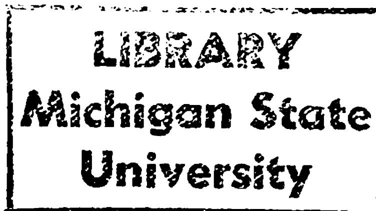
THESIS



LIBRARY
Michigan State
University

This is to certify that the

thesis entitled

THE ASSESSMENT OF THE EUROPEAN FERRET AS A MODEL SPECIES FOR ORGANOPHOSPHATE-INDUCED DELAYED NEUROTOXICITY

presented by

ANNE M. KOGUT

has been accepted towards fulfillment of the requirements for

M.S. degree in Animal Science

Richard J. Aulerich
Major professor

Date May 9, 1986

O-7639

MSU is an Affirmative Action/Equal Opportunity Institution

MICHIGAN STATE UNIVERSITY LIBRARIES
3 1293 00688 6869

RETURNING MATERIALS:
Place in book drop to remove this checkout from your record. FINES will be charged if book is returned after the date stamped below.

NOV. 0 6 1995
1996
NOV 1 9 1998

ASSESSMENT OF THE EUROPEAN FERRET AS A MODEL SPECIES FOR ORGANOPHOSPHATE-INDUCED DELAYED NEUROTOXICITY

by

Anne M. Kogut

A Thesis

Submitted to Michigan State University
in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE

Department of Animal Science

1986

ABSTRACT

THE ASSESSMENT OF THE EUROPEAN FERRET AS A MODEL SPECIES FOR ORGANOPHOSPHATE-INDUCED DELAYED NERUOTOXICITY

by

Anne M. Kogut

Organophosphate-induced delayed neurotoxicity was studied in the European ferret (M. putorius furo) to show species sensitivity, assess clinical signs and histopathologic lesions, and to evaluate the use of the ferret as a model species. In dermal and oral tests, adult male ferrets were given a single dose of tri-o-toyl phosphate (TOTP). In each test, 10 ferrets per group were treated with 0, 250, 500, or 1000 mg TOTP/kg body weight. At 48 hours post-treatment, half the animals in each group were sacrificed to assess whole brain neurotoxic esterase activity. The remaining ferrets were observed and received neurologic examinations for 58 days. All ferrets treated dermally with 1000 mg TOTP developed clinical signs of delayed neurotoxicity ranging from ataxia to partial paresis. Ferrets administered 250 and 500 mg TOTP developed variable degrees of ataxia. None of the animals dosed orally developed progressive delayed neurotoxic signs. Axon degeneration in the dorsal spinocerebellar tracts was the most consistent lesion. Neurotoxic esterase activity was maximally inhibited at 46% of control activity 48 hours post-exposure. Results suggest that the ferret can be used as a model for organophosphate-induced delayed neurotoxicity.

ACKNOWLEDGEMENTS

I wish to thank the members of my family, my mom, and my sister Monica, and my fiance Jim. Thanks for all your support, love, and understanding. Jim's constant encouragement, love, and emotional support (not to mention all his help with the ferrets) kept my hopes and goals in sight.

I would also like to thank Dr. Richard Aulerich, my major professor, whose constant guidance and insight allowed me to keep my chin up and finally finish my research. I extend this gratitude to the other two members of my committee, Dr. Steven Bursian and Dr. Duke Tanaka. Dr. Bursians knowledge and interest in my area of research helped me solve some of the roadblocks I encountered along the way. Dr. Tanaka not only encouraged me to develop my talents as a student and individual, but also taught me invaluable histopathological techniques and interpretations.

Many others have made this accomplishment possible. I would not have been able to conduct all my laboratory work without the assistance of Kay Trosko. Thanks also goes to Angelo Napolitano and the rest of the people at the Experimental Fur Farm for their assistance in animal care, and to Ms. Carol Daniel for typing this manuscript.

Finally, I would like to extend my deepest gratitude to the Office of Radiation, Chemical, and Biological Safety,

not only for financial assistance, but mostly for the friendships of everyone associated with the ORCBS.

TABLE OF CONTENTS

	Page
Acknowledgements	ii
List of Tables	vi
List of Figures	v
Introduction	1
Review of Literature	2
The Ferret	2
Natural History and Care	2
Use in Research	3
Tri-ortho-Toyl Phosphate	5
Organophosphate-Induced Delayed Neurotoxicity	7
Historic Background	7
Species Susceptibility and Variability	9
Mechanism of Action	12
Clinical and Pathological Manifestations	13
Materials and Methods	16
Animal Selection and Care	16
Test Procedures	17
Single Dermal Dose	19
Single Oral Dose	20
Weights and Measurements	21
Clinical Evaluations and Tests	21
General Observations of Gait, Posture, and Habits	21
Spinal Cord Reflexes	22
Postural Reaction	24
Histopathological Techniques	25
Thionin Stain	26
Modified Fink-Heimer Technique	26
Speilmeyer Method	27
Statistical Analysis	28
Results	28
Body Weight Changes and Feed Consumption	29
Neurotoxic Esterase (NTE) Activity	34
Clinical Observations	34
Histopathology	46
Discussion	55

Page

Conclusions 65

References 66

LIST OF TABLES

		Page
Table 1.	Susceptible and non-susceptible species to organophosphate-induced delayed neurotoxicity	11
Table 2.	Ferret diet	18
Table 3.	Description of rating scale and criteria used in assessing organophosphate-induced delayed neurotoxicity	23
Table 4.	Effect of dermal and oral administration of TOTP on neurotoxic esterase activity in the ferret	35
Table 5.	Description of clinical response ratings	36
Table 6.	Clinical assessment of ferrets administered tri-ortho-tolyl phosphate	42
Table 7.	Neurological evaluations of ferrets administered tri-orthotolyl phosphate	44

LIST OF FIGURES

		Page
Figure 1.	Reaction of TOTP to form the toxic metabolite saligenin cyclic phosphate	6
Figure 2.	Effect of dermal administration of TOTP on feed consumption	30
Figure 3.	Effect of oral administration of TOTP on feed consumption	31
Figure 4.	Effect of dermal administration of TOTP on body weight	32
Figure 5.	Effect of oral administration of TOTP on body weight	33
Figure 6.	Control ferret displaying characteristic posture	37
Figure 7.	Arched back of ferret administered 1000 mg TOTP	38
Figure 8.	Sliding gait and sprawled legs of a TOTP-treated ferret	39
Figure 9.	Hindlimbs of control and TOTP-treated ferrets	41
Figure 10.	Cross section of lumbar spinal cord of a ferret administered 1000 mg TOTP	47
Figure 11.	Dorsal spinocerebellar tracts of a ferret administered 1000 mg TOTP	47
Figure 12.	Longitudinal section of dorsal spinocerebellar tracts in the lumbar region of a spinal cord in a control ferret	49

Page

Figure 13. Longitudinal section of dorsal spinocerebellar tracts in the lumbar region of the spinal cord in a control ferret 49

Figure 14. Longitudinal section of dorsal spinocerebellar tracts in the lumbar spinal cord of ferret number F-2 administered 1000 mg TOTP 50

Figure 15. Longitudinal section of dorsal spinocerebellar tracts in the lumbar spinal cord of ferret number F-2 administered 1000 mg TOTP 50

Figure 16. Longitudinal section of dorsal spinocerebellar tracts of ferret number F-13 administered 1000 mg TOTP 51

Figure 17. Longetudinal section of dorsal spinocerebellar tracts of ferret number F-13 administered 1000 mg TOTP 51

Figure 18. Cross section of lumbar spinal cord of a control ferret 52

Figure 19. Cross section of lumbar spinal cord of a ferret administered 1000 mg TOTP/kg 52

Figure 20. Cross section of cervical spinal cord of a control ferret 53

Figure 21. Neuron from ventral horn of cervical spinal cord from a control ferret 53

Figure 22. Neuron in the ventral horn cervical spinal cord of a ferret treated with 1000 mg TOTP/kg 54

Figure 23. Neuron in cervical spinal cord of a ferret treated with 1000 mg TOTP/kg 54

INTRODUCTION

The increased concern about the effects of chemicals and environmental contaminants on human health has prompted many toxicological evaluations. Organophosphates are some of the most widely used compounds in agriculture and industry and have been related to potential health problems. Organophosphate-induced delayed neurotoxicity (OPIDN) is just one possible consequence of exposure to organophosphates. This delayed neurotoxicity is defined as nerve damage with delayed onset or toxic distal axonopathy (Shaumberg and Spencer, 1979). The clinical effect is characterized by an initial delay period of 14 to 21 days, after which development of ataxia that progresses to paralysis is observed. Morphological changes in animals that exhibit this condition include axonal degeneration with subsequent Wallerian degeneration of the central and peripheral nervous system. Compounds such as tri-ortho-toyl phosphate (TOTP), phenylphosphonothionate (leptophos), mipafox, trichlofon, and 0-ethyl 0-4 nitrophenyl phenylphosphonothioate (EPN) have been implicated in the production of OPIDN in animals and humans.

