	1819.2	1709.8	-109.5
	F	12	877.8	743.2	-134.6
16.0	M	4	1728.0	1658.0	- 70.0
	F	12	869.8	777.1	- 92.7
64.0	M	4	1779.8	1689.2	-127.8
	F	12	909.7	833.1	- 76.6*

* Significantly different from control ($P \leq .05$).

Table 53. Reproductive performance of female ferrets fed various concentrations of thiram for 23 weeks.

Concentration (ppm)	♀'s bred/ total	♀'s whelped/ ♀'s bred	Gestation (days)	Live lits/ ♀ whelped	Total kits ♀ whelped
0	12/12	12[a] /12	41.3 ± 0.98[b]	10.6 ± 2.58	11.2 ± 2.66
4.0	12/12	9/12	41.3 ± 0.71	11.3 ± 1.94	11.7 ± 2.00
16.0	12/12	8/12	41.5 ± 0.76	8.2 ± 3.41	9.2 ± 4.37
64.0	7/12	0/7	---	0	0

[a] One female died from problems associated with parturition.

[b] Means ± S.D.

Table 54. Average kit body and litter weights and kit survival for dams fed various concentrations of thiram for 23 weeks.

Concentration (ppm)	Average kit body weight (g)			Average litter weight (g)			% survival	
	Birth	3 week	6 week	Birth	3 week	6 week	3 week	6 week
0	8.9 ± 1.53[a]	86.3 ± 17.87	320.3 ± 55.10	94.2 ± 18.61	886.9 ± 85.80	3232 ± 422.6	95.8	94.1
4.0	8.8 ± 1.45	82.2 ± 17.30	292.9** ± 56.96	99.2 ± 16.45	867.7 ± 159.27	2996 ± 583.2	93.1	89.2
16.0	8.7 ± 1.50	76.7** ± 26.54	300.4 ± 61.24	69.7* ± 23.60	604.2** ± 210.47	1990** ± 995.4	96.9	81.5
64.0	---	---	---	---	---	---	--	--

[a] Means ± S.D.

* Significantly different from control ($P \leq .05$).

** Significantly different from control ($P \leq .01$).

Table 55. Blood parameters of ferrets fed various concentrations of thiram for 6 months.

Concentration (ppm)	n	RBC ($x10^6$)	WBC ($x10^3$)	Hb (g/dl)	Hct (%)
0	8	12.08[a] ± 1.069	11.5 ± 3.65	22.4 ± 1.71	57.3 ± 2.54
4	8	11.00 ± 0.457	12.3 ± 2.47	21.4 ± 1.43	56.2 ± 3.98
16	8	10.91 ± 0.826	13.1 ± 4.47	21.5 ± 2.46	55.8 ± 6.37
64	8	8.92** ± 1.587	14.8 ± 6.84	18.8** ± 1.66	49.2** ± 5.59

[a] Mean ± S.D.

** Significantly different from control ($P \leq .01$).

Table 56 . Body and organ weights of male (M) and female (F) ferrets fed various concentrations of thiram for 6 months.

Concentration (ppm)		Body wgt. (g)	Brain (% body)	Liver (% body)	Spleen (% body)	Kidney (% body)	Lung (% body)	Heart (% body)
0	M	1549.5	.535	4.003	.663	.689	.625	.551
	F	916.0	.731	4.138	.506	.602	.669	.532
	C[a]	---	--	---	.584	.646	--	--
4	M	1433.8	.555	3.916	.688	.664	.654	.528
	F	792.0	.824	4.211	.670	.685	.742	.591
	C	---	--	---	.679	.674	--	--
16	M	1454.0	.527	4.047	1.130	.647	.618	.511
	F	792.0	.810	4.380	.792	.625	.732	.594
	C	---	--	---	.961**	.636	--	--
64	M	1470.8	.541	3.873	1.245	.643	.700	.553
	F	738.2	.823	4.388	1.272	.699	.812**	.572
	C	---	--	---	1.258**	.671	--	--
		Organs expressed as a percent of brain weight						
0	M			760.0	128.8	128.9	118.0	104.2
	F			573.6	71.4	83.8	92.5	74.1
	C			---	---	---	---	---
4	M			712.8	124.0	120.6	118.0	95.5
	F			513.7	81.6	83.7	90.1	72.1
	C			---	---	---	---	---
16	M			770.5	213.1*	122.7	117.1	96.9
	F			541.2	98.3	77.3	90.6	73.2
	C			---	---	---	---	---
64	M			724.2	233.1**	119.8	130.3	103.3
	F			535.9	154.7	85.3	98.9	69.8
	C			---	---	---	---	---

[a] Sexes are combined where no significant difference ($P \leq .05$) was found between them.

* Significantly different from control ($P \leq .05$).

** Significantly different from control ($P \leq .01$).

No obvious birth defects were observed. Reproduction was prevented in the 64 ppm diet, and the reproductive indices (Tables 53 and 54) revealed effects on the growth of the kits at 4 and 16 ppm as well.

The hematologic parameters (Table 55) showed decreases in RBC count, hemoglobin concentration, and hematocrit at the highest dietary concentration, as in the mink reproduction test. Likewise, the organ weight data (Table 56) revealed increased spleen weight at the highest concentration, as in the mink reproduction test, as well as at the middle level. An increase in the lung weight (as a percentage of body weight) of females fed 64 ppm was also seen.

DISCUSSION

Thiram affected feed consumption and body weight gain in mink at dietary concentrations of 82 ppm and greater and in ferrets at 50 ppm and greater. Feed consumption and body weight have been reported to be negatively affected in rats (Lee et al., 1978; Lowy et al., 1980), chickens (Waibel et al., 1957; Rasul and Howell, 1974), turkeys, and geese (Waibel et al., 1957) during subacute exposure to 150 to 400 ppm of thiram. It is interesting to note that at 20 ppm male ferret body weight was significantly affected, even though feed consumption during the test was approximately equal to controls. This illustrates:

1) the greater sensitivity of ferrets to thiram than the rat, chicken, turkey, and goose, and
2) the possibility that thiram affects body weight in a manner not entirely due to feed intake.

The effect of 20 ppm dietary thiram was also more severe in the males, illustrating the fact that, since the average male body weight is approximately twice the average female body weight (in both ferrets and mink), the male has more weight (as body fat) to lose during a subacute poisoning.

It is difficult to draw strong conclusions from the mink LC_{50} data, or to make comparisons to the ferret data based on such fragmentary results. It is apparent that 82 ppm thiram added in water was detrimental to mink, while the results from the 45 ppm group indicate that this concentration may be safe for mink (it must be remembered that only four animals were exposed in both of these groups). In stark contrast to these results, the eight mink fed 1543 ppm thiram added in oil consumed a normal amount of feed over 25 days (with a cumulative intake of > 12000 mg of thiram) and regained all the weight lost during the week of exposure to 1543 ppm thiram added in water. (During this week, the mink's feed consumption averaged only 25 g/d, and two animals died). From these results, it is apparent that the claim that the toxicity of thiram is enhanced in the presence of fats and oils (Merck Index, 1976) does not hold for mink. In fact, the opposite was true in this test.

With regard to the reproduction tests, an inspection of the body weight data reveals a dose-dependent decrease in weight change for male mink, with a significant decrease at 40 ppm ($P \leq .05$), although the average weight of the males in this group was within the range of normal body weights for male mink. No such pattern was evident for the ferrets. In fact,

the weight loss for female ferrets fed 64 ppm was significantly less than the loss by controls ($P \leq .05$). This may be indicative of a disruption in the normal pre-estrus pattern of female ferrets, which is marked by reduced feed consumption and weight loss.

Further evidence of a disruption of the normal reproductive cycle of female ferrets was evident from the number of females bred. In the 64 ppm group, only 7 of 12 females were judged to be in estrus (as determined by the extent of vulvar swelling) at the termination of the 8-week exposure period, and none of the 12 whelped. Evidence of disruption of the normal reproductive cycle of female mink is more difficult to quantify, since female mink do not normally exhibit decreased feed consumption and weight loss of the same magnitude as female ferrets, nor do they exhibit noticeable vulvar swelling during estrus. Thiram has been associated with disruptions of the estrus cycle in previous experiments. Davydova (1973) found an extension of the estrus cycle at the expense of the resting cycle in rats exposed to 3.8 mg thiram/m^3 of air for 5 hr/day, 5 days/wk for 4.5 months. A prolonging of the diestrus phase has been reported in rats fed 400 ppm thiram in the diet for 14 days prior to mating. Wedig *et al.* (1968) reported inhibition of ovulation in bobwhite quail at oral doses as low as 8.8 mg/kg/day.

The only reproductive effect of thiram on female mink was a significant decrease in the average kit birth weight at 40 ppm ($P \leq .05$). All other reproductive indices were within the

range of normal values (although the number of females whelped and the number of live kits per female at 40 ppm was quite low). Actually, the 3 week and 6 week body weights of kits on the 10 ppm diet were significantly greater than controls, and were somewhat above the normal range. Since the litter size was not decreased at 10 ppm this high body weight was not due to fewer but larger kits. The reason for this increase is unknown.

The reproductive effect of thiram on female ferrets was more severe. Reproduction was prevented at 64 ppm, and kit body weight at 3 weeks and litter weight at birth, 3, and 6 weeks are all significantly less than controls ($P \leq .05$) at 16 ppm. Six week survival was also less than control ($P \leq .10$). (The same trend of decreased 6 week survival was apparent in the mink reproduction test, although the decrease at 40 ppm was not significant). From the results of these two reproduction tests, it would appear that female ferrets are more sensitive to thiram than female mink.

Decreases in the number of offspring per female, birth weight, and/or offspring viability have been reported in several studies with thiram. Robens (1969) has reported increased resorption of fetuses, low birth weight, and decreased viability of offspring in hamsters given 125 or 250 mg/kg thiram orally from days 5-15 of gestation. Short et al. (1976) found a significant decrease in the number of implants and number of pups/female in rats fed 400 ppm in the diet for 14 days prior to mating. These authors also reported no reproduction due to 100% resorption at oral doses of 164 and 200 mg/kg, decreased

number of pups/female at 136, 164, and 200 mg/kg, and decreased live birth weight at all doses (40, 90, and 136 mg/kg) for rats given thiram po during days 6-15 of gestation. In similar studies with mice, these authors noted no changes in litter sizes, incidence of resorption, or birth weight at oral doses of 100 or 300 mg/kg administered during days 6-14 of gestation. Finally, these authors found no effect on viability or growth with rats fed 300 ppm of thiram in the diet from day 16 of gestation through day 21 post partum, but 100% mortality of pups by day 21 post partum at a dietary concentration of 1000 ppm.

Examination of the blood and organ weight data of the four tests revealed a consistent pattern of response in mink and ferrets upon dietary exposure to thiram. The hematologic parameters showed an anemic condition at the highest dietary concentrations for all tests, while the spleen weights (as a percentage of brain weight) at these same levels were increased over controls. Whether these results are related would require further tests. Increased spleen weight has been reported by Lee and Peters (1976) in rats fed 400 and 1000 ppm thiram in the diet for 80 weeks, but not in rats fed 100 ppm for this length of time. These authors also reported increased weights of liver, kidney, and brain at 1000 ppm, results seen only in the ferret LC_{50} test among these experiments. They also reported no changes in blood chemistry in this study, while decreases in RBC count, hemoglobin concentration, and hematocrit occurred at the highest dietary concentration in

all tests in which these parameters were determined. Increased lung weight was occasionally seen in these experiments, although the cause and significance of these findings are unclear.

The dietary no effect level of thiram for rats has been reported to be 38 ppm (Lowy et al., 1979; 1980) or 48 ppm (WHO, 1965). In the LC_{50} tests of this experiment, a dietary no effect level was not found for mink (since a decrease in hematocrit was seen at 45 ppm), while the no effect level for ferrets may have been 8 ppm (if the increase in brain weight of females at this dietary concentration is regarded as not biologically significant). These results again illustrate the relative sensitivity of mink and ferrets to toxic compounds. In fact, the results of an 80 week chronic feeding study with rats (Lee and Peters, 1976) show just how sensitive mink and ferrets are to thiram in relation to rats. In this study, some rats survived the highest dietary concentration, 1000 ppm, for the 80 weeks of the study, while in the 28-day LC_{50} tests, mink were removed from the test at concentrations of $\geq$ 147 ppm (to prevent the animals from starving) and all ferrets fed 312 ppm had died by the 16th day of the test.

CONCLUSIONS

The following conclusions may be drawn from tests performed with thiram on mink and ferrets:

1) Mink reject feed containing $\geq$ 82 ppm thiram.
2) The dietary LC_{50} for ferrets lies between 125 and 312 ppm.

3) Thiram added to the diet with water as the carrier affects mink much more severely than thiram added in corn oil.
4) Signs of thiram intoxication in mink and ferrets include reduced feed consumption or feed rejection, weight loss, tarry stools, listlessness, and occasional convulsions accompanied by high-pitched vocalizations.
5) Reproduction is prevented in ferrets at 64 ppm. Interference with the estrus cycle is the probable cause of the reproductive problem. 16 ppm of thiram in the diet of ferrets causes low birth weight, as does 40 ppm in the diet of mink.
6) Ferrets appear to be more sensitive to the reproductive effects of thiram than mink.
7) Mink and ferrets appear to be more sensitive to the effects of thiram in the diet than rats.

Experiment IV - Results

Two dietary LC_{50} tests were conducted with Aroclor 1254. One of the objectives of these tests was to determine differences in the effects of PCB intoxication on young, rapidly growing animals and older animals whose body weight changes are primarily due to fat deposition. The ages of animals used in these tests were approximately 13 to 17 weeks old for the younger animals and > 1 year old for the older animals.

Based on results of previous experiments conducted in this laboratory, in which an approximate LC_{50} for Aroclor 1254 over a 9 month study was calculated to be 6.65 mg/kg

(Ringer et al., 1981), dietary concentrations of 0, 10.0, 18.0, 32.4, 58.3, and 105.0 ppm were chosen for the 28-day studies. The Aroclor was dissolved in acetone, mixed with a small amount of ground mink cereal, and evaporated to dryness. The pre-mix was then added to the basal diet. Actual dietary concentrations of Aroclor 1254 have not been determined.

A 21-day acclimation period was begun on 29 July, 1982 for young animals. The 28-day LC_{50} trial began on 19 August, 1982, and ended on 16 September, 1982, followed by a 7-day withdrawal period. The test was terminated on 23 September, 1982. A 21-day acclimation period was begun on 7 October, 1982 for older animals. The 28-day LC_{50} trial began on 28 October, 1982, and ended on 25 November, 1982, followed by a 7-day withdrawal period. The test was terminated on 2 December, 1982.

Signs of intoxication were first noted on day 12 for the young animals, when 2 animals fed the 105.0 ppm diet were noted to be listless, and also on day 12 for the older animals, when several animals on the two highest diets were observed to have tarry stools. Feed consumption was affected earlier in both groups, since some animals in both tests exhibited reduced feed consumption by day 4. Reduced feed consumption appeared to be more pronounced among the older animals, being noted earlier and at lower dietary concentrations than for the young animals. Body weight changes and feed and compound consumption are summarized in Tables 57 and 58 for young animals and Tables 59 and 60 for older animals.

Table 57. Average body weight changes and feed[a] and compound[b] consumed by young mink fed various concentrations of Aroclor 1254 for 28 days.

Concentration (ppm)	Parameter measured	Acclimation	Week 1	Week 2	Week 3	Week 4	Withdrawal	4 week cumulative	5 week cumulative
0	Weight change (g)	+54.5	+41.6	+ 59.4	- 15.1	+ 18.6	+ 1.9	+104.5(10)[c]	+106.4(10)[c]
	Feed consumption (g/d)	250	288	278	293	223	260	---	---
	Compound consumption (mg/d)	0	0	0	0	0	0	---	---
	Compound consumption (mg/wk)	0	0	0	0	0	0	0	0
10.0	Weight change (g)	+76.5	+48.1	+ 17.9	- 37.5	- 2.9	-63.7	+ 25.6(10)[c]	- 38.1(10)[c]
	Feed consumption (g/d)	260	274	230	233	184	204	---	---
	Compound consumption (mg/d)	0	2.74	2.30	2.33	1.84	0	---	---
	Compound consumption (mg/wk)	0	19.16	16.06	16.32	12.92	0	64.46	64.46
18.0	Weight change (g)	+88.7	+30.1	- 25.9	- 68.2	- 14.2	-23.9	- 78.2(10)[c]	-102.1(10)[c]
	Feed consumption (g/d)	260	281	212	196	190	200	---	---
	Compound consumption (mg/d)	0	5.05	3.82	3.53	3.41	0	---	---
	Compound consumption (mg/wk)	0	35.37	26.75	24.70	23.90	0	110.72	110.72
32.4	Weight change (g)	+83.3	+ 8.2	- 55.1	- 89.0	- 41.2	-61.9	-177.1(10)[c]	-208.1(9)[c]
	Feed consumption (g/d)	258	228	145	151	164	164	---	---
	Compound consumption (mg/d)	0	7.37	4.71	4.89	5.32	0	---	---
	Compound consumption (mg/wk)	0	51.62	32.99	34.21	37.25	0	156.07	156.07
58.3	Weight change (g)	+72.4	-33.7	-114.8	-116.2	- 90.0	-49.8	-296.2(8)[c]	-328.4(5)[c]
	Feed consumption (g/d)	291	197	110	107	94	152	---	---
	Compound consumption (mg/d)	0	11.50	6.39	6.24	5.50	0	---	---
	Compound consumption (mg/wk)	0	80.52	44.73	43.69	38.48	0	207.42	207.42
105.0	Weight change (g)	+94.2	-71.7	-149.4	-157.3	-126.2	--	-473.6(5)[c]	--- (0)[c]
	Feed consumption (g/d)	290	162	94	81	52	--	---	---
	Compound consumption (mg/d)	0	17.06	9.89	8.55	5.50	--	---	---
	Compound consumption (mg/wk)	0	119.42	69.24	59.86	38.49	--	287.01	287.01

[a] Feed consumption based on the average of two consecutive days consumption.

[b] Daily compound consumption calculated from average feed consumption times dietary concentration; weekly compound consumption from daily consumption times 7.

[c] Body weight change based on the number of live animals shown in parentheses.

Table 58. Initial and final weights of young male (M) and female (F) mink fed various concentrations of Aroclor 1254 for 28 days and after a 7-day withdrawal period.

Concentration (ppm)	Sex	n	Initial wgt. (g)	Final wgt. (g)	Change (g)	Change after withdrawal (g)
0	M	5	1232.6	1376.7	+144.0	-158.0
	F	5	860.8	925.8	+ 65.0	+ 63.6
10.0	M	5	1310.0	1381.0	+ 71.0	+ 23.6
	F	5	908.8	889.0	- 19.8	- 99.8
18.0	M	5	1296.6	1231.4	- 65.2**	- 85.8**
	F	5	912.4	821.2	- 91.2	-118.4*
32.4	M	5	1279.4	1075.4*	-204.0**	-295.6**
	F	5	826.8	676.6	-150.2**	-182.4**
58.3	M	5	1330.6	848.8**	-463.4**	-507.6**
	F	5	910.2	643.6*	-266.6**	-272.2**
105.0	M	5	1324.8	741.2**	-583.6**	-583.6**
	F	5	915.0	536.6**	-378.4**	-378.4**

* Significantly different from control ($P \leq .05$).

** Significantly different from control ($P \leq .01$).

Table 59. Average body weight changes and feed[a] and compound[b] consumed by older mink fed various concentrations of Aroclor 1254 for 28 days.

Concentration (ppm)	Parameter measured	Acclimation	Week 1	Week 2	Week 3	Week 4	Withdrawal	4 week cumulative	5 week cumulative
0	Weight change (g)	+72.6	+ 64.5	+ 3.3	+ 31.8	+ 4.8	-29.6	+104.4(10)[c]	+ 74.8(10)[c]
	Feed consumption (g/d)	377	323	320	315	241	199	---	---
	Compound consumption (mg/d)	0	0	0	0	0	0	---	---
	Compound consumption (mg/wk)	0	0	0	0	0	0	0	0
10.0	Weight change (g)	+46.6	- 21.8	- 30.8	- 51.6	- 43.4	+35.6	-147.6(10)[c]	-100.4(9)[c]
	Feed consumption (g/d)	342	199	196	180	148	203	---	---
	Compound consumption (mg/d)	0	1.99	1.96	1.80	1.48	0	---	---
	Compound consumption (mg/wk)	0	13.95	13.69	12.62	10.37	0	50.63	50.63
18.0	Weight change (g)	+71.8	- 41.8	-108.9	- 74.3	- 46.8	+19.3	-271.8(10)[c]	-252.5(10)[c]
	Feed consumption (g/d)	357	157	181	177	198	192	---	---
	Compound consumption (mg/d)	0	2.82	3.25	3.19	3.57	0	---	---
	Compound consumption (mg/wk)	0	19.76	22.77	22.33	24.97	0	89.83	89.83
32.4	Weight change (g)	+62.7	- 84.6	- 63.3	- 83.3	- 74.4	+ 8.9	-305.6(10)[c]	-299.3(9)[c]
	Feed consumption (g/d)	322	148	158	154	137	176	---	---
	Compound consumption (mg/d)	0	4.78	5.12	4.98	4.44	0	---	---
	Compound consumption (mg/wk)	0	33.45	35.83	34.84	31.12	0	135.24	135.24
58.3	Weight change (g)	+37.6	-125.9	-100.8	-143.0	-132.4	-39.8	-527.6(5)[c]	-606.2(4)[c]
	Feed consumption (g/d)	274	100	142	95	105	136	---	---
	Compound consumption (mg/d)	0	5.84	8.30	5.54	6.11	0	---	---
	Compound consumption (mg/wk)	0	40.89	58.07	38.81	42.77	0	180.54	180.54
105.0	Weight change (g)	+15.8	-176.2	- 87.6	-197.9	-167.5	-24	-626.5(3)[c]	-714 (1)[c]
	Feed consumption (g/d)	291	54	105	58	70	138	---	---
	Compound consumption (mg/d)	0	5.71	11.01	6.06	7.40	0	---	---
	Compound consumption (mg/wk)	0	39.98	77.10	42.41	51.74	0	211.23	211.23

[a] Feed consumption based on the average of two consecutive days consumption.

[b] Daily compound consumption calculated from average feed consumption times dietary concentration; weekly compound consumption from daily consumption times 7.

[c] Body weight change based on the number of live animals shown in parentheses.

Table 60. Initial and final weights of older male (M) and female (F) mink fed various concentrations of Aroclor 1254 for 28 days, and after a 7-day withdrawal period.

Concentration (ppm)	Sex	n	Initial wgt. (g)	Final wgt. (g)	Change (g)	Change after withdrawal (g)
0	M	5	1753.6	2000.2	+246.6	+210.4
	F	5	1055.0	1017.2	- 37.8	- 60.8
10.0	M	5	1711.0	1557.0**	-150.8**	- 98.6**
	F	5	1080.4	939.2	-141.2	-130.6
18.0	M	5	1703.8	1370.2**	-333.6**	-290.0**
	F	5	1103.0	893.0	-210.0	-215.0
32.4	M	5	1592.6	1215.2**	-377.4**	-392.0**
	F	5	1076.0	842.2	-235.8*	-211.2
58.3	M	5	1570.4	978.0**	-594.4**	-605.2**
	F	5	954.2	575.2**	-379.0**	-402.2**
105.0	M	5	1710.6	983.8**	-719.6**	-731.6**
	F	5	953.0	494.8**	-458.2**	-458.2**

* Significantly different from control ($P \leq .05$).