Factors that influence the neurotoxic effect involve the form and purity of the test compound, dosage, frequency and duration of exposure, route of administration, metabolism, and toxicokinetics (Abou-Donia, 1981). Species sensitivity

is another factor that influences the occurrence of organophosphate-induced delayed neurotoxicity. Common laboratory animals such as the mouse and rat have been found to be relatively insensitive to OPIDN. The chicken is presently the species of choice for OPIDN testing, but the validity of extrapolation of data to man has been questioned. The cat has also been utilized for OPIDN testing, but the increased disfavor towards the use of pet species in research has led to the search for more suitable species for toxicological evaluations.

The necessity for another species sensitive to OPIDN prompted this study on the European ferret (*Mustela putorius furo*). The objectives of this study were:

1. To assess the sensitivity of the ferret to OPIDN by clinical observations.
2. To determine histopathological changes related to OPIDN.
3. To assess the use of the ferret as a model species for OPIDN.

LITERATURE REVIEW

The Ferret

Natural History and Care

The European ferret belongs to the order Carnivora and the family Mustelidae. Two color strains are found, one of which is the fitch color, characterized by a brown and yellow patterned coat. The second, the ruby-eyed albino,

is recessive to the fitch variety. The ferret was introduced into the Americas in the 19th century where it came from its native lands of Northern Africa, Europe, and Asia (Willis and Barrow, 1971). Ferrets have been used in the past for rabbit hunting but have become a popular choice in recent years for laboratory animals and pets. However, some states have laws that prohibit possessing ferrets because of a concern about proliferation of this species in the wild and possible damage to poultry farms (Williams, 1976).

Male ferrets weigh between 1350 and 2100 gms, but show a marked seasonal weight change; there is a loss in weight during the breeding season and a gain during the fall (Williams, 1976). Their body length is approximately 30 cm and they have a lifespan in commercial breeding operations of 2-5 years. Since the ferret is a carnivore, it requires a high amount of protein in its diet. A large variety of foods such as commercial and conventional mink diets or commercial dog and cat food provide suitable nourishment. The ferret has a seasonal breeding period that extends from March to August. The female exhibits delayed ovulation (Hahn and Wester, 1969). The gestation period is 42 days and two litters of 3 to 13 kits per year are normal (Asdell, 1946; Farris, 1950).

Use in Research

The ferret has become a popular choice for a research animal and has been used in such research areas as virology,

with an emphasis on canine distemper (Green, 1945), influenza (Perrin and Oliphant, 1940), gastroenterology (Pfeiffer and Weibel, 1973), and endocrinology (Rieger and Murphy, 1977). The occurrence of spontaneous genital malformations has made the ferret a desirable species for teratological research (Haddad _et al_., 1975; Elizan _et al_., 1964). In a recent investigation of the ferret's haematology, serum chemistry, pathology, and reproduction has concluded that the ferret is a suitable species for toxicological evaluations of new and experimental drugs (Thornton _et al_., 1979). The ferret has also become a potential animal model for cardiological research on anatomical relationships of the aortic arch vessels (Willis and Barrow, 1971).

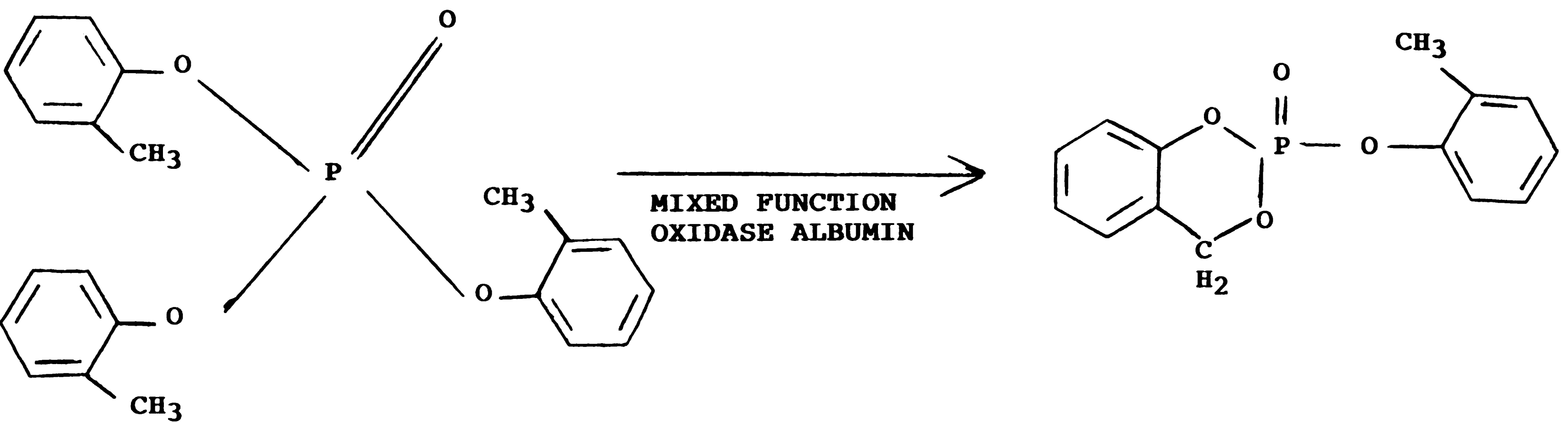
The increasing use of non-rodent species and consequent demands and controversies over the use of primates and dogs in research has led to the possible use of the ferret as a non-rodent species. The ferret is also desirable as a laboratory animal because it is very adaptable to human handling, can be housed in standard laboratory cages, requires no exercise space, and can be raised in colonies (Hahn and Wester, 1969). Ferrets are also excellent lab animals because of their relatively short gestation period and large litter size and because their breeding season can be regulated through manipulation of the photoperiod (Willis and Barrow, 1971).

Tri-Ortho-Toyl Phosphate (TOTP)

TOTP is a tri-aromatic organophosphorus ester which is used as a plasticizer in lacquers and varnishes, and also as a gasoline additive, pesticide and flame retardant (Fassett, 1967). Other synonyms for this compound are: tri-ortho-cresyl phosphate (TOCP), tri-2-toyl phosphate, o-cresyl phosphate, and tri-2-methylphenyl phosphate (Sax, 1984). TOTP is a colorless liquid with a molecular weight of 368.39 and melting and boiling points of -30°C and 410°C, respectively. It is insoluble in water but is soluble in alcohol and ether.

Cavanagh (1973) noted that the presence of 2 or more 2-methyl substituted benzene rings was necessary for toxicity and that the liver played an important role in activating the parent compound. TOTP is a relatively inactive parent molecule that does not induce acute cholinergic or neurotoxic effects, but requires bioactivation to produce neurotoxicity (Casida et al., 1961). This conversion is a two step reaction which involves a mixed-function oxidase hydroxylation of a methyl group and then a cyclization reaction, catalyzed by albumin (Figure 1). TOTP and its metabolite, saligenin cyclic phosphate, are used as model chemicals against which other compounds are tested for delayed neurotoxicity potential (Baron, 1981). Saligenin cyclic phosphate was found to be five times more neurotoxic than TOTP after oral administration to chickens (Bleiberg and Johnson, 1965).

FIGURE 1. Reaction of TOTP to form the toxic metabolite saligenin cyclic phosphate.



O
P
CH3
O
O
O
CH3
CH3
MIXED FUNCTION
OXIDASE ALBUMIN
CH3
O
O
P
O
O
O
C
H2
TOTP
SALIGENIN CYCLIC
PHOSPHATE

Organophosphate-Induced Delayed Neurotoxicity

Historic Background

OPIDN has been recognized in both humans and animals for over 50 years. The extensive use of organophosphates in industry and agriculture has increased the chances of toxicity to man, however, most of the human outbreaks of delayed neurotoxicity have been accidental. OPIDN was first recognized by Lorot in 1899 following the treatment of tubercular patients with phosphocresote (Metcalf, 1982). During the late 1920's and 1930's, an outbreak of polyneuritis existed throughout the midwest and southwest United States. The causal agent was found to be TOTP (Smith *et al*., 1930). An 80% alcoholic extract of Jamaican ginger was adulterated with 2.5% TOTP and was sold over the counter during the U.S. Prohibition Era. The condition that developed from the ingestion of the alcohol extract became known as "ginger jake syndrome" and was characterized by a delay period of 10-21 days, after which symptoms of weakness in fingers, foot drop, and partial paralysis developed (Baron, 1981). Over 20,000 people experienced various degrees of paralysis resulting from an average exposure to 30-40 mg TOTP/kg body weight.

Various other incidents of poisoning from ingestion of food or drink adulterated with cresyl phosphates have been described. In 1936, 60 people were left permanently

paralyzed in Napal after lubricating oil containing 0.4% TOTP was accidently added to soybean oil (Metcalf, 1982). Also in the 1930's, a widely sold abortifacient of parsley extract (Apiol) produced OPIDN in 90 women in Holland, Germany, France, Yugoslavia, and Sweden (Davies, 1963). This extract contained 20-50% TOTP. Between 1939 and 1945, many German and Swiss soldiers became affected when lubricating oil or contaminated cooking oil was used for cooking (Metcalf, 1982). Another large epidemic of delayed neurotoxicity poisoning occurred in Morocco in 1959 where over 10,000 people became affected after using cooking oils adulterated by aircraft engine oils containing approximately 3.0% cresyl phosphate (Smith and Spaulding, 1959). Dogs also reportedly showed neurotoxic symptoms in this incident.