** Significantly different from control ($P \leq .01$).

Clinical signs included reduced feed consumption, weight loss, listlessness, and unconsciousness prior to death. Some animals also had bloody or tarry stools near death. At the end of the 28-day exposure period for young animals, 2 of 10 animals had died on the 58.3 ppm diet and 7 of 10 had died on the 105.0 ppm diet. After the withdrawal period, 1 of 10 animals had died on the 32.5 ppm diet, 5 of 10 died on the 58.3 ppm diet, and 10 of 10 died on the 105.0 ppm diet. The mortality pattern is described in Table 61. For the older animals, 5 of 10 animals on the 58.3 ppm diet and 8 of 10 animals on the 105.0 ppm diet had died by the end of the 28-day exposure period. After the withdrawal period, 1 of 10 animals had died on the 10.0 and 32.5 ppm diets, 6 of 10 died on the 58.3 ppm diet, and 9 of 10 died on the 105.0 ppm diet. The mortality pattern is described in Table 62. Blood-filled intestines were usually found at necropsy of mortalities, and ascites and/or hydropericardium was occasionally noted. Occasional gastric ulcers were also noted at necropsy of mortalities and at the terminal kill.

The data yielded LC_{50}'s of 105.0 ppm, with a 95% confidence interval of 79.4 to 139.0 ppm, and a slope of 1.57, with a 95% confidence interval of 1.45 to 1.70, for the 28-day exposure, and 58.3 ppm, with a 95% confidence interval of 39.1 to 87.0 ppm, and a slope of 1.58, with a 95% confidence interval of 1.16 to 2.15, for the 28-day exposure period plus the withdrawal period for the young mink. For the older animals, the data yielded LC_{50}'s of 84.0 ppm, with a 95% confidence interval

Table 61. Mortality pattern of young mink fed Aroclor 1254 during a 28-day LC_{50} test.

Concentration (mg/kg)	No of animals dying/day of test																																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
0																																			
10.0																																			
18.0																																			
32.4																																			1
58.3																			1								1		1	1				1	
105.0																	1						1		1	1	1	2	1	1		1			

Table 62. Mortality pattern of older mink fed Aroclor 1254 during a 28-day LC_{50} test.

Concentration (mg/kg)	No of animals dying/day of test																																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
0																																			
10.0																														1					
18.0																																			
32.4																																1			
58.3																				1	1				1		1	1				1			
105.0																				1	1				3	1		2				1			

Table 63. Body and organ weights of growing male (M) and female (F) mink fed various concentrations of Aroclor 1254 for 28 days.

Concentration (ppm)		Body wgt. (g)	Brain (% body)	Liver (% body)	Spleen (% body)	Kidney (% body)	Lung (% body)	Heart (% body)
0	M	1390.6	0.77	4.03	0.29	0.62	0.67	0.74
	F	924.5	0.93	4.13	0.29	0.59	0.68	0.69
	C[a]	---	--	4.08	0.29	--	0.68	0.72
10.0	M	1333.6	0.79	5.34	0.26	0.56	0.73	0.63
	F	809.0	1.17	5.17	0.32	0.67	0.84	0.67
	C	---	--	5.25**	0.29	--	0.79	0.65
18.0	M	1210.8	0.86	5.75	0.37	0.65	0.87	0.70
	F	794.0	1.12	5.89	0.36	0.69	0.98	0.68
	C	---	--	5.82**	0.36	--	0.92*	0.69
32.4	M	983.8**	1.12	5.45	0.33	0.76*	1.18	0.75
	F	644.4*	1.34*	6.09	0.43	0.87**	1.00	0.83
	C	---	--	5.77**	0.38	--	1.09**	0.79
58.3	M	804.6**	1.43**	5.57	0.34	0.91**	1.11	0.72
	F	625.6*	1.45**	6.46	0.36	0.95**	1.19	0.73
	C	---	--	6.01**	0.35	--	1.15**	0.73
105.0	M	739.4**	1.51**	5.47	0.31	0.93**	1.25	0.76
	F	491.0**	1.77**	5.25	0.35	1.11**	1.22	0.78
	C			5.36**	0.33	--	1.24**	0.77
		Organs expressed as a percent of brain weight						
0	M			520.9	37.2	80.4	86.6	96.3
	F			441.2	31.1	64.0	73.9	73.0
	C			---	34.1	--	--	--
10.0	M			678.9	33.6	71.8	93.4	80.2
	F			466.2	28.9	58.3	73.4	59.1
	C			---	31.2	--	--	--
18.0	M			676.8	44.2	76.7	100.6	81.8
	F			525.5	31.8	61.7	87.5	60.7
	C			---	38.0	--	--	--
32.4	M			496.7	31.4	69.1	105.2	67.6**
	F			455.5	32.6	65.4	75.7	61.9
	C			---	32.0	--	--	--
58.3	M			443.2	29.1	68.4	84.4	56.9**
	F			470.0	27.1	67.7	85.2	52.5*
	C			---	28.1	--	--	--
105.0	M			366.0	20.3	62.8*	84.9	51.4**
	F			296.7	19.6	62.5	68.4	43.8**
	C			---	19.9*	--	--	--

[a] Sexes are combined where no significant difference ($P \leq .05$) was found between them.

* Significantly different from control ($P \leq .05$).

** Significantly different from control ($P \leq .01$)

Table 64. Body and organ weights of fully grown male (M) and female (F) mink fed various concentrations of Aroclor 1254 for 28 days.

Concentration (ppm)		Body wgt. (g)	Brain (% body)	Liver (% body)	Spleen (% body)	Kidney (% body)	Lung (% body)	Heart (% body)
0	M	1964.0	0.52	4.16	0.20	0.48	0.61	0.52
	F	994.2	0.79	5.29	0.34	0.59	0.81	0.66
	C[a]	---	--	--	--	0.53	--	0.59
10.0	M	1610.4*	0.61	5.73	0.31	0.60	0.92	0.65
	F	1038.8	0.80	5.95	0.35	0.60	0.91	0.70
	C	---	--	--	--	0.60	--	0.68
18.0	M	1413.8**	0.72	6.41**	0.28	0.69	1.04*	0.91
	F	888.0	0.96	7.64**	0.50	0.85	1.01	0.83
	C	---	--	--	--	0.77**	--	0.87**
32.4	M	1200.6**	0.83*	6.87**	0.32	0.79	1.13**	0.77
	F	864.8	1.01	6.96*	0.38	0.76	1.07	0.77
	C	---	--	--	--	0.78**	--	0.77**
58.3	M	965.2**	1.02**	6.02*	0.28	0.87	0.98*	0.68
	F	552.0*	1.45**	6.00	0.34	0.95	1.32**	0.67
	C	---	--	--	--	0.91**	--	0.67
105.0	M	979.0**	1.15**	5.14	0.25	0.89	0.99*	0.69
	F	494.8**	1.72**	5.53	0.29	0.96	1.43**	0.78
	C	---	--	--	--	0.92**	--	0.73*
		Organs expressed as a percent of brain weight						
0	M			816.9	39.6	92.6	119.3	101.7
	F			662.1	42.3	74.6	102.1	83.0
	C			---	40.9	--	---	---
10.0	M			930.6	50.4	98.7	149.3	106.9
	F			744.8	43.7	75.0	113.5	88.4
	C			---	47.4	--	---	---
18.0	M			890.4	38.5	95.4	145.2	127.0
	F			809.8	50.5	87.6	106.5	87.6
	C			---	44.5	--	---	---
32.4	M			845.1	38.9	97.2	139.6	95.8
	F			716.9	38.0	76.6	112.1	79.6
	C			---	38.4	--	---	---
58.3	M			630.4	28.8	89.2	97.4	68.0**
	F			413.8*	22.7	65.2	91.3	45.9**
	C			---	25.7*	--	---	---
105.0	M			480.7**	24.5	80.6	89.2	63.8**
	F			325.0**	16.5	56.1	84.7	45.4**
	C			---	20.5**	--	---	---

[a] Sexes are combined where no significant difference ($P \leq .05$) was found between them.

* Significantly different from control ($P \leq .05$).

** Significantly different from control ($P \leq .01$).

of 53.5 to 131.9 ppm, and a slope of 2.44, with a 95% confidence interval of 1.43 to 4.16, for the 28-day exposure, and 47.0 ppm, with a 95% confidence interval of 29.6 to 74.6 ppm, and a slope of 2.11, with a 95% confidence interval of 1.34 to 3.33, for the 28-day exposure period plus the withdrawal period.

Hematologic parameters were not determined for these tests. The organ weight data, summarized in Table 63 for young mink and Table 64 in older mink, revealed increases in liver weight (as a percentage of body weight) for all dietary concentrations for the young mink and all but the 10 and 105 ppm diets for older mink. Increases in brain, kidney, and lung (as percentages of body weight) were seen in the young mink at several concentrations, while these same organs plus the heart were increased in older mink. When the organs were expressed as percentages of brain weight, only the spleen, kidney, and heart were affected (reduced) in the younger animals, while the liver, spleen, and heart were affected (again reduced) in the older animals.

DISCUSSION

Several factors lead to the conclusion that the young, rapidly growing mink were less sensitive to the effects of Aroclor 1254. Obviously, the LC_{50} values for the older animals were lower at the end of the 28-day exposure and after the withdrawal period, although not dramatically different. It must be noted, however, that the initial body weights of the younger animals were considerably smaller than the initial

body weights of the older animals, especially for the males, and therefore the dose, on a mg/kg of body weight basis, for the younger animals was relatively higher. Thus, the difference between the corresponding LC_{50}'s was actually somewhat greater than it would appear.

Other factors that point to an increased sensitivity for the older animals are: (1) a more pronounced decrease in feed consumption, both in the magnitude of the differences between controls and exposure groups (e.g. for week 1, young controls consumed 288 g/d while young 105.0 ppm-fed animals consumed 162 g/d, and for week 1, older controls consumed 323 g/d while older 105.0-fed animals consumed 54 g/d), and in the number of dietary groups affected (e.g. for week 1, feed consumption was noticeably reduced from controls at 32.4, 58.3, and 105.0 ppm among the young animals, while feed consumption is noticeably reduced at all exposure groups among the older animals); (2) a more pronounded effect on cumulative weight change, again both in the magnitude of the differences and in the extent of the effect among the various exposure groups (although this effect was mediated somewhat by the fact that the older animals had more weight to lose); (3) a faster time to death for older mink (e.g. 26.5 days for young mink and 25.6 days for older mink fed 105.0 ppm, and 27.8 days for young and 25.5 days for older mink fed 58.3 ppm); and (4) a larger cumulative intake of Aroclor 1254 over the 28-day exposure period for all dietary concentrations of young mink versus older mink, which did not produce correspondingly larger weight losses or mortality.

Several other noteworthy results were obtained from these tests. It was apparent from the cumulative weight changes that a dose-dependent response was elicited upon exposure to Aroclor 1254. It also appears that males were more severely affected, although this was probably due to the fact that, in mink, the average body weight of the male is approximately double that of the female, and therefore, the male has more weight (as body fat) to lose. From the data for the withdrawal period, it would appear that a limited recovery was occurring in those animals still able to eat at the end of the 28-day exposure period, since feed consumption increased in nearly all treatment groups over week 4 levels, although in most instances the animals continued to lose weight. It was also clear from the data of these tests that a withdrawal period is necessary in LC_{50} tests with certain substances to obtain an accurate picture of the toxicity of the test substance.

The organ weight data revealed an increase in liver weight (as a percentage of body weight) in both age groups, as has been reported previously for mink (and most other animals) exposed to PCBs (Ringer et al., 1981). However, the weight of the organ (as a percentage of brain weight) increased up to 18 ppm in the younger animals and 32.4 ppm in the older animals, then decreased at higher concentrations. This response was probably a reflection of the fact that the animals fed the higher concentrations were either dead or dying at necropsy, and had probably used most or all of the glycogen store in the liver, leading to the lower liver weights.

Other effects noted in these studies are consistent with previous research with mink fed PCBs. Reduced feed consumption, reduced growth rate, enlarged kidneys, hemorrhaigc gastric ulcers, tarry stools, and hydropericardium/ascites have been reported by Ringer et al. (1981), and these results were also seen in these tests.

It is interesting to note that, had a reproduction test been performed based on the results of the LC_{50} test with the older mink, dietary concentrations of approximately 0.5, 2.0, and 8.0 ppm would have been chosen, based on the adverse effects noted at 10 ppm on body and liver weight. Since reproduction tests very similar to those conducted in these experiments have been conducted by this laboratory with Aroclor 1254, it is useful to review the findings of those experiments (Aulerich and Ringer, 1977). These authors tested the reproductive effects of 0, 1, 2, 5, 10, and 15 ppm of Aroclor 1254 in the diet, and found severe effects on reproduction at $\geq$ 2 ppm and outright mortality at 15 ppm. No reproductive effect was noted at 1 ppm. The dietary concentrations that would have been chosen based on the LC_{50} test results would have found both a dietary no effect level and an adverse dietary concentration in the reproduction test conducted according to the protocol presented here, based on the results of Aulerich and Ringer.

CONCLUSIONS

The following conclusions may be drawn from the tests performed with Aroclor 1254 on mink:

1) The 28-day dietary LC_{50} for young mink is 105 ppm and 84 ppm for older mink. Following a 7-day withdrawal period, these values are 58.3 and 47.0 ppm, respectively.
2) A dose-dependent weight loss occurs upon dietary exposure to Aroclor 1254.
3) Clinical signs of exposure include inanition, lethargy, and occasional unconsciousness and tarry stools. Enlarged liver and kidneys, gastric ulcers, ascites and/or hydropericardium, and blood filled intestines are often seen at necropsy.
4) Young mink appear to be less sensitive to the effects of Aroclor 1254 in the diet than older mink.
5) A withdrawal period is important in assessing the toxicity of a test substance if mortality or other signs of intoxication are still occurring at the end of the 28-day exposure.

Experimental Protocols - Results

The protocols developed for 28-day dietary LC_{50} and reproduction tests for wildlife mammalian carnivores as a result of theses tests have been submitted to the Environmental Protection Agency, and appear in Appendices A and B, respectively.

DISCUSSION

Various sections of the protocols are discussed within the final drafts submitted to EPA. This discussion will focus on an overview of the results of the experiments as they pertain to the development of the protocols.

The protocols developed for dietary LC_{50} and reproduction tests in these experiments allow the testing of a wide range of test substances (theoretically, any substances that can be mixed uniformly in the diet). The test substances used in these experiments covered a wide range of physico-chemical and biological properties, including: solid and liquid substances; water soluble, lipid soluble, and relatively insoluble compounds; volatile and non-volatile substances; and extremely toxic to relatively non-toxic test materials in acute oral exposure. The mode of action of the test substances ranged from metabolic poisons to neurotoxins to compounds of unknown biological mode of toxicity. The protocols as submitted thus appear to be capable of assessing the subacute or reproductive toxicity of most test substances (if they are not too unstable in air), providing an estimate of either a toxic level or a no effect level of the test substance.

The experiments have been conducted indoors under controlled conditions, suggesting that dietary LC_{50} and reproduction tests may be conducted in a reproducible fashion by properly equipped laboratories. It is interesting to note that, in

experiments initiated in this laboratory to assess the secondary toxicity of certain test substances, Aroclor 1254 was used as the test substance in 28-day dietary LC_{50} tests. In the primary toxicity test conducted in conjunction with these tests, using the protocols developed here, a dietary LC_{50} of > 74.8 ppm was found after 28 days' exposure, and an LC_{50} of 48.5 ppm, with a confidence interval of 36.5 to 64.5 ppm and slope of 1.92, was calculated after a 10-day withdrawal period (unpublished research, Michigan State University). These results are in very close agreement with the values calculated from the LC_{50} test with older mink in this study, even though the tests were conducted during different seasons (summer vs. late fall), different years, and had different protein sources in the diet (20% chicken vs. 40% rabbit).

The value of an accurate estimate of the acute oral LD_{50} of a test substance, obtained from either the literature or range-finding techniques, cannot be overstated. As evidenced by the ferret LC_{50} test with Compound 1080, a statistically valid dietary LC_{50} can be calculated with as few as 32 animals, using 3 dietary concentrations plus control and 8 animals per concentration, if an accurate LD_{50} is available from which to specify the test concentrations. Similarly, a good estimate of the dietary LC_{50} (or more specifically, a dietary no effect level in an LC_{50} test) is very helpful in conducting a reproduction test. A good estimate of the no effect level can provide valuable information in establishing dietary concentrations which will maintain the animals in a physical condition

such that they will be able to mate. This permits testing the reproductive effects of a test substance rather than other physiological effects.

An excellent example of the consequences of choosing dietary concentrations for a reproduction test not based on the no effect level of an LC_{50} test is seen in the ferret reproduction test with thiram. In this test, dietary concentrations of 0, 4, 16, and 64 ppm were chosen in spite of adverse effects at 20 ppm in the ferret LC_{50} test. These concentrations were chosen in part because dietary concentrations of 0, 2.5, 10, and 40 ppm had been selected for the concurrent mink reproduction test, based on a no effect level of 45 ppm in the mink LC_{50} test. The dietary concentrations for the ferret reproduction test thus reflected the repeated experience of this laboratory that ferrets are less sensitive than mink to toxic substances. As the results showed, 64 ppm thiram in the diet proved to be too high a dietary concentration, resulting in no reproduction and only 7 of 12 females coming into estrus. If the LC_{50} test data had been employed, dietary concentrations of approximately 0, 1, 4, and 16 ppm would have been tested (4 and 16 ppm actually were tested), and statistically valid differences would have been found at 16 ppm. Since the lowest concentration actually tested, 4 ppm, resulted in decreased kit body weight at 6 weeks postpartum, as well as a reduction in the whelping percentage, a no effect level may not have been found in this reproduction test.

Several other problems occurred during these tests. The importance of a palatability test was underscored by the results of the mink and ferret LC_{50} tests with thiram. Several animals were "wasted" in the mink test when they had to be removed from the test due to feed rejection. This could have been avoided if a palatability test was conducted properly, as in the ferret test where no such problems were encountered. This test also points out, in a rather drastic manner, the importance of the carrier in a test, since vastly different results were obtained with distilled water or corn oil as the carrier. Similar results have been reported by Gile et al. (1983), who noted carrier-related differences in the toxicities of some pesticides in avian LC_{50} tests.

The age of the test animals at the start of an LC_{50} test can also have a dramatic effect, as witnessed by the ferret LC_{50} tests with Compound 1080. In this test, young, rapidly growing ferrets were capable of "outgrowing" a lethal dietary concentration during the 28 days of the test, since the body weight gain during the test far outdistanced the increase in feed consumption, thus "diluting" the dose on a mg/kg of body weight basis. On the other hand, the LC_{50} tests with Aroclor 1254, using young and old mink, showed only slight differences in the toxicity of the PCB to the two age groups, with the younger group being less sensitive. It may be that young, rapidly growing animals can be successfully used with lipid soluble compounds that accumulate in the body, while this age group is unsuitable for testing water soluble substances.

Hill and Camardese (1981) have found an increase in the subacute dietary LC_{50} with increasing age of test birds for four different classes of pesticides, starting the tests with birds with ages ranging from one day to 21 days old.

The length of the LC_{50} test was set at 28 days for several reasons. A 28-day test permits absorption, distribution, metabolism, enzyme induction, redistribution, bioconcentration, and elimination to occur in the body, similar to that which might occur in animals subacutely exposed to a substance in the environment via the daily diet. A 28-day test allows the testing of slow-acting or bioaccumulating substances, especially if a withdrawal period is employed. This is exemplified by the LC_{50} tests with Compound 1080 and Aroclor 1254, in which prolonged and delayed mortality were seen (Tables 6, 11, 61, and 62). These results may not have been recorded in tests of shorter duration. In some instances, it may be possible to achieve satisfactory results with a shorter test using higher dietary concentrations, although the possibility of feed avoidance or rejection, as occurred in the 1080 and thiram tests, becomes greater with increased dietary concentrations. Also, certain substances cause delayed effects and/or mortality, whether administered as a single acute dose or as multiple subacute doses. Increasing the concentration of the substance does not necessarily shorten the time to death. Examples of this phenomenon include the PCBs (as seen in the Aroclor 1254 LC_{50} tests) and some delayed neurotoxins.

The standard avian dietary LC_{50} test length is 5 days' exposure in birds of a very young age. This test is designed

to test the substance with birds at their most susceptible age. It also guarantees that the compound will be tested via the diet, since very young birds are not able to survive 5 days without eating. Such a protocol would be impractical if applied to mink and ferrets, however, since weanling mink and ferrets can vary widely in body weight (see, for example, the standard deviations of 6-week body weights in the reproduction tests of this study), and can probably survive 5 days without eating. As previously mentioned, young mink can be less sensitive to some substances than older mink (e.g. Aroclor 1254).

In regard to the reproduction tests, especially with mink, it is important to note some reproductive anomalies which may occur in a test, especially if first-year animals are used. A small percentage of first-year females will be found in a cohort which will not accept males during the normal reproductive season, are barren, or which do not have normal maternal instincts, while a small percentage of males will not attempt to mate or will produce no viable spermatozoa. Each of these anomalies was seen at least once during the course of these experiments, and points out the importance of assigning at least 12 females and 4 males per dietary concentration if inexperienced breeders are used. If proven breeders are used, it may be possible to conduct a statistically valid reproduction test with as few as 8 females and 2 males per concentration.

Several advantages can be envisioned in the use of mink and/or ferrets as the species of choice for toxicological

tests with carnivores. First, these two species have been shown to be among the most sensitive of the wildlife species to compounds of environmental interest, as stated previously. Mink, and to a lesser extent, ferrets may dispel part of the growing opposition to the use of "pet" species as test subjects in scientific investigations. Mink and ferrets can be purchased, generally, for approximately one-half the cost of standard laboratory beagles, and in the case of mink may permit recovery of part of the cost in the pelt. Also, mink and ferrets generally require less cost for maintainence than the dog. Negative factors that may arise in the use of mink and/or ferrets as toxicological models include: handling problems, especially with mink; a limited reproduction season, especially with mink; the natural range of these species, which may preclude their use in certain hot climates; and, of course, the limited background data for these species in the literature.

CONCLUSIONS

The following conclusions may be drawn from tests performed with several test substances on mink and ferrets:

1) Statistically valid dietary LC_{50} tests may be performed with mink and ferrets, using 32-60 animals assigned to 3-5 dietary concentrations plus a control, with 8-10 animals per concentration.
2) Statistically valid reproduction tests may be performed with mink and ferrets, using 64 animals assigned

to 3 dietary concentrations plus a control, with 4 males and 12 females per concentration.

3) Factors which may affect the results of these tests include the age of the animals at the beginning of the test, the carrier used to mix the test substance into the diet, the accuracy of the estimate of the toxicity of the test substance, and the palatability of the test substance.
4) A wide range of test substances can be assessed using these protocols.
5) The tests can be conducted indoors, allowing reproducible results in properly equipped laboratories.