The outbreaks of OPIDN were not confined only to TOTP, in that other chemicals were found to induce neurotoxic symptoms. In 1953 workers manufacturing a systemic insecticide called mipafox developed acute organophosphate poisoning symptoms of vomiting, nausea, cramps, and general muscular weakness (Bidstrup et al., 1953). These acute symptoms subsided after 4 days, but were subsequently followed by leg weakness and flaccid paralysis characteristic of OPIDN. During the 1950's and 1960's, thousands of chemicals had been evaluated for use as pesticides and some were found to cause delayed neurotoxicity in animals and man. One of

these compounds, leptophos, continued to be marketed even after tests confirmed the production of OPIDN in hens (Metcalf, 1982). After large applications of substantial quantities of leptophos in Egypt, over 1300 water buffalo died from OPIDN and widespread human poisoning developed (Abou-Donia et al., 1974). The problem with leptophos prompted the U.S. Environmental Protection Agency to revoke all tolerance levels for residues of this pesticide in foods (Casida and Baron, 1976).

Testing requirements for delayed neurotoxicity have been in effect in the U.S. since August 1978 (Federal Register, 1978). This testing applies to all pesticides registered for use in the United States if the active ingredients, metabolites, degradation products, or impurities cause acetylcholinesterase depression or are structurally related to substances that are known to induce delayed neurotoxicity. The World Health Organization (1976) has classified the hazards of delayed neurotoxicity as a dose response relationship that involve exposure of individuals to occupationally or accidentally high doses for short periods of time or exposure to low levels for an extended period of time. Investigations are now in progress to estimate "no effect levels" of exposure to these compounds.

Species Susceptibility and Variability

The production of OPIDN in animal studies designed to simulate human exposure is difficult due to marked species

differences in susceptibility. The White Leghorn hen (Gallus gallus domesticus) has been used as a model species because of its adaptability and availability. Abou-Donia (1981) has concluded that the attributes of the use of the chicken as a model for OPIDN include a complete correlation with human response, in that the delay period, pathology and clinical signs are similar to humans, both sexes are susceptible, clinical signs are standardized and easy to grade, and there is an availability of extensive structure-activity data. The domestic cat (Felis domestica) has also been suggested as a model species but three differences in response when compared to the chicken have been noted. These differences include a difficulty in protecting against acute poisoning, slower progression of delayed neurotoxicity, and the propensity for improvement in some animals (Abou-Donia et al., 1983b).

The list of susceptible and non-susceptible species, shown in Table 1, was compiled by Johnson (1975) and Baron (1981). The slow loris (Nycticebus coucany coucany) was found to be affected by repeated dermal applications of tricresyl phosphate (TCP) (Ahmed and Glees, 1971). Triaryl phosphate poisoning was exhibited in cattle exposed both naturally and experimentally (Beck et al., 1977). Rats, guinea pigs, and Japanese quail are all insensitive to known delayed neurotoxins when administered in single doses (Johnson,

Table 1. Susceptible and non-susceptible species to organophosphate-induced delayed neurotoxicity (Johnson, 1975; Baron, 1981).

Susceptible	Non-susceptible
Man	Rat
Baboon	Mouse
Squirrel Monkey	Rabbit
Water Buffalo	Guinea Pig
Horse	Hamster
Cow	Gerbil
Sheep	Japanese Quail
Pig	
Dog	
Cat	
Slow Loris	
Chicken	
Duck	
Pheasant	
Turkey	
Partridge	

1975). The differences in susceptibility may be due to inadequate absorption of the neurotoxin, enhanced metabolism and elimination, and/or the unique structure of the nervous system of individual species (Bursian et al., 1983). Immature animals of a sensitive species have been found to be less susceptible than adults to OPIDN (Cavanagh, 1973).

Mechanism of Action

The initial studies on delayed neurotoxicity showed that the site of action was a defined area in the nervous system. Modell et al. (1946) and Koelle and Gilman (1946) hypothesized that the paralytic effects seen after administration of di-isopropyl fluorophosphate (DFP) were due to prolonged inhibition of acetylcholinesterase. Another esterase besides acetylcholinesterase was implicated by the studies of Baron and Cassida (1962). Subsequently, phosphorylation sites present in the brain and spinal cord were identified (Johnson, 1969). This "neurotoxic esterase" (NTE) was found to be inhibited by known delayed neurotoxins in vivo and was convincingly determined to be the site for initial neurotoxic activity when certain protective compounds were bound to the esterase and prevented delayed neurotoxic effects (Johnson, 1970). Thus, it was determined that the nature of the group binding to the active site and the binding site itself resulted in the neurotoxic response.

Johnson (1974) further demonstrated that the binding to NTE by a delayed neurotoxic compound induced a hydrolysis

of an ester or amide bond, resulting in an ionized acidic group. This time dependent process was termed "aging" and was found not to occur with non-delayed neurotoxic compounds. It was hypothesized that the charged group could alter membranes, thus inducing initial biochemical lesions. A recent interpretation of the mechanism of action suggests that initiation of OPIDN can be monitored by the inhibition of catalytic ability (Johnson, 1982). Two events can occur after this initiation. Either the inhibited NTE "ages" and the modified protein has a deleterious degenerating effect on the long axons, or the binding of NTE does not occur, resulting in no aging effect and a normal response. The processes following initiation which lead to development of neuropathy have not been established. Assays have been used to predict the delayed neurotoxic potential by the amount of NTE inhibition. In hens, it was found that for single dose tests, 75% inhibition initiated neuropathy and 45-65% inhibition produced neuropathy in chronic tests (Johnson, 1982).

Clinical and Pathological Manifestations

The acute toxic clinical signs of OPIDN poisoning are characteristic of cholinergic poisoning and include tremors, lacrimation, salivation, diarrhea, and other effects of parasympathetic stimulation, OPIDN is characterized by three significant features unrelated to the acute signs (Baron,

1981). First, a delay occurs in the interval following acute poisoning. During this time, no evidence or signs of poisoning exists, but, following this period, a progressive ataxia occurs. Second, species differ in their susceptibility to delayed neurotoxins. Finally, a large variety of organophospahtes and other chemicals may cause the same or similar lesions. Clinical signs are similar in all susceptible species. Not only are the effects dose dependent, but route of administration also plays a major role in delayed neurotoxicity (Baron, 1981). Clinical signs are not seen until 8 to 10 days or up to 21 days after exposure (Stuart and Oehme, 1982). Weakness and ataxia develop in the lower limbs and may progress to paralysis. In severe cases, the upper limbs may also be affected (Johnson, 1980; Gordon, 1983). The weakness can then be followed by what is described as "toe drop" in man, which produces a characteristic high stepping gait (Smith _et al._, 1930). A progressive flaccid paresis of the legs (hindlimbs in quadrapeds) with possible effects on the forelimbs has been observed (Stuart and Oehme, 1982). The onset of paralysis in man is accompanied by abnormal sensory experiences such as a burning and tingling sensation in the feet (Cavanagh, 1973). The hindlimbs are always more affected than the forelimbs in all species. Certain species have been noted to express characteristic responses in relation to OPIDN. For example, cats were noted

to have an abnormal response to the stroking reflex and a reluctance to jump (Taylor, 1967). Recovery may occur in less severe cases and proceeds in the reverse order from which the motor deficits occurred (Stuart and Oehme, 1982). In man, recovery is slow and often, in severe cases, seldom complete, with residual ataxia present (Metcalf, 1982; Takade, 1982).

The morphological lesion most associated with OPIDN is best described as a "dying-back" process or Wallerian degeneration of the long axons of the peripheral nerves and both ascending and descending tracts of the spinal cord (Bradley, 1976; Miller, 1983).

Demyelination was initially thought to be the primary lesion (Lillie and Smith, 1932) but it was later demonstrated that myelin degeneration was secondary to axon degeneration (Cavanagh, 1954). The degenerative lesions are most frequently observed in the larger diameter axons and can be detected histologically at the same time as clinical signs are observed (Baron, 1981). Further, axonal changes appear to be well advanced before the myelin begins to disrupt (Baron, 1981). Abou-Donia and Graham (1979a) noted in chickens that lesions in the peripheral nerves occurred earlier than those in the spinal cord and that evidence of regeneration in the peripheral nerves was present. Also, the most consistent histological changes observed were degeneration of the anterior columns

of the thoracic and lumbar spinal cord. In the cat, histological changes consisted of swelling, vacuolation, and fragmentation of axons and myelin sheath (Abou-Donia et al., 1983b). These lesions were located in the gracile and spinocerebellar ascending tracts of the posterior columns of the cervical spinal cord and also in the lateral corticospinal descending tracts of the thoracic and lumbar regions. Although evidence has been presented suggesting that no nerve cell damage occurs (Cavanagh and Koller, 1979), other evidence indicates nerve cell damage. Large numbers of chromalytic neurons in the lumbar regions of the spinal cord taken from a slow loris treated with TCP have been observed (Ahmed and Glees, 1971). These nerve cells are characterized by a pale cytoplasm, eccentric position of the nucleus, and the peripheral arrangement of the nissl substance.

MATERIALS AND METHODS

Animal Selection and Care

Eighty adult male, agouti-colored, European ferrets (*Mustela putorius furo*) were obtained from Marshall Research Animals, North Rose, New York. The animals were immunized against canine distemper and housed indoors at the Michigan State University Experimental Fur Farm. Adult (1-3 years old) male ferrets were used because of the greater sensitivity of adult animals to delayed neurotoxicity (Barnes and Denz,

1953) and to eliminate any sex differences in response to the neurotoxin.

In the test room, the ferrets were housed two per cage in galvanized wire cages (61 (L) x 30 (W) x 46 (H) cm) with attached feed and water cups. The cages were suspended 69 cm above the floor. The ferrets were subjected to ambient temperature and a 12 hour light/dark schedule. Ventilation was provided by wall fans and ceiling vents. Wood shavings, changed periodically, were spread under the cages to facilitate animal waste disposal. The ferrets were fed the diet shown in Table 2, which in previous studies (Bleavins and Aulerich, 1981) has been demonstrated to sustain ferrets in good health. Food and water were provided *ad libitum*.

Test Procedures

Pre-test procedures for both the dermal and oral trials included a two week acclimation period during which the animals were weighed weekly and observed daily. Only healthy ferrets were used in the tests.

A total of 40 animals were used for each trial. Since the animals were housed two per cage, cages were randomly assigned to a treatment group. Both animals in each cage were given the same treatment but the animals were assigned a unique number which was identified by the ferrets weight and distinctive color markings. There were four treatment groups consisting of 10 animals per group. The treatments

Table 2. Ferret diet.

Ingredients	Percentage
Commercial Mink Cereal	20%
Duck By-Product[a]	24%
Ocean Fish Scrap	15%
Beef Tripe	8%
Beef Trimmings	4%
Beef Liver	4%
Beef Lungs	4%
Added Water	21%

[a] For the oral test, 24% chicken by-product was substituted for the duck by-product.

in both tests consisted of a control (vehicle only), 250, 500, and 1000 mg TOTP/kg body weight. Animals in groups were cared for in the same way.

The day prior to dosing, the animals were weighed to obtain an approximate weight in order to determine the quantity of TOTP required for dosing. Approximately 20 minutes before dosing and daily for 10 days thereafter all animals were given 1.5 mg of atropine sulfate (Professional Veterinary Laboratories, Minneapolis, MN) by subcutaneous injection in the back of the neck to prevent possible illness or death due to the initial cholinergic effects of TOTP (Baron, 1981).

Single Dermal Dose

The amount of TOTP (Eastman Kodak Company, Rochester, New York) required to yield the desired dose levels of 250, 500, and 1000 mg TOTP/kg body weight was calculated as follows. Using a density factor of 0.862 for TOTP, 5.4, 10.8, and 21.6 ml of TOTP were dissolved in 19.6, 14.2, and 3.4 ml of 95% ethyl alcohol to yield the desired concentrations of 250, 500, and 1000 mg TOTP/ml, respectively. One ml/kg body weight of TOTP was applied to a shaved area (approximately 10 cm^2) on the back of the neck of each ferret. Ethyl alcohol was applied to the controls. The solution was allowed to be absorbed through the skin before the animals were returned to their cages to ensure that the compound was not consumed or rubbed from the skin. After dosing, the animals were

observed closely for 24 hours to note any abnormal behavior or adverse health effects.

Five animals (one per cage) per treatment group were sacrificed by cervical dislocation 48 hours post-treatment for determination of whole brain neurotoxic esterase (NTE) activity. Brains were removed, weighed, and immediately frozen in dry ice, and subsequently analyzed for NTE activity, as described by Johnson (1977). The remaining 20 animals were observed daily for development of clinical signs typical of OPIDN and were given neurological examinations during the next 58 days.

Single Oral Dose

TOTP was mixed as described for the dermal trial, except corn oil replaced ethyl alcohol as the vehicle. The animals were dosed by gavage by placing a 3 (W) x 10 (L), 0.3 thick cm piece of clear plastic, with a 4 mm hole in the center, behind the canine teeth to hold the animals mouth open (Hornshaw, 1984). A 1.4 (ID) x 1.57 (OD) mm piece of polyethylene tubing, attached to an 18 gauge needle on a 3.0 ml syringe was inserted through the hole in the plastic, down the esophagus, and into the animal's stomach. This procedure prevented the ferret from biting the tubing and also assured that the animal was administered the entire dose. As in the dermal trial, 20 ferrets (one from each cage) were killed 48 hours post-dosing and the brains removed

for analysis of NTE activity. The remaining ferrets were observed daily for development of clinical signs of OPIDN and were given neurologic examination during the next 58 days.

Weights and Measurements

All animals were weighed on a digital balance (Fisher Scientific Co., Model #EWO-4010) when first obtained and at weekly intervals throughout the trial.

To account for variations in daily feed consumption, feed intake was measured for 2 consecutive days each week during the test period. During the days feed consumption was measured, feed was placed in containers (12 oz. tuna cans) secured to the bottom of the cages, rather than provided on the feed grids, to avoid wastage. An amount of feed, in excess of what a ferret would normally consume in a day, was placed in each container. These filled containers were weighed and then placed in the cages. At approximately the same time the next day, the containers were removed, weighed, amount of feed consumed recorded, and any uneaten feed discarded. Fresh feed was placed into the containers and the procedure repeated. In the event of spilled or wasted feed, the data were discarded.

Clinical Evaluations and Tests

General observation of gait, posture and habits

Beginning approximately a week after administration of TOTP, the animals were evaluated three times a week for

development of clinical signs typical of delayed neurotoxicity. Each animal was allowed to walk freely on a flat level wooden surface for 3-5 minutes. During this "walking time" the animals were observed for abnormalities in posture, gait, and general behavior. Normal ferrets walked in a crouched position with their heads pointed towards the ground, sniffing. Their gait was slow or fast, but very coordinated. Those animals showing any abnormalities during the observation period were subjected to further neurological tests similar to those used to evaluate the cat for delayed neurotoxicity (Abou-Donia et al., 1983b).

Each animal was subjectively rated on a scale from 0-6 for general observations. Those animals that received a score of R_1 or more were subjected to the neurological tests described below. The animal's response was also subjectively rated for each individual test using a similar scale. Descriptions of the rating scales are shown in Table 3.

Spinal Cord Reflexes

Flexor reflex - A toe on each hindfoot was pinched in an attempt to elicit a response. Normal animals retracted their limbs quickly; both hindlimbs were retracted in most cases.

Extensor thrust reflex - This procedure was used to test hindlimb functions associated with the femoral tibial nerve. In this test, the palm of the hand was placed against

Table 3. Description of rating scale and criteria used in assessing organophosphate-induced delayed neurotoxicity.

	General observations (gait, posture, habits)	Characteristics	Neurological examination
R_0	Normal	----	Normal response
R_1	Slight leg weakness	Slight change in usual walking behavior	Slightly abnormal response
R_2	Leg weakness	Some reluctance to walk and leg stiffness	Variable abnormal response
R_3	Mild ataxia	Change in gait and movement with minor loss of coordination	Abnormal response
R_4	Severe ataxia	Unsteadiness with loss of coordination and animal falling over	Little response
R_5	Slight paresis	Inability to stand and dragging of hindfeet	Little to no response
R_6	Severe paresis	Inability to walk on hind-limb	No response

the hind foot pads of the animal to apply pressure with quick short thrusts while the animal was supported by the axillae and hindlimbs above the examining table. The normal response is for the animal to resist the pressure and extend its limbs and claws.

Postural Reactions:

The following tests are designed to show complex responses that involve higher nerve centers as well as the spinal cord and the peripheral nerves.

Wheelbarrow - The animals' hindlimbs were picked up off the table so that the animal was forced to walk on its forelimbs. Normal animals walk with coordinated movements.

Extensor postural thrust and hindlimb walking - The animals were supported by the axillae while their hindfeet were slowly lowered to touch the table. Normal animals show hindlimb and claw extension while also supporting their weight on their legs. The animals were then walked backward and forward, using only the hindlimbs, to test coordination.

Hanging/Grasping reaction - In this test, the animals were supported by the axillae and above the table. The normal response of the animal is to spread and extend its hindlimbs to the side and extend the claws (i.e. grasping effect). This behavior was very characteristic of the ferret.

The last two neurological tests, proprioreceptive positioning and righting reaction, were only conducted on

animals in the oral trial. Through working with the ferrets on the dermal trial, it was found that these two tests were appropriate for detecting neurological deficits in the ferret.

Proprioreceptive positioning - The animals hindlimb paws are "turned over" so that the dorsal surface is facing the table surface. The normal response is for the animal to position the paws in the plantar position or to fully extend the paws.

Righting reactions - The animals were placed on their sides and backs. The normal response is for the animal to right itself.

Histopathological Techniques

Fifty eight days after treatment with TOTP, two ferrets per treatment group from the dermal trial were selected for evaluation of histopathological lesions of the nervous system. Following anaesthesia via intraperitoneal injections of sodium pentobarbital (30-40 mg/kg), they were perfused transcardially with a 10% formalin saline solution for approximately 15 minutes. The brain, spinal cord, and sciatic nerves were removed and tissue samples were preserved separately in a 10% formalin solution. Tissue blocks were taken from the cervical and lumbar spinal cord of each animal and placed in a 30% sucrose solution 48 hours prior to sectioning. The tissue blocks were frozen with dry ice and sectioned horizontally and cross sectioned at 50 μm, using a sliding microtome.