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APPENDIX A

MAMMALIAN WILDLIFE (MINK AND FERRET) DIETARY LC_{50} TEST

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MAMMALIAN WILDLIFE (MINK AND FERRET) DIETARY LC_{50} TEST

1. Scope

1.1. This protocol describes a method for determining the subacute dietary toxicity of a test substance (that can be mixed uniformly into the diet) administered to animals in their daily diet.

Toxicity is expressed as the median lethal concentration of the test substance (LC_{50}) and the slope of the dose-response curve.

1.2. This protocol is intended for use with carnivorous species, such as the mink (Mustela vison) and European ferret (Mustela putorius furo). Other carnivorous species may be used with appropriate modifications.

2. Summary

2.1. Groups of animals of the same species and age (both sexes) are fed diets containing a test substance in a geometric series of concentrations for 28 days to measure lethality. This exposure period may be followed by a withdrawal period during which lethality is also measured.

2.2. Daily observations for signs of toxicity and mortality are reported.

2.3. Data derived from treatment and control groups are compared statistically to detect changes in body weight and feed consumption.

3. Significance

3.1. This protocol provides a means of measuring the toxicity of a test substance in the daily diet of a carnivore under controlled conditions. The use of a 28-day dietary exposure period allows metabolic transformations of the test substance to occur.

3.2. This protocol provides data for assessing the potential adverse effects of chemicals to mammalian carnivores exposed through dietary intake, the normal exposure route in the environment. The mammalian carnivore occupies a position high on the food chain, thus it may be subject to the effects of bioaccumulation of chemicals.

3.3. This protocol permits collection of data on signs of toxicity in addition to mortality.

3.4. The dose-response curve provides additional information about the susceptibility of carnivores to a test substance.

3.5. This test provides a basis for deciding whether additional toxicity testing should be conducted.

4. Definitions

4.1. LC_{50}: The calculated concentration of a test substance which causes 50 percent lethality of a test animal population under the conditions of the test.

4.2. Test substance: The element, chemical compound, formulation, known mixture, or material mixed in diets and fed for the purpose of determining an LC_{50}.

4.3. Concentration: The weight of the test substance per unit weight of the diet (expressed as mg/kg of diet).

4.4. Acclimation period: A period of at least 7 days immediately preceeding the exposure period during which the test animals are housed in the test facilities under test conditions and fed the untreated diet and drinking water to be used during the exposure period.

4.5. Exposure period: The 28-day period during which the test animals are fed diets containing the test substance.

4.6. Withdrawal period: The period following an exposure period during which all animals are fed an untreated diet to allow for observation of delayed mortality.

4.7. Conventional diet: Feed consisting of both fresh and dried ingredients with water added to provide a semi-solid consistency.

4.8. Dry diet: Feed consisting of only dried ingredients fed in pelleted form.

5. Precautions

5.1. Contact with all test substances, solutions, and mixed diets should be minimized with appropriate protective clothing, gloves, eye protection, etc. The use of fume hoods and increased ventilation in test rooms is necessary when handling volatile substances. Information on other mammalian toxicity and special handling procedures should be known before this protocol is used.

5.2. Disposal of excess test substances, solutions, mixed diets, excreta, and treated animals should be done with consideration for health and environmental safety, and in accordance with all federal, state, and local regulations.

5.3. Cleaning and rinsing of glassware, feeders, and other equipment with volatile solvents should be performed only in well-ventilated areas.

5.4. Since this protocol addresses the use of carnivores, appropriate precautions should be used in the handling of test animals.

6. Test Animals

6.1. This protocol is intended for use with carnivorous species, such as the mink (Mustela vison) and European ferret (Mustela putorius furo). Other carnivorous species may be used with appropriate modifications.

6.2. All animals for a given test must come from one source and strain and be of approximately the same age to minimize variability. Test animals may be obtained from commercial sources or may be reared in laboratory colonies, but they must not have been used in a previous test. It is recommended that test animals be immunized against diseases commonly afflicting the test species (for mink and ferrets, immunization should include: canine distemper, virus enteritis, and botulism). Animals that are deformed, injured, emaciated, or phenotypically different from normal animals must not be used

as test subjects. The population of animals from which the test subjects (treated and control) are selected shall be considered unsuitable for testing if mortality exceeds 5% during the acclimation period.

6.3. It is preferable to use animals that have approached their mature body size (for mink and ferrets this is about 18-20 weeks of age). Older animals can also be used to determine the LC_{50}. The use of younger animals may yield a distorted LC_{50} value because the change in body weight far exceeds the change in feed consumption resulting in a decreased amount of test substance consumed per unit of body weight over the 28-day period.

7. Facilities

7.1. Space requirements for most carnivores have not been standardized. However, adherence to general guidelines and principles of good laboratory animal care (1) in addition to literature published on individual species (see Table 1) should provide a basis for adequate space requirements. Cages must be constructed so as to prevent cross-contamination and contact between animals. Species not conducive to colony rearing, such as mink, must be caged individually.

7.2. Construction materials in contact with animals should not be toxic, nor be capable of excessively adsorbing or absorbing test substances. Stainless steel, galvanized steel, or materials coated with perfluorocarbon plastics are acceptable materials, but other construction materials may also be useful.

7.3. Adequate ventilation should be provided at all times (1).

7.4. If test is conducted indoors, the photoperiod should simulate ambient daylight conditions at the date of initiation of the definitive test.

8. Diets

8.1. Diets must be formulated in accordance with the nutrient requirements of the test species (2). Any unmedicated commercial diet that meets the

minimum nutritional standards of the test species is acceptable.

8.2. Fresh diets and water must be provided daily and fed ad libitum.

9. Diet Preparation

9.1. Knowledge of the physical, chemical, and biological properties of the test substance is important in test diet preparation.

9.2. Test diets can be prepared by mixing the test substance directly into the feed or by dissolving or suspending the test substance in a solvent or carrier prior to mixing with the feed. The use of solvents or carriers may be necessary to achieve uniformity. If solvents or carriers are used, they must also be added to the control diet.

9.3. When conventional diets are used, sufficient diet should be mixed to provide adequate feed for the 28-day exposure period. The amount of feed to be mixed may be based upon the feed consumption data obtained during the acclimation period. When mixed, the diet should be stored frozen in containers large enough to hold 1-2 day's feed. In testing volatile substances, sealable containers must be used.

When dry diets are used, they should be stored so as to maintain the stability of the test substance in the diet. The frequency with which the feed is mixed is dependent upon the physical/chemical properties of the test substance.

9.4. It is recommended that all diets be analyzed for the concentration of the test substance in the diet.

10. Procedure

10.1. Range finding test: In order to determine the test concentrations to be used in the definitive test, a range finding test may be conducted using several widely spaced concentrations.

10.2. Acclimation period: All animals shall be conditioned to the test facilities, including: photoperiod, temperature, and caging for a minimum

of 7 days. During this period all animals shall be given the untreated (control) diet and drinking water, as used during the definitive test.

Test animals should be weighed at the start of the acclimation period. It is recommended that feed consumption be measured during the latter part of the acclimation period.

10.3. Definitive test:

10.3.1. Each test animal shall be randomly assigned to a specific test diet concentration and be uniquely identified.

10.3.2. The test diets must be fed for 28 days. For some test sub-substances, it may be necessary to include a withdrawal period, during which the test diets are replaced with untreated feed, in order to observe prolonged or delayed toxicity.

10.3.3. Individual body weights must be recorded at the initiation of the definitive test and at weekly intervals thereafter, and on the day of death. Feed consumption must be measured weekly for the exposure and withdrawal periods, and should be based on a minimum of two consecutive day's feed consumption.

10.3.4. Mortality, behavioral abnormalities, and other signs of toxicity shall be recorded daily during the test.

10.3.5. For tests conducted indoors, the photoperiod shall be maintained at the same schedule in effect at the conclusion of the acclimation period.

10.3.6. A minimum of eight animals for each test concentration shall be used. The test concentrations should be geometrically spaced so as to result in at least 2 dietary concentrations yielding 10-90% mortality. These results usually can be obtained with 4-6 dietary concentrations, including a control.

10.3.7. A test shall be considered invalid if more than 12.5% of the control animals die during the definitive test.

10.3.8. It is strongly recommended that a dietary concentration group should be removed from testing when food consumption measurements indicate that 10% or less feed, compared to controls and/or acclimation period values, is consumed daily for the first two week's feed consumption measurements.

10.3.9. Necropsies shall be performed on all mortalities. At the termination of the test all surviving test animals shall be killed by accepted humane methods and necropsies performed.

11. Quality Assurance

11.1. In order to assure the quality and reliability of data developed using this protocol, good laboratory practices should be followed (3,4).

12. Reporting Requirements

12.1. Name of the investigator(s), laboratory, laboratory address, location of raw data, and date of initiation and termination of test.

12.2. Name of species tested, including scientific name, source, and age of the animals at the beginning of the test.

12.3. A detailed description of the test substance including its chemical name, synonyms, structure, formulations, purity, source, batch, lot number, and physical/chemical properties and name of solvent or carrier, if used.

12.4. Description of the test facilities and housing conditions, including test cages, temperature, and photoperiod.

12.5. Name and source of feed, including description and proximate analysis of diet.

12.6. The dietary concentrations; number of animals per concentration; body weights; feed consumption; signs of toxicity; behavioral changes; % mortality for each concentration; significant necropsy findings; calculated LC_{50} values and 95% confidence limits, slope of the dose-response curve and 95% confidence limits, and the name and reference of the statistical method used; highest

dietary concentration at which no signs of toxicity were observed; anything unusual about the test; any deviations from the protocol; and other relevant information.

13. Discussion of Protocol by Section

3.1. It is recommended that, if possible, tests be conducted indoors. Indoor tests allow greater control of test conditions, and therefore, greater reproducibility. Indoor facilities, if heated, allow conducting tests at any time of the year. Indoor facilities also make the possibility of escape of test animals less likely.

3.5. The 28-day dietary LC_{50} test can be used as a basis for further tests. Results from the 28-day test may indicate the need for subsequent reproduction or chronic tests with the test species. These results might also indicate the need for other types of tests, such as aquatic, inhalation, secondary toxicity, etc., or tests designed for a target organ or organ system.

4.4;10.2. An acclimation period is required to condition the animals to the test facilities, diet, water, temperature, and lighting system to be used in a test. A minimum of 7 days is required, but a longer period may be necessary, especially if the animals to be used in a test are changed from outdoor to indoor housing (or vice-versa) or if the diet or water to be used in a test is different from that which the animals are accustomed to consuming. It is important to measure feed consumption during this period as these measurements can provide an indication of how much feed the animals should consume once the test starts, how much diet to mix for the test, and can also serve as a "control" value for each group.

4.5;10.3.7. The prescribed length of the mammalian dietary LC_{50} test is 28 days for several reasons. A 28-day test allows time for absorption, distribution, metabolism, enzyme induction, redistribution, bioconcentration, and elimination to occur, similar to that which might occur in animals subacutely

Table 2. Mortality pattern of mink fed sodium monofluoroacetate (Compound 1080) during a 28 day LC_{50} test.

Concentration (mg/kg)	No. of animals dying/day of test																											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
0																												
0.05																												
0.90																												
1.62																												
2.90																							1	2			1	
5.25					1			1			1				1				1	1	1					1	1	

Table 3. Mortality pattern of ferrets fed sodium monofluoroacetate (Compound 1080) during a 28 day LC50 test.