Three stains were used to evaluate the tissues. These included a modified thionin stain for nissl substance, a modified Fink-Heimer technique for degenerating axons, and the Speilmeyer method for normal myelin. Procedures for each stain are summarized below.

Thionin Stain (Clark, 1978)

1. Mount sections on subbed slides with an acetate mounting medium and allow to dry thoroughly.

2. Place slides in thionin for 1 minute.

3. Transfer to distilled water for 5 minutes.

4. Dehydrate the sections through a gradual series of 70%, 95%, and 100% for 5 minutes each.

5. Transfer to two changes of xylene for 10 minutes each.

6. Coverslip sections and view under bright-field illumination.

This method stains the nissl substance blue.

Modified Fink-Heimer Technique (Tanaka, 1976)

1. Wash sections briefly in distilled water and then transfer to a 0.025% potassium permanganate solution for 10 minutes.

2. Rinse briefly in distilled water and then decolorize completely in a 0.5% hydroquinone and 0.5% oxalic acid mixture.

3. Wash thoroughly in 4 volumes of distilled water for 5 minutes each.

4. Transfer sections to a 2.5% uranyl nitrate solution for 10 minutes.

5. Wash in 3 volumes of distilled water for 5 minutes each.

6. Transfer to a 2.5% silver nitrate solution for 10 minutes.

7. Transfer sections one at a time to a freshly prepared ammoniacal silver stain (45% silver nitrate, 75 ml; 80% ethanol, 25 ml; concentrated ammonia, 6 ml; 2.5% sodium hydroxide, 30 ml). Time in this solution may range from 1 to 1½ minutes.

8. Transfer individual sections to a Nauta reducing solution (10% formalin, 27 ml; 1% citric acid, 27 ml; 95% ethanol, 90 ml; distilled water, 800 ml) for approximately 30 seconds or until tissue turns a light brown. Transfer to a fresh reducing solution for 30 seconds.

9. Rinse tissues in distilled water and place in a 0.5% sodium thiosulfate solution for 1 minute.

10. Wash sections thoroughly in 4 changes of distilled water for 5 minutes each.

11. Mount sections on slides with a gel alcohol mounting medium, dehydrate, and coverslip as described above for the above thionin stain.

This method stains degenerating axons black.

Speilmeyer Method (Ambrogi, 1960)

1. Wash sections thoroughly in distilled water.

2. Put sections in 2.5% ferric ammonium sulfate solution overnight.

3. Wash in distilled water for approximately 1 minute.

4. Place sections in 70% ethanol for 10 minutes.

5. Transfer sections to hematoxylin stain for 30 minutes.

6. Wash twice in distilled water.

7. Transfer to a fresh ferric ammonium sulfate solution until blue fibers are clear and sharp (approximately 15 minutes). Watch sections closely as they differentiate.

8. Wash twice in distilled water.

9. Mount sections on slides using the same technique described for the Fink-Heimer method.

The myelin should stain black.

The sections that were stained by the thionin method were used to observe the neuronal cell bodies of the ventral motor horn in the cervical and lumbar spinal cord. Cross sections through the neuronal cell bodies of both treated and control animals were drawn at a magnification of X250 using a drawing tube on the microscope to detect any differences in nerve cell size between the treated and control ferrets. Only nerve cells showing a nucleus and a nucleolus were drawn so the complete area of individual cells was measured and not duplicated. The cells were grouped into lateral and medial cells for the cervical region and into large (dorsal) and small (ventral) cells for the lumbar region. The areas of the drawn cells were calculated using a Bioquant Image Analysis program on an Apple IIE Computer.

Statistical Analysis

Weekly feed consumption and body weight data were analyzed by one-way analysis of variance (Gill, 1978). For the data obtained from the Bioquant Image Analysis, controls and treated groups were statistically analyzed using the standard t-test and F-test. The $P \leq 0.05$ level was used as the criteria for significance.

RESULTS

The dermal trial began on October 18, 1984 and ended on December 13, 1984. The oral trial was started on August

14, 1985 and terminated on October 11, 1985. Results from the two trials are presented together to show comparisons.

Body Weight Changes and Feed Consumption

One-way analysis of variance (ANOVA) of the weekly feed consumption and body weight data for both the oral and dermal trials yielded no significant differences between the groups, although several trends were observed as shown in Figures 2-5. All treatment groups in the dermal trial exhibited increased feed consumption at week 2, followed by a decrease before becoming stable for all subsequent weeks (Figure 2). The animals administered 1000 mg TOTP displayed the greatest feed consumption. The animals on the oral trial showed a stable feed consumption throughout the trial, except for the unexplained marked increase by the control group at week 3 (Figure 3).

Ferrets in the dermally-treated groups exhibited an initial weight loss following treatment with the largest loss recorded for the 1000 mg TOTP group (Figure 4). All of these animals showed a body weight increase from the initial weight after week 3 except for the highest dose level. The 1000 mg TOTP-treated group gained weight over the initial weight by week 5 and had a final weight change similar to the other groups. As shown in figure 5, the orally-treated groups also exhibited initial body weight losses, but not all groups showed a final weight gain over their initial

FIGURE 2. Effect of dermal administration of TOTP on feed consumption.

Control
250 mg TOTP
500 mg TOTP
1000 mg TOTP

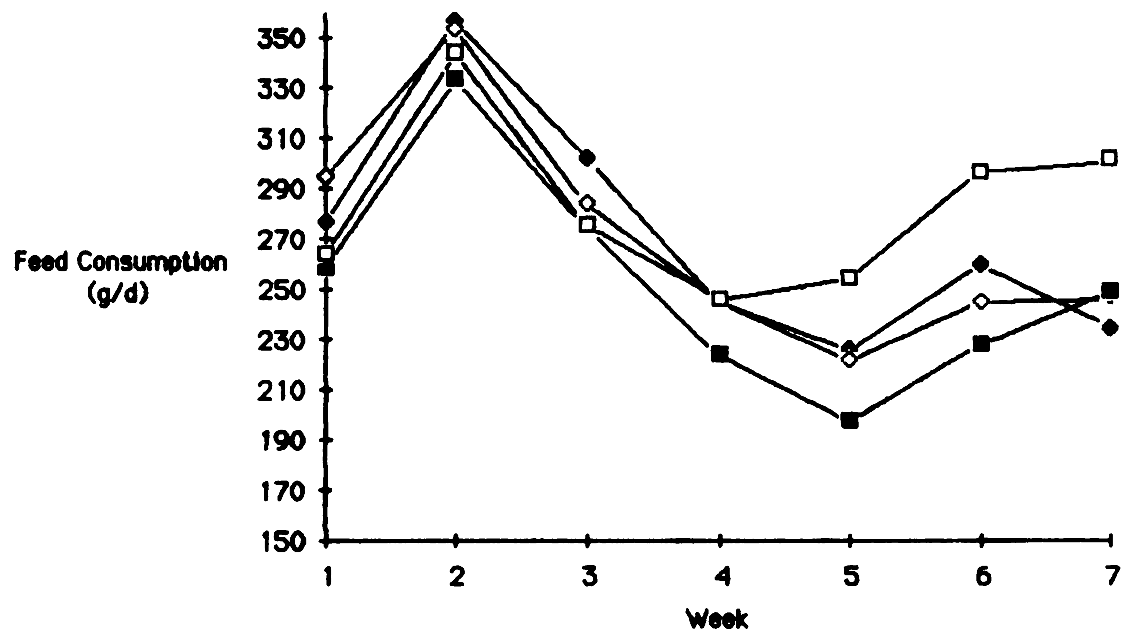
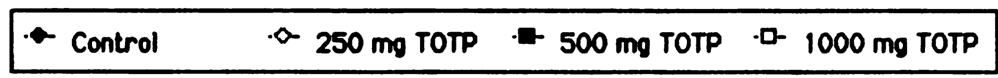

Feed Consumption
(g/d)
350
330
310
290
270
250
230
210
190
170
150
1
2
3
4
5
6
7
Week

FIGURE 3. Effect of oral administration of TOTP on feed consumption.