Concentration (mg/kg)	No. of animals dying/day of test																										
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
0																												
4.76										1																		
8.56														1														
15.40										2											1	1	1			1	1	

exposed to a substance via diet in the environment. A 28-day test also allows testing of slow-acting or bioaccumulating substances. Such tests could prove negative or misleading in a test of shorter duration. For example, prolonged mortality patterns were observed in 28-day tests with mink (Table 2) and ferrets (Table 3) fed Compound 1080, in which mortalites were observed up to the end of the test. Delayed mortalities were observed in a 28-day test with mink fed Aroclor 1254 (Table 4), in which mortalities were observed during a 7-day withdrawal period as well as during the exposure period. In some instances, it may be possible to achieve satisfactory results with a test of shorter duration using higher concentrations of the test substance, but the possibility of feed rejectoin or avoidance becomes greater with increasing concentrations. For example, in the 1080 tests already noted, signs of feed avoidance appeared in the first week of both tests in a dose-related manner. Increasing the concentration in these tests would have resulted in nearly complete avoidance of the feed and subsequent removal of the highest dietary concentrations from the test for humane reasons. Also, certain substances cause delayed mortality, whether administered as a single dose or multiple dosages. Increasing the concentration of the substance does not necessarily shorten the time to death. An example of this phenomenon is seen in the Aroclor 1254 test.

4.6;10.3.2. A withdrawal period is recommended when animals are still exhibiting signs of toxicity at the end of the exposure period. This period provides a more accurate estimation of the true toxicity of a test substance, especially if the substance causes delayed or cumulative injury. By observing the animals and measuring feed consumption during this peirod, the permanence of the injury can also be estimated. It is recommended that a withdrawal period not exceed 14 days.

5.4. Several precautions must be mentioned if the researcher is to use the mink as a test species. Mink are by nature extremely aggressive and may attack

a handler if given the opportunity. Appropriate cautions, such as leather gloves and arm-coverings, should be used. Mink can also transmit certain diseases, most importantly tetanus, and also tuberculosis and rabies if contracted from outside sources, so researchers should take precautions for these diseases. The possibility of TB infection can be reduced to nearly zero by avoiding the use of pork products in the diet, and proper caging and isolation of test facilities from wild animals can similarly reduce the possibility of rabies infections to nearly zero.

7.1. While space requirements for mink and ferrets have not been determined, individual cages measuring 61 (L) x 76 (W) x 46 (H) cm (24 x 30 x 18 in) have proven adequate for tests performed in conjunction with the development of this protocol, and are within the range of cage dimensions widely used in the fur industry.

In designing a caging system for carnivores, and especially for mink, it is important to prevent both cross-contamination of treatment groups and contact between individual animals. As stated previously, mink are extremely aggressive and may attack neighboring animals, if contact can occur. This can be prevented by providing adequate space between adjoining cages if wire mesh cage material is used throughout the cage, or by use of solid dividers between adjoining cages.

7.1;10.2;10.3.5. Photoperiod is maintained at the schedule in effect near the conclusion of the acclimation period because a changing photoperiod subjects mink and ferrets to slight adjustments in normal body rhythms with respect to feeding, sleeping, fat mobilization or deposition, coat development, and hormonal activity. It should be noted that the effects of the length of the light/dark cycle on toxicity have not been investigated in these species.

9.2. It is very important to assure uniform distribution of a test substance in the diet. In many instances, this will be more easily accomplished using the conventional diet, since many substances can be mixed into a diet more

uniformly if the diet is semisolid and capable of being machine-mixed. For some test substances, especially water soluble ones, this may be the only method of assuring uniform distribution, since pelleted diets are not conducive to being coated with aqueous solutions, but rather tend to become a mash.

No matter which type of diet is used it is recommended that innocuous solvents or carriers, such as distilled water or corn oil, be used if possible. (It is recommended that, unless the amount of test substance to be added is large, solvents or carriers be used to introduce the substance to the diet to ensure uniform distribution). If an innocuous solvent or carrier cannot be found, it is recommended that a volatile solvent, such as acetone or hexane, be used to dissolve the test substance, if possible, and the resultant solution be added to a small amount of either the dry diet or a dry ingredient (e.g. cereal) of the conventional diet. After the solvent is evaporated the pre-mix can then be mixed with the rest of the diet uniformly. (If this procedure is used, it must likewise be used on the control diet).

9.3. If the researcher chooses to use the conventional diet, it is important not to freeze the diets in containers too large, since the diets will not remain fresh under refrigeration for more than 2-3 days.

10.1. In most cases, LD_{50} estimates for mink or ferrets will not be available to aid in setting dietary concentrations for the LD_{50} test. Therefore, range-finding procedures can be employed to save both time and animals by reducing errors or miscalculations in setting these concentrations. LC_{50} estimates for other species may be helpful in setting dietary concentrations, although in general mink and ferrets are more sensitive to toxic compounds than laboratory animals. For this reason, if LD_{50} estimates are available for other species, these values can be used as the upper limit of doses in the range-finding procedure. This procedure can be a geometrically spaced series of doses (e.g. in multiples of 2 or ½) administered by gavage to 2 animals per dose, in

which case the approximate LD_{50} is the dose at which 1 or 2 animals die after an appropriate period of observation (often one week). (It is suggested that, when administering an oral dose to mink or ferrets by gavage, a piece of plastic large enough to force the animal's mouth open, with a small hole in the center, be used. The tube can then be inserted through the opening without the animal biting it - see Figure 1). If LD_{50} estimates are not available for other species, widely-spaced doses (e.g. 1, 10, 100, and 1000 mg/kg) can be administered to one animal to find a lethal dose. The range-finding procedure described above can then be employed, centering on the lethal dose.

If range-finding procedures yield an approximate LD_{50} value, the highest dietary concentration should then be set to ensure that an animal will consume the equivalent of an LD_{50} dose in one day's feed. If a lethal dose is not found, the highest dietary concentration should then be set at 5000 mg/kg, since concentrations above this value are assumed to be non-toxic.

Palatability tests may also be employed to save time and animals. It is recommended that the highest proposed dietary concentration be fed for several days to 2-4 animals to determine if they will eat the diet at this concentration of the test substance. If not, it may be necessary to reduce the highest concentration to a level at which the diet will be eaten.

10.3.3. In estimating feed consumption by mink or ferrets, several precautions are necessary. Since feed consumption can be adversely affected on a short-term basis by temperature, weather, and other factors, estimates should be based on at least two consecutive day's consumption. These days should also be days in which the animals are not handled (e.g. during weighing, moving, etc.), since handling can produce a temporary reduction in feed consumption.

10.3.6. It is possible to conduct an LD_{50} test with as few as 3 dietary concentrations and 8 animals per concentration if a good estimate of the LD_{50} for the test species is available. In many instances, however, accurate results

can be achieved with 5 dietary concentrations and 10 animals per concentration if a good estimate of the LD_{50} is available from range-finding procedures.

10.3.9. It is suggested that necropsies be performed on all test animals, either on the day of death or at the termination of the test. Valuable information on the mode of action and target organs or organ systems of the test substance can sometimes be gained from gross observation of the test animals at necropsy, and histopathological examination can sometimes provide more information. Weights of internal organs of control and treated animals can be compared statistically to determine effects of the substance, although the effects of starvation can sometimes be confounded with effects of the substance.

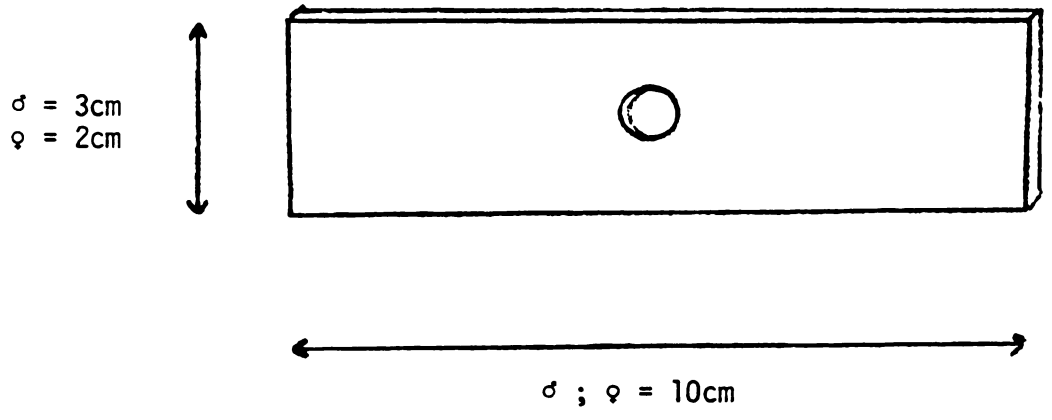

Figure 1. Device for gavage.

Table 1. Cage dimensions as reported in the literature for mink and ferrets.

Species	Cage dimensions (L X W X H in cm)						Reference
Mink	76.2 to 121.9	X	45.7 to 61.0	X	38.1 to 61.0		5
	91.4	X	45.7	X	38.1		6
	76.2 to 91.4	X	38.1 to 61.0	X	22.9 to 45.7		7
Ferret	76	X	76	X	46		8

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APPENDIX B

MAMMALIAN WILDLIFE (MINK AND FERRET) REPRODUCTION TEST

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MAMMALIAN WILDLIFE (MINK AND FERRET) REPRODUCTION TEST

1. Scope

1.1. This protocol describes a method for determining the reproductive toxicity of a test substance (that can be mixed uniformly into the diet) administered to animals in their daily diet. Reproductive toxicity may be expressed as an adverse effect on: a) adult survival; b) oogenesis and/or spermatogenesis; c) embryo or fetus development; d) reproductive indices; or e) offspring growth and survival.

1.2. This protocol is intended for use with carnivorous species, such as the mink (Mustela vison) and European ferret (Mustela putorius furo). Other carnivorous species may be used with appropriate modifications.

2. Summary

2.1. Groups of animals of the same species and age (both sexes) are fed diets containing a test substance in a series of concentrations, plus a control, for 8 weeks prior to breeding, during breeding, gestation, and parturition, and for 3 weeks of lactation (approximately 23 weeks) to measure reproductive toxicity.

2.2. Animals are observed daily and mortalities are reported.

2.3. Data derived from treatment and control groups are compared statistically to detect changes in body weight; feed consumption; length of gestation; percent of females bearing offspring; total offspring born per female (live and dead); average birth weight of offspring; average live litter weight; average weight of offspring at 3 weeks; and percent offspring survival to 3 weeks.

3. Significance

3.1. This protocol provides a means of measuring the reproductive toxicity of a test substance in the daily diet of a carnivore under controlled conditions.

3.2. This protocol provides data for assessing the potential adverse effects of chemicals to mammalian carnivores exposed through dietary intake, the normal exposure route in the environment. The mammalian carnivore occupies a position high on the food chain, thus it may be subject to the effects of bioaccumulation of chemicals.

3.3. This protocol permits collection of data on signs of toxicity and mortality over an extended period of dietary exposure, such as may occur in nature.

3.4. This test provides a basis for deciding whether additional toxicity testing should be conducted.

4. Definitions

4.1. Test substance: The element, chemical compound, formulation, known mixture, or material mixed in diets and fed for the purpose of determining reproductive toxicity.

4.2. Concentration: The weight of the test substance per unit weight of the diet (expressed as mg/kg of diet).

4.3. Acclimation period: A period of at least 7 days immediately preceding the exposure period during which the test animals are housed in the test facilities under test conditions and fed the untreated diet and drinking water to be used during the exposure period.

4.4. Conventional diet: Feed consisting of both fresh and dried ingredients with water added to provide a semi-solid consistency.

4.5. Dry diet: Feed consisting of only dried ingredients fed in pelleted form.

5. Precautions

5.1. Contact with all test substances, solutions, and mixed diets should be minimized with appropriate protective clothing, gloves, eye protection, etc. The use of fume hoods and increased ventilation in test

rooms is necessary when handling volatile substances. Information on other mammalian toxicity and special handling procedures should be known before this protocol is used.