Control
250 mg TOTP
500 mg TOTP
1000 mg TOTP

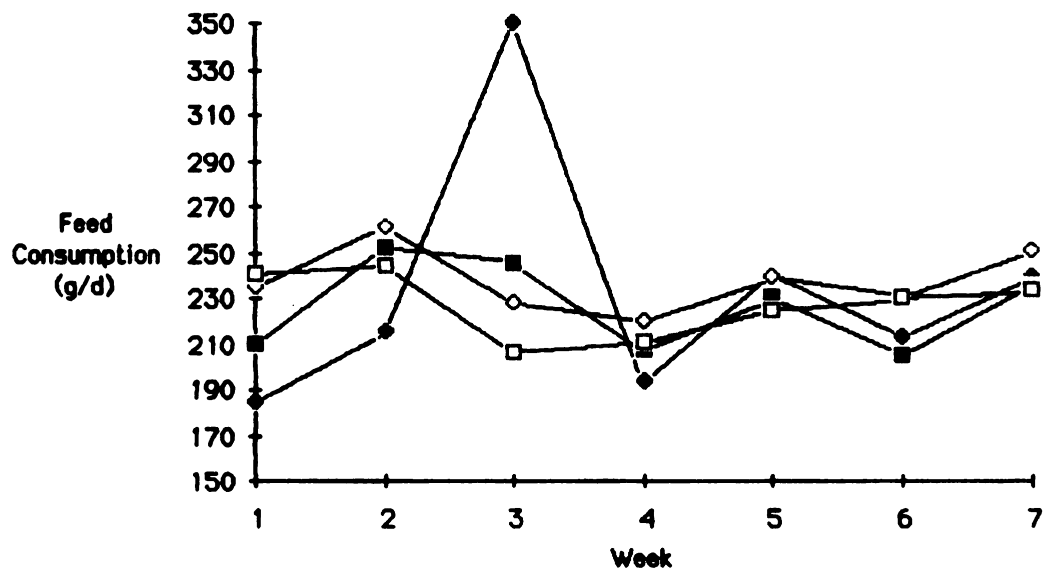

Feed
Consumption
(g/d)
350
330
310
290
270
250
230
210
190
170
150
1
2
3
4
5
6
7
Week

FIGURE 4. Effect of dermal administration of TOTP on body weight.

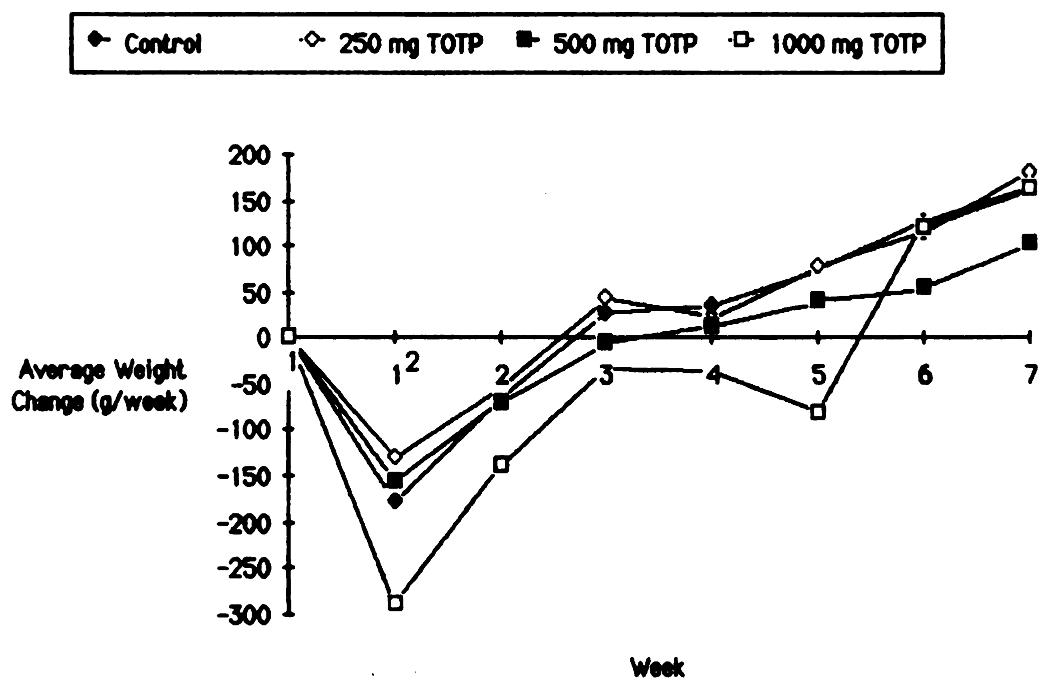
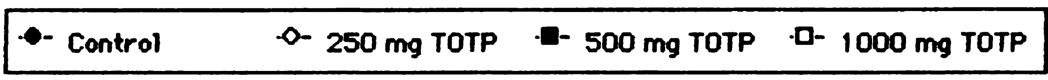
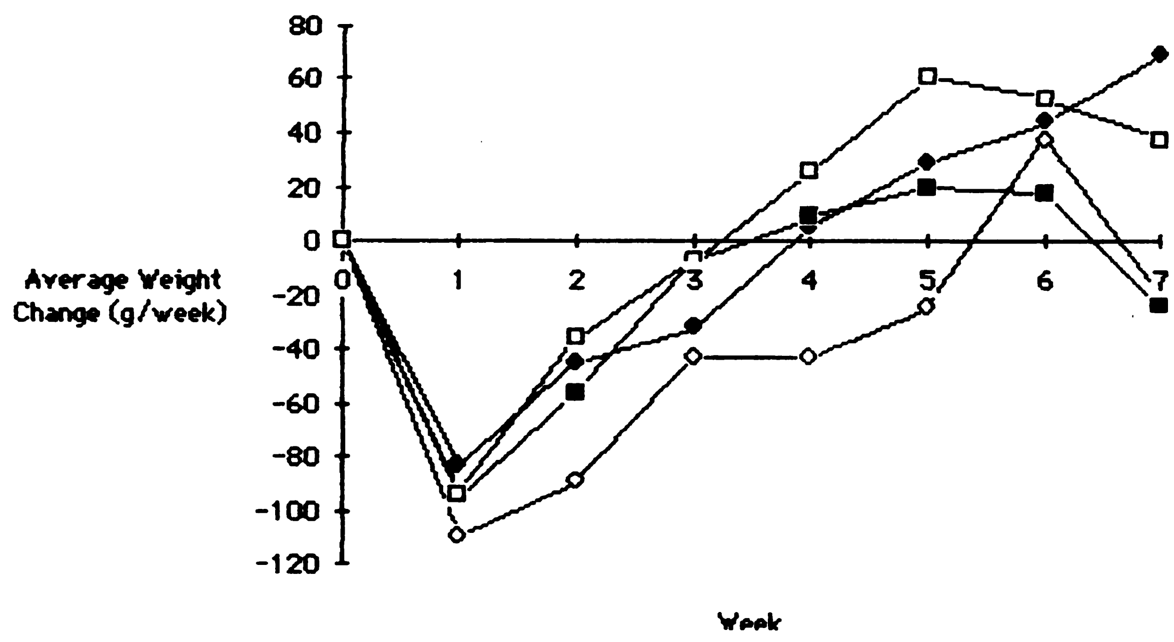

Control
250 mg TOTP
500 mg TOTP
1000 mg TOTP
200
150
100
50
0
-50
-100
-150
-200
-250
-300
Average Weight
Change (g/week)
1
1 2
2
3
4
5
6
7
Week

FIGURE 5. Effect of oral administration of TOTP on body weight.


Control
250 mg TOTP
500 mg TOTP
1000 mg TOTP


Average Weight Change (g/week)
80
60
40
20
0
-20
-40
-60
-80
-100
-120
0
1
2
3
4
5
6
7
Week

body weight. Comparison of the results of the two trials showed the initial body weight loss of the dermal groups was larger than for the oral groups.

Neurotoxic Esterase (NTE) Assay

The results of the NTE assays are shown in Table 4. The 1000 mg TOTP group exhibited the maximum inhibition of NTE for the dermal trial, 46%. The maximum inhibition for the oral trial was 37% and was associated with the animals in the 500 mg TOTP group.

Clinical Observations

Animals administered 500 and 1000 mg TOTP in both the dermal and oral trials, exhibited typical cholenergic poisoning signs of diarrhea, with the feces being tarry black in color. These signs diminished within one week after treatment and no other clinical signs of cholinergic poisoning were noted. The neurological examinations of the TOTP-treated ferrets revealed neurologic deficits in only the hindlimbs. The neurologic dysfunctions seen in the ferret were rated in 6 stages as summarized in Table 5. All animals with these dysfunctions were alert and active despite these neurologic problems. The normal posture of the ferret; head down, back flat, and legs positioned under the body is shown in Figure 6. Compared to the control ferret, the treated animal shown in Figure 7 has an arched back, characteristic of the R_3 response. Figure 8 shows a ferret with a sprawled, sliding

Table 4. Effect of dermal and oral administration of TOTP on neurotoxic esterase activity in the ferret.

Dose (mg/kg)	Neurotoxic esterase activity nmole product/min/gram tissue)	
	Dermal	Oral
0	1351[a] ± 53[b]	1257 ± 112
250	1142 ± 39 (18%)[c]	966 ± 176 (23%)
500	1019 ± 63 (25%)	797 ± 148 (37%)
1000	735 ± 77 (46%)	931 ± 46 (26%)

[a] Mean ± standard error. Based on 5 animals per treatment group. Numbers in parentheses denote percent inhibition of NTE activity when compared to the appropriate control.

Table 5. Description of clinical response ratings.

Response rating	Observed clinical signs
R_1	Muscle stiffness Slowed movements and careful placement of hindfeet Hopping movements instead of walking Some reluctance to walk
R_2	Uncoordinated movements Stiff wobble walk High stepping walk
R_3	Back arched during walking, with uncoordinated movements (Figure 3) Mild ataxia Unsteadiness in walk
R_4	Sliding walk with sprawled legs Sits on haunches and falls over Occasional falling over during walking Moderate ataxia
R_5	Sprawling walk (Figure 4) Falls over during walking Occasional dragging of hindfeet Difficulty in righting itself Severe ataxia
R_6	Complete paralysis

FIGURE 6. Control ferret displaying characteristic posture.