5.2. Disposal of excess test substances, solutions, mixed diets, excreta, and treated animals should be done with consideration for health and environmental safety, and in accordance with all federal, state, and local regulations.

5.3. Cleaning and rinsing of glassware, feeders, and other equipment with volatile solvents should be performed only in well-ventilated areas.

5.4. Since this protocol addresses the use of carnivores, appropriate precautions should be used in the handling of test animals.

5.5. Since mink and ferrets are known to be sensitive to handling and other disturbances during the first 2 weeks post-partum, contact and outside disturbances should be minimized during this period.

6. Test Animals

6.1. This protocol is intended for use with carnivorous species, such as the mink (Mustela vison) and European ferret (Mustela putorius furo). Other carnivorous species may be used with appropriate modifications.

6.2. All animals for a given test must come from one source and strain and be of approximately the same age to minimize variability. Test animals may be obtained from commercial sources or may be reared in laboratory colonies, but they must not have been used in a previous test. It is recommended that test animals be immunized against diseases commonly afflicting the test species (for mink and ferrets, immunization should include: canine distemper, virus enteritis, and botulism). Animals that are deformed, injured, emaciated, or phenotypically different from normal animals must not be used as test subjects. The population of

animals from which the test subjects (treated and control) are selected shall be considered unsuitable for testing if mortality exceeds 5% during the acclimation period.

6.3. It is preferable to use animals that are proven breeders. However, availability and cost may dictate that animals in their first breeding season be used.

7. Facilities

7.1. Space requirements for most carnivores have not been standardized. However, adherence to general guidelines and principles of good laboratory animal care (1) in addition to literature published on individual species (see Table 1) should provide a basis for adequate space requirements. Cages must be constructed so as to prevent cross-contamination and contact between animals. Species not conducive to colony rearing, such as mink, must be caged individually.

7.2. Construction materials in contact with animals should not be toxic, nor be capable of excessively adsorbing or absorbing test substances. Stainless steel, galvanized steel, or materials coated with perfluorocarbon plastics are acceptable materials, but other construction materials may also be useful.

7.3. A nest area and nesting material must be provided for all females prior to the parturition period. All materials must be free of contaminants.

7.4. Adequate ventilation should be provided at all times (1).

7.5. If test is conducted indoors, the photoperiod should simulate ambient daylight conditions throughout the acclimation period and definitive test. This protocol addresses the use of mink and ferrets during their natural breeding seasons. Photoperiodic manipulation may permit the use of this protocol at other seasons. Since very low intensities of light may alter the reproductive cycle, care must be taken to ensure that total darkness is maintained during the appropriate periods.

If test is conducted outdoors, care must be taken to ensure that the photoperiod is not altered by extraneous light sources.

8. Diets

8.1. Diets must be formulated in accordance with the nutrient requirements of the test species (2). Any unmedicated commercial diet that meets the minimum nutritional standards of the test species is acceptable.

8.2. Fresh diets and water must be provided daily and fed ad libitum.

9. Diet Preparation

9.1. Knowledge of the physical, chemical, and biological properties of the test substance is important in test diet preparation.

9.2. Test diets can be prepared by mixing the test substance directly into the feed or by dissolving or suspending the test substance in a solvent or carrier prior to mixing with the feed. The use of solvents or carriers may be necessary to achieve uniformity. If solvents or carriers are used, they must also be added to the control diet.

9.3. When conventional diets are used, sufficient diet should be mixed to provide adequate feed for approximately 4 weeks. The amount of feed to be mixed may be based upon the feed consumption data obtained during the acclimation period. When mixed, the diet should be stored frozen in containers large enough to hold 1-2 day's feed. In testing volatile

substances sealable containers must be used. Feed should be similarly mixed and frozen as needed for the duration of the test.

When dry diets are used they should be stored so as to maintain the stability of the test substance in the diet. The frequency with which the feed is mixed is dependent upon the physical/chemical properties of the test substance.

9.4. It is recommended that all diets be analyzed for the concentration of the test substance in the diet.

10. Procedure

10.1. Dietary concentrations of test substance.

10.1.1. Establishing the dietary concentrations of a test substance for a reproductive study is a difficult but essential first step in determining an environmental effect of a chemical substance upon reproduction. A number of procedures exist for establishing the dietary concentrations to be used. Three are presented in this protocol.

10.1.1.1. If a mammalian wildlife dietary LC_{50} test (see pp - of this volume) has been conducted with the species under consideration, the highest dietary concentration at which no signs of toxicity were observed should approximate the highest of a series of geometrically spaced dietary concentrations, plus a control.

10.1.1.2. Another method of establishing dietary concentrations utilizes known or expected environmental concentrations of the test substance. Two or more dietary concentrations, plus a control, should be used. Examples of series of concentrations that may be used include 1X, 3X, and 5X or 1X, 3X, and 10X, where X equals the environmental concentration.

10.1.1.3. If LC_{50} data are lacking, it is useful to conduct a preliminary study with several widely spaced dietary concentrations of the test substance. The dietary concentrations may be established from these preliminary studies. It is recommended that 3 or more dietary concentrations plus a control be tested in the definitive test if this procedure is followed.

10.2. Experimental Design

10.2.1. This protocol is intended for use with individually caged animals only. Males and females will be paired only during breeding attempts, and one male will be assigned to a treatment group for each 3 or 4 females. Thus, this protocol is primarily designed to test female reproductive effects, and provides only limited data on male reproductive effects. If data on male reproductive effects are desired, a different experimental design will be necessary.

10.2.2. A minimum of 12 females is recommended per dietary concentration and an equal number for the control group. In addition, a minimum of 3 males should be housed per treatment group. It is recommended that breeding attempts be made only between males and females within the same treatment group. Use of proven breeders may reduce the number of animals per treatment group, but not to less than 8 females and 2 males per group.

10.2.3. If the experimental design is selected as per Section 10.2.2., one of the following criteria must be met:

1) One dietary concentration must produce an effect.

2) The highest dietary concentration must contain at least 1000 mg/kg.

3) The highest dietary concentration must be at least 100 times the highest known or expected environmental concentration.

If the researcher selects an experimental design based on considerations of Type I and Type II error, the number of females per treatment group may be specified by the researcher's levels of power, significance, and difference between means to be detected.

10.2.4. Each test animal shall be randomly assigned to a specific test diet concentration and be uniquely identified.

10.3. Acclimation Period

10.3.1. All animals shall be conditioned to the test facilities, including: photoperiod, temperature, and caging for a minimum of 7 days. During this period all animals shall be given the untreated (control) diet and drinking water, as used during the definitive test.

Test animals should be weighed at the start of the acclimation period. It is recommended that feed consumption be measured during the latter part of the acclimation period.

10.4. Definitive Test

10.4.1. The test diets must be fed daily throughout the pre-breeding, breeding, gestation, parturition, and lactation periods, a duration of approximately 23 weeks.

10.4.1.1. Pre-breeding period: Individual body weights must be recorded at the initiation of the definitive test and bi-weekly (once every other week) thereafter for the 8 weeks of the pre-breeding period. Feed consumption must also be measured bi-weekly during the pre-breeding period, and should be based on a minimum of two consecutive day's feed consumption.

10.4.1.2. Breeding period: This period lasts approximately 3-4 weeks, during which the females are presented to the males

within treatment groups for breeding. Matings are confirmed by microscopic examination of vaginal aspirations for viable spermatozoa, and recorded by date. Females with confirmed matings are given the opportunity for a second mating either 1 or 8 days following the initial confirmed mating.

Generally, body weight changes and feed consumption measurements are not recorded during this period. These measurements may be performed if animals are not excessively disturbed.

10.4.1.3. Gestation period: This period lasts approximately 6 weeks for ferrets and 7-8 weeks for mink. During this period animals should not be weighed, handled, or unduly disturbed.

10.4.1.4. Parturition period: This period lasts up to 3 weeks, depending on species. During this period females are checked daily for newborn. All newborn are counted, weighed, and recorded within 24 hours post-partum.

10.4.1.5. Lactation period: Individual body weights of all surviving newborn are recorded at the end of this 3 or more week period. This period should not extend beyond 6 weeks, the normal weaning time for mink and ferret offspring. During this period, offspring may come in contact with or eat (after 3 weeks) the maternal diet.

10.4.1.6. Termination: At the termination of the test all males and an equal number of females chosen at random from each dietary group should be killed by accepted humane procedures and necropsies performed. Tissue residue analyses and histopathological examination may be helpful. Measuring organ weights may also provide useful information about the test substance.

10.4.2. General considerations

10.4.2.1. All animals must be observed daily. All overt clinical signs and any abnormal behavior must be recorded when observed. If mortality occurs, the date and body weight must be recorded and necropsy performed.

10.4.2.2. A test may be considered invalid if more than 20% of the control animals die during the definitive test.

10.5. Reproductive indices

10.5.1. The following reproductive indices must be calculated:

1) Length of gestation: The time, in days from the last confirmed mating until parturition.

2) Number whelped, not whelped: The number of females giving birth and not giving birth in a treatment group. Number whelped includes females that die during the process of whelping from problems associated with parturition. This value is expressed as the number of females whelped or not whelped per the number of females with confirmed matings in a treatment group.

3) Live newborn/female whelped: The average number of live newborn produced by all females that give birth in a treatment group. This value does not include females that die during the process of whelping from problems associated with parturition.

4) Average birth weight: The average weight of all live newborn born in a treatment group, weighed to the nearest tenth of a gram within 24 hours post-partum.

5) Average litter weight: The average weight of all litters (live newborn only) born in a treatment group, weighed to the

nearest tenth of a gram within 24 hours post-partum.

6) Percent newborn survival to 3 weeks: The number of live newborn in a treatment group surviving to 21 days of age, expressed as a percentage of all live newborn born in a treatment group.

7) Average 3 week body weight: The average weight of all live newborn in a treatment group, weighed to the nearest gram on the 21st day after birth.

10.5.2. The following reproductive indices may also be useful:

1) Total newborn/female whelped: The average number of all newborn (alive and dead) produced by all females that give birth in a treatment group. This value includes females that die during the process of whelping from problems associated with parturition.

2) Percent newborn survival to 6 weeks: Identical to 21 day survival, but extended to 42 days.

3) Average 6 week body weight: Identical to 21 day weights, but measured at 42 days of age.

10.6. Statistical analysis

10.6.1. The following variables may be analyzed by analysis of variance (3) and significant differences may be tested by Dunnett's method for comparison with control (4):

1) Body weight changes

2) Feed consumption

3) Length of gestation

4) Live offspring/female whelped

5) Total offspring/female whelped

6) Average birth weight

7) Average litter weight

8) Average 3 week body weight

9) Average 6 week body weight

10.6.2. The following variables may be analyzed by contingency tables (5) and significant differences may be tested by Bonferroni's Chi-square test (6):

1) Number whelped, not whelped

2) Percent newborn survival to 3 weeks

3) Percent newborn survival to 6 weeks

10.6.3. Other valid statistical procedures may be used to analyze data.

11. Quality Assurance

11.1. In order to assure the quality and reliability of data developed using this protocol, good laboratory practices should be followed (7,8).

12. Reporting Requirements

12.1. Name of the investigator(s), laboratory, laboratory address, location of raw data, and date of initiation and termination of test.

12.2. Name of species tested, including scientific name, source, and age of the animals at the beginning of the test.

12.3. A detailed description of the test substance including its chemical name, synonyms, structure, formulations, purity, source, batch, lot number, and physical/chemical properties and name of solvent or carrier, if used.

12.4. Description of the test facilities and housing conditions, including test cages, temperature, and photoperiod. If conducted outdoors, adverse weather conditions may alter test results, especially during the parturition period, and should be reported.

12.5. Name and source of feed, including description and proximate analysis of diet.

12.6. The dietary concentration; number of males and females per concentration; body weights; feed consumption; signs of toxicity; abnormal behavior; mortality; reproductive indices; statistical methods employed; significant necropsy findings (including organ weights, if recorded); anything unusual about the test; any deviations from the protocol; and other relevant information.

13. Discussion of Protocol by Section

3.1. It is recommended that, if possible, tests be conducted indoors. Indoor tests allow greater control of test conditions, and therefore, greater reproducibility. Indoor facilities, if heated, allow more accurate measurements of feed consumption than outdoors, especially during sub-freezing conditions. Indoor facilities also make the possibility of escape of test animals less likely.

3.4. The reproduction test can be used as a basis for further tests. Results from a reproduction test may indicate the need for subsequent chronic tests with the test species. These results might also indicate the need for other types of tests, such as aquatic or inhalation, or tests designed for a target organ or organ system.

This protocol can provide limited data on the effects of a substance on male reproductive performance. However, if such effects are noted, it would be necessary to conduct further tests employing a different experimental design than the one described in the protocol to quantify male effects.

5.4. Several precautions must be mentioned if the researcher is to use the mink as a test species. Mink are by nature extremely aggressive and may attack a handler if given the opportunity. Appropriate cautions, such as leather gloves and arm-coverings, should be used. Mink can also transmit certain diseases, most importantly tetanus, and also tuberculosis and rabies if contracted from outside sources, so researchers should take precautions for these diseases. The possibility of TB infection can be reduced to nearly zero by avoiding the use of pork

products in the diet, and proper caging and isolation of test facilities from wild animals can similarly reduce the possibility of rabies infections to nearly zero.

7.1. While space requirements for mink and ferrets have not been determined, individual cages measuring 61 x 76 x 46 cm (24 x 30 x 18 in) and nest boxes measuring 38.1 x 27.9 x 26.7 cm (15 x 11 x 10.5 in) have proven adequate for tests performed in conjunction with the development of this protocol, and are within the range of cage and nest box dimensions widely used in the fur industry.

In designing a caging system for carnivores, and especially for mink, it is important to prevent both cross-contamination of treatment groups and contact between individual animals. As stated previously, mink are extremely aggressive and may attack neighboring animals, if contact can occur. This can be prevented by providing adequate space between adjoining cages if wire mesh cage material is used throughout the cage, or by use of solid dividers between adjoining cages.

7.3. It is very important to ensure that newborn are protected from toxic compounds. Of special importance is the nest material provided for the females. The researcher should be sure to use nest materials which are free of toxicants. A particular area of concern is wood by-products which may be contaminated with compounds to which mink are suspected to be sensitive.

7.5. In order to bring mink and ferrets into breeding condition indoors, it is necessary to gradually increase the length of daylight during the test. If the animals are held indoors for an extended period of time prior to the test, it is also necessary to gradually decrease daylight prior to the acclimation period to provide a necessary quiescent period of sexual development for the animals.

9.2. It is very important to assure uniform distribution of a test substance in the diet. In many instances, this will be more easily accomplished using the conventional diet, since many substances can be mixed into a diet more uniformly if the diet is semisolid and capable of being machine-mixed. For

some test substances, especially water soluble ones, this may be the only method of assuring uniform distribution, since pelleted diets are not conducive to being coated with aqueous solutions, but rather tend to become a mash.

No matter which type of diet is used it is recommended that innocuous solvents or carriers, such as distilled water or corn oil, be used if possible. (It is recommended that, unless the amount of test substance to be added is large, solvents or carriers be used to introduce the substance to the diet to ensure uniform distribution). If an innocuous solvent or carrier cannot be found, it is recommended that a volatile solvent, such as acetone or hexane, be used to dissolve the test substance, if possible, and the resultant solution be added to a small amount of either the dry diet or a dry ingredient (e.g. cereal) of the conventional diet. After the solvent is evaporated the pre-mix can then be mixed with the rest of the diet uniformly. (If this procedure is used, it must likewise be used on the control diet).

9.3. If the researcher chooses to use the conventional diet, it is important not to freeze the diets in containers too large, since the diets will not remain fresh under refrigeration for more than 2-3 days.

10.1.1.3. If an estimate of a dietary concentration at which signs of toxicity are not observed is lacking, it is recommended that a preliminary study be conducted to aid in establishing dietary concentrations for the definitive test. This study may be patterned after the protocol for mammalian dietary LC_{50} tests, using several widely spaced concentrations over a short period (e.g. 7-14 days) to determine an approximate no effect concentration. Since the data which will be generated from a study such as this would be expected to be fragmentary, it is suggested that at least 3 dietary concentration's be tested in the definitive test in order to maximize the possibility of meeting the criteria for an acceptable test while minimizing the possibility of wasting time, money, and animals.

10.2.2. Since relatively few reproduction tests have been conducted with mink and ferrets, and experimental procedures have varied in those tests, few background data are available to aid in determining the proper number of females to use to detect a significant difference for a given reproductive index. Thus, the minimum number of females per dietary concentration specified in this protocol was based on reproduction tests performed in conjunction with the development of this protocol and on other reproduction tests with mink and ferrets.

Due to considerations of cost and availability of proven breeders, it generally will be necessary to use animals which have not had breeding experience. If this is the case, it is recommended that a minimum of 12 females per treatment group be used, to provide a margin of safety against females which will not accept males, are barren, or which do not have proper maternal instincts (each of these reproductive anomalies will be exhibited by a small percentage of first year females within a cohort). Since the male's only function in reproduction is the mating act, it is not necessary to house equal numbers of males and females, unless male reproductive effects are expected. Thus, it is only necessary to house one male for every 3 or 4 females per dietary concentration. Again, if first year animals are used, it is suggested that the male:female ratio be 1:3, to provide a margin of safety against males which will not attempt to mate or which produce no viable spermatozoa.

If proven breeders are used, it may be possible to meet the criteria for an acceptable test with as few as 8 females and 2 males per dietary concentration.

10.3. An acclimation period is required to condition the animals to the test facilities, diet, water, temperature, and lighting system to be used in a test. A minimum of 7 days is required, but a longer period may be necessary, especially if the animals to be used in a test are changed from outdoor to indoor housing (or vice-versa) or if the diet or water to be used in a test is different

from that which the animals are accustomed to consuming. It is important to measure feed consumption during this period as these measurements can provide an indication of how much feed the animals should consume once the test starts, how much diet to mix for the test, and can also serve as a "control" value for each group.

10.4.1. The suggested length of the mammalian reproduction test of approximately 20-23 weeks is designed to conform to the normal reproductive seasons of mink (March through June) and European ferrets (April through July), with an 8 week exposure period prior to the reproductive season. The total length of the mink exposure period can be expected to be somewhat longer than the ferret exposure period because mink exhibit a variable delay in implantation of fertilized ova, while ferrets do not. Thus, the gestation period for mink can range naturally from approximately 40 to 60 days, whereas for ferrets the gestation period will normally be approximately 42 days.

The length of this test allows ample time for absorption, distribution, metabolism, enzyme induction, re-distribution, bioconcentration, and elimination to occur, and for tolerance to be acquired, similar to that which might occur to animals chronically exposed to a substance in the environment.

10.4.1.1. In estimating feed consumption by mink or ferrets, several precautions are necessary. Since feed consumption can be adversely affected on a short-term basis by temperature, weather, and other factors, estimates should be based on at least two consecutive day's consumption. These days should also be days in which the animals are not handled (e.g. during weighing, moving, etc.), since handling can produce a temporary reduction in feed consumption.

10.4.1.2. Under natural conditions, mating attempts are begun at the beginning of March for mink and the end of April for ferrets. In breeding mink, a female is presented to a male and, if receptive, is allowed to mate. If not receptive, the female is removed and presented to a male approximately 4 days later. Once a successful mating occurs (as verified by the presence of viable

spermatozoa in a vaginal aspiration taken just after copulation), the female is given the opportunity to mate a second time, either 8 days after the initial mating or the next day (if the first mating occurs late in the breeding season). In breeding ferrets, females are presented to males when they are judged to be in estrus (determined by the extent of vulvar swelling) and left overnight. Vaginal aspirations are not normally taken from female ferrets, and they are not given the opportunity for additional matings. If the researcher has reason to suspect male reproductive effects, vaginal aspirations may be taken for examination of spermatozoa.

Generally, it is advisable to discontinue recording body weights and measuring feed consumption once breeding attempts are begun. The increased handling of the animals during the breeding period causes perturbations in the animals' daily routines, resulting in decreased feed consumption by some animals. In addition, some animals respond to increased handling by becoming excitable. Repeated breeding attempts, coupled with routine weighings, can produce some females that are so excitable that breeding them becomes extremely difficult. Once the breeding period is over, it is best that the animals are left undisturbed as much as possible, especially during the first 2 weeks post-partum.

10.4.1.4. It is suggested that in checking for newborn, care is taken not to excessively disturb the females. If a nest box is not employed, visual inspection often is sufficient to determine whether a litter has been born. If a nest box is employed, it may be necessary to exclude the female from the nest box while checking the nest for newborn. If the female refuses to leave the nest box, this is often an indication that parturition has occurred.

10.4.1.6. It is suggested that necropsies be performed on selected test animals at the termination of the test. Valuable information on the mode of action and target organs or organ systems of the test substance can sometimes be gained from gross observation of the test animals at necropsy, and histopathological

examination of tissues can sometimes provide more information. Weights of internal organs and blood parameters of controls and treated animals can also be compared statistically to determine effects of the substance.

10.4.2.2. It is highly unlikely, based on the results of tests conducted in conjunction with the development of this protocol and on general mortality patterns derived from the fur industry, that more than 20% of a population of healthy mink or ferrets would die over the course of a 23 week reproduction test. If a researcher suffers the loss of greater than 20% of control animals in a test, it is possible that problems may exist in his diet or husbandry practices, or that disease has affected his stock.

10.5. The reproductive indices required in this protocol are selected based on features of the reproductive performance of mink and ferrets. Weights of all offspring (live and dead) are not required to be tested and reported because mink and ferrets are known to consume dead or stillborn young, thus, testing this reproductive index may produce incorrect or misleading results. Percent survival and weights of offspring are required at 3 weeks to allow minimal disturbance of dams and offspring during the critical period after birth and to ensure that nourishment received by offspring is almost totally of maternal origin. Percent survival and weights of offspring at 6 weeks is not required because the young usually begin consuming at least some solid feed by 4 weeks of age.

As mentioned previously, mink are known to exhibit a variable delay in implantation of fertilized ova, thus the length of gestation may not be useful in assessing effects of a substance on gestation in mink. It may, however, be very useful in assessing these effects in ferrets.

10.6. The statistical procedures suggested are only a few of the valid statistical methods which may be used. Use of more advanced methods may prove more powerful in detecting significant differences. Certain procedures may permit testing two or more combined reproductive indices to assess the true effect

of a substance on reproductive performance, even though none of the indices by themselves are statistically significant (15).

Table 1. Cage and nest box dimensions as reported in the literature for mink and ferrets.

Species	Dimensions (L X W X H in cm)												Reference
	Cage						Nest box						
Mink	76.2 to 121.9	X	45.7 to 61.0	X	38.1 to 61.0		30.5 to 45.7	X	25.4	X	30.5 to 35.6		(9)
	91.4	X	45.7	X	38.1		30.5	X	30.5	X	25.4 to 30.5		(10)
	76.2 to 91.4	X	38.1 to 61.0	X	22.9 to 45.7		----------						(11)
Ferret	76	X	76	X	46		38	X	29	X	27		(12)
			----------				12.5	X	15.2	X	15.2		(13)
			----------				48.3	X	39.8	X	30.0		(14)

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