FIGURE 7. Arched back of ferret administered 1000 mg TOTP.

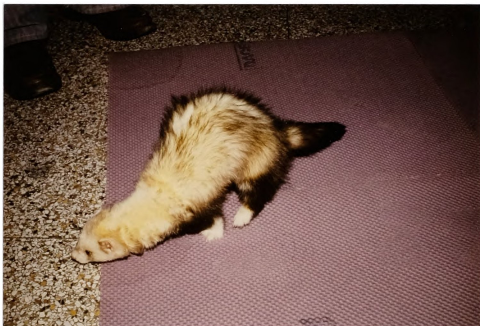
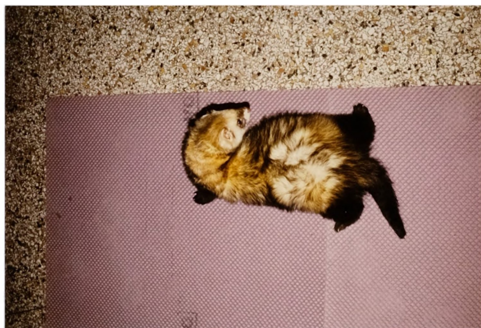


FIGURE 8. Sliding gait and sprawled legs of a TOTP-treated ferret.



gait, characteristic of the R_4 response. The differences in hindlimb support between a control ferret and a 1000 mg TOTP dermally-treated ferret is illustrated in Figure 9. The control animal on the right shows normal, strong hindlimb support, while the treated animal on the left exhibits minimal hindlimb support.

The individual animal rating responses for both trials are summarized in Table 6. There was an 11-day delay period before the initial onset of any clinical signs. Two ferrets (F2 and F13) administered dermal doses of 1000 mg TOTP showed the earliest clinical signs and progressed the quickest to advanced stages of neurologic deficits. The days showing the onset of responses indicate a slow progression of increased neurological deficiencies over time. None of the animals in the dermal trial, showing progressive responses, exhibited any signs of recovery from these deficits. Only three animals on the oral trial showed delayed neurotoxic signs (Table 6). However, these signs did not progress beyond an R_2 response and diminished after 55 days.

Results obtained from the additional neurologic examinations are shown in Table 7. The treated animals did not display abnormal locomotor or reflex signs until at least 10 days after general clinical signs were observed. Also, the animals did not show an abnormal response to the neurologic examination until the general observation response was at

FIGURE 9. Hindlimbs of control and TOTP-treated ferrets.

Table 6. Clinical assessment of ferrets administered tri-ortho-tolyl phosphate.

Dose (mg TOTP/kg body weight)	Ferret number	Response ratings (days post-treatment)				
		R_1	R_2	R_3	R_4	R_5
Dermal						
0	4	--	--	--	--	--
	40	--	--	--	--	--
	25	--	--	--	--	--
	6	--	--	--	--	--
	5	--	--	--	--	--
250	27	--	--	--	--	--
	39	--	--	--	--	--
	24	--	--	--	--	--
	20	53	--	--	--	--
	15	51	--	--	--	--
		(52 ± 1)[b]				
500	23	--	--	--	--	--
	16	23	49	--	--	--
	10	27	49	--	--	--
	37	27	--	--	--	--
	14	25	42	--	--	--
		(26 ± 1)				
1000	22	21	--	23	39	49
	35	16	23	25	37	--
	2	11	16	--	18	39
	38	21	27	--	35	--
	13	11	13	16	29	39
		(16 ± 2)	(20 ± 3)	(21 ± 3)	(32 ± 4)	(42 ± 3)

(Table 6, con't)

(Table 6, con't)

Dose (mg TOTP/kg body weight	Ferret number	Response ratings (days post-treatment)				
		R_1	R_2	R_3	R_4	R_5
Oral						
0	No animals showed abnormal responses.					
250	No animals showed abnormal responses.					
500	No animals showed abnormal responses.					
1000	26	--	--	--	--	--
	40	--	--	--	--	--
	23	21	30	--	--	--
	18	23	--	--	--	--
	37	27	30	--	--	--
		(24 ± 2)	(30)			

[a] See Table 3 for description of response ratings.

[b] Mean ± standard error, days post-treatment, 5 animals per treatment group.

[c] Responses diminished after 55 days.

Table 7. Neurological evaluations of ferrets administered tri-ortho-toyl phosphate[a,b].

Dose (mg TOTP/kg body weight)	Ferret number	Response ratings (days post-treatment R_1	R_2	R_3	R_4	R_5
Dermal		Flexor reflex				
1000	2	22	24	--	44	--
	13	22	24	44	--	--
	35	24	42	--	--	--
	22	49	53	57	--	--
	38	42	47	51	--	--
		Extensor-postural thrust and hindlimb walking				
	2	22	24	26	38	55
	13	22	24	44	49	--
	35	34	42	53	--	--
	22	42	51	53	--	--
	38	38	40	51	--	--
		Extensor-thrust reflex				
	2	22	24	26	38	55
	13	22	24	44	49	--
	35	34	42	53	--	--
	22	42	51	53	--	--
	38	38	40	51	--	--
		Hanging and grasping reflex				
	2	--	28	30	34	--
	13	22	28	32	57	--
	35	42	53	--	--	--
	22	49	--	53	--	--
	38	38	47	57	--	--

(Table 7, con't)

(Table 7, con't)

Dose (mg TOTP/kg body weight)	Ferret number	Response ratings (days post-treatment) R_1	R_2	R_3	R_4	R_5
Oral		Flexor reflex				
1000	37	36	--	--	--	--
	23	38	--	--	--	--
		Extensor-postural thrust and hindlimb walking				
	37	38	--	--	--	--
	23	43	--	--	--	--
		Extensor-thrust reflex				
	37	38	--	--	--	--
	23	43	--	--	--	--
		Hanging and grasping reflex				
	37	33	--	--	--	--
	23	38	--	--	--	--

[a] Animals exposed to TOTP at doses below 1000 mg/kg body weight gave normal responses in the neurological tests performed and thus are not included in the table.

[b] See Table 3 for description of response ratings.

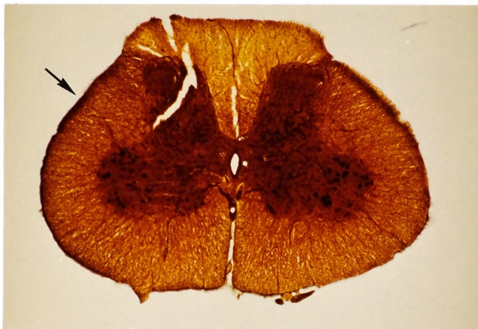
least an R_3. Animals that had abnormal flexor reflex responses showed a decrease in response time after the stimuli was administered. Abnormal extensor postural thrust and hindlimb walking response was characterized by the animal's tendency to set on its haunches (instead of standing on the hindlimbs), falling over, and showing a reluctance to walk. Extensor-thrust responses were the same as postural-thurst responses (Table 7). These animals exhibited little to no resistance to exerted pressure on the footpads of the hindlimb. Abnormal responses to the hanging/grasping test were generally characterized by the lack of extraction of the claws and a limp posture of the hindlimbs. The animals in all treatment groups for both trials had normal responses to the wheelbarrow test. No abnormal responses to the proprioreceptive positioning and righting reactions were observed in the ferrets on the oral trial.

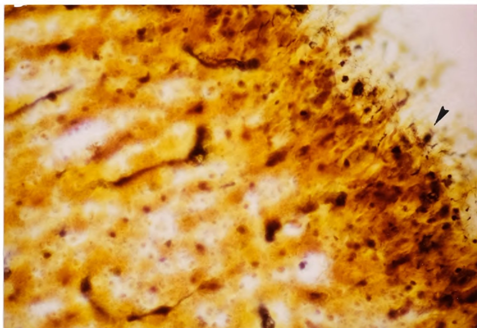
Histopathology

The most distinctive differences noticed in the tissues were obtained by the Fink-Heimer method. Degeneration was found in both animals administered dermal doses of 1000 mg TOTP and was located in the dorsal spinocerebellar tracts of the lumbar spinal cord (Figure 10). Degeneration of the long axons in these tracts was characterized by the presence of black, silver impregnated, fragmented axons (Figure 11). For comparison axons of the dorsal spinocerebellar tracts

FIGURE 10. Cross section of lumbar spinal cord of a ferret administered 1000 mg TOTP (84X, Fink-Heimer).

FIGURE 11. Dorsal spinocerebellar tracts of a ferret administered 1000 mg TOTP (350X, Fink-Heimer).





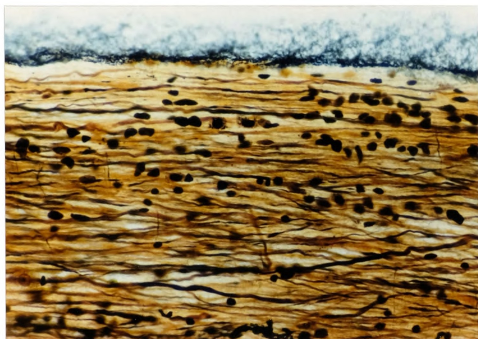
from controls are shown in Figures 12 and 13. The axon fibers are intact and lack the fragmented appearance of degenerating fibers. Longitudinal sections of this same area in a 1000 mg TOTP/kg dermally-treated animal (F-2) are shown in Figures 14 and 15. The axon degeneration is denoted by the silver impregnation of fragmented nerve fibers. Animal F-13, also treated dermally with 1000 mg TOTP/kg, exhibited more extensive degeneration in these tracts (Figures 16 and 17). Lesions were not detected at the cervical levels of the spinal cord nor in animals administered 500 and 250 mg TOTP/kg.

Observations of the tissue stained by the Speilmeyer method, which stains myelin, did not yield any discernible differences between treated and control animals. Myelin degeneration was not evident in any of the treatment groups in either the cervical or lumbar regions of the spinal cord (Figures 18 and 19).

The cells of control ferrets located in the ventral horn of both the cervical and lumbar regions of the spinal cord stained with thionin are shown in Figure 20. A higher magnification of a nerve cell from the cervical spinal cord of a control animal is shown in Figure 21. Nerve cells from the same region but obtained from animals administered 1000 mg TOTP/kg (Figures 22 and 23) showed no detectable qualitative differences when compared to those of the controls (Figures 22 and 23). Although most of the cells appeared normal in

FIGURE 12. Longitudinal section of dorsal spinocerebellar tracts in the lumbar region of a spinal cord in a control ferret (300X, Fink-Heimer).

FIGURE 13. Longitudinal section of dorsal spinocerebellar tracts in the lumbar region of the spinal cord in a control ferret (700X, Fink-Heimer).



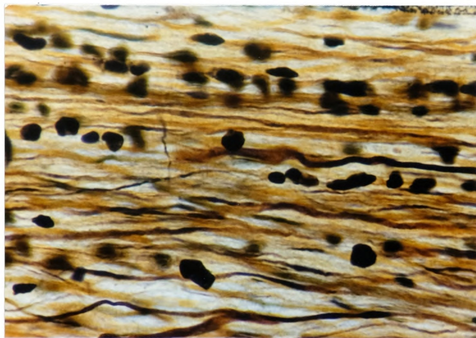


FIGURE 14. Longitudinal section of dorsal spinocerebellar tracts in the lumbar spinal cord of ferret number F-2 administered 1000 mg TOTP (100X, Fink-Heimer).

FIGURE 15. Longitudinal section of dorsal spinocerebellar tracts in the lumbar spinal cord of ferret number F-2 administered 1000 mg TOTP (200X, Fink-Heimer).

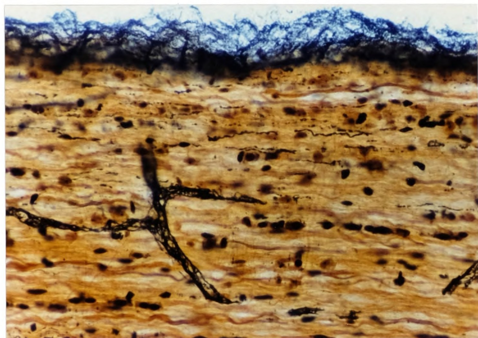
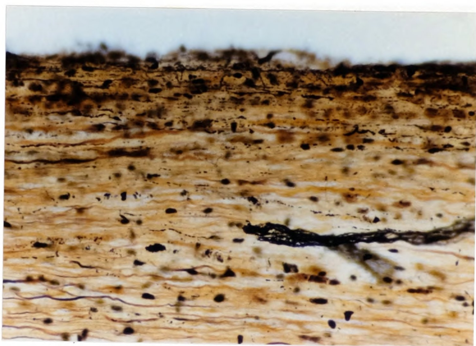


FIGURE 16. Longitudinal section of dorsal spinocerebellar tracts of ferret number F-13 administered 1000 mg TOTP (300X, Fink-Heimer).

FIGURE 17. Longitudinal section of dorsal spinocerebellar tracts of ferret number F-13 administered 1000 mg TOTP (600X, Fink-Heimer).



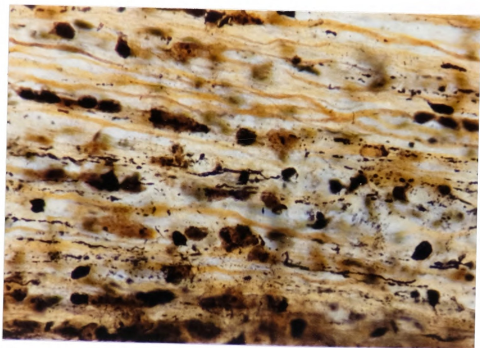
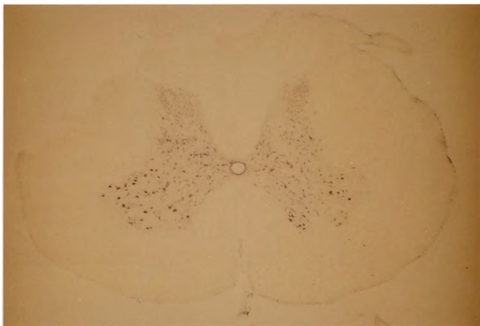


FIGURE 18. Cross section of lumbar spinal cord of a control ferret (66X, Speilmeyer).

FIGURE 19. Cross section of lumbar spinal cord of a ferret administered 1000 mg TOTP/kg (66X, Speilmeyer).

FIGURE 20. Cross section of cervical spinal cord of a control ferret (87X, Thionin).

FIGURE 21. Neuron from ventral horn of cervical spinal cord from a control ferret (700X, Thionin).



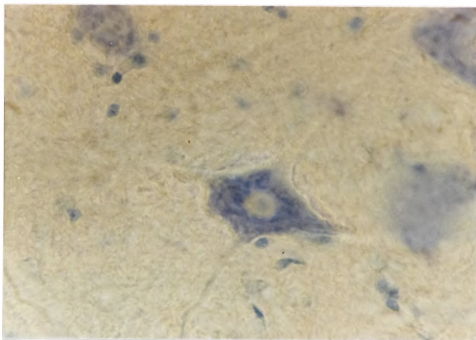
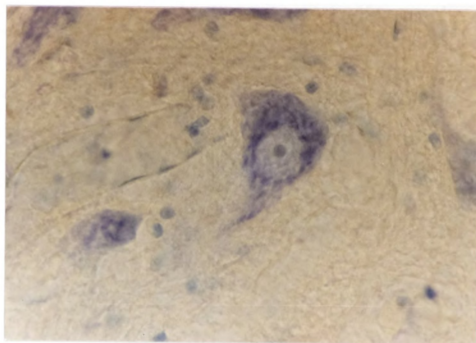
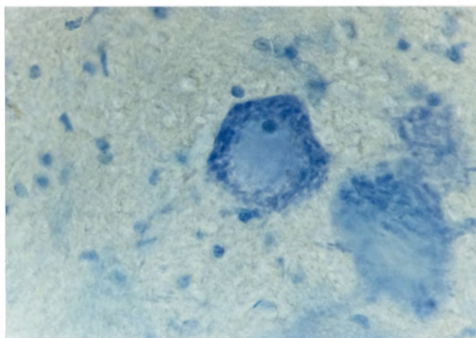


FIGURE 22. Neuron in the ventral horn of the cervical spinal cord, of a ferret treated with 1000 mg TOTP/kg (700X, Thionin).

FIGURE 23. Neuron in cervical spinal cord of a ferret treated with 1000 mg TOTP/kg (700X, Thionin).





the treated animal, some swelling of the cells was noticed. The Bioquant area measurements provided evidence that some changes in cell size were present. Neurons located in the lateral part of the ventral horn of the cervical spinal cord in the 1000 mg/kg TOTP-treated ferrets were significantly larger than those of the controls. No significant size difference was evident between cells located in the medial part of the ventral horn in 1000 mg/kg TOTP-treated animals and controls.

The cells located in the lateral part of the ventral horn of the lumbar spinal cord in the TOTP-treated animals were not significantly different in size from those of the controls. However, individual animal differences were observed. The nerve cells of ferret F-2 were significantly larger than those from either control animal. Another 1000 mg/kg TOTP-treated animal, F-13, did not statistically show a significant size difference but did have a smaller calculated mean area than the controls. No significant size differences were found in the area of small neurons located in the medial part of the ventral horn in the lumbar region between treated and control animals.

DISCUSSION

Three parameters were evaluated in assessing the neurotoxic effects of TOTP on the ferret. These included biochemical changes, functional deficiencies, and

histopathological manifestations. The animals on the dermal trial exhibited no statistical significant differences in feed consumption throughout the trial, but several trends were observed. The differences in feed consumption may be attributed to the consistency of the diet, environmental factors, and individual animal differences. The reported average daily feed consumption of 232 g/day for adult male ferrets fed a similar diet by Bleavins and Aulerich (1981) was attained by week 4 for all dermal treatment groups. The ferrets administered dermal doses of 1000 mg TOTP showed the highest feed consumption of any group, which correlates with the increased body weights of the same group during the test. Feed consumption for the animals on the oral trial remained relatively constant throughout the study. The presence of only minor cholinergic effects in orally-treated animals supports the findings of no great increase in feed consumption after cholinergic effects should have diminished (Figure 3). The reason for the apparent trend for increased feed consumption of the control animals in the oral trial during week 2 is unknown.

The substantial initial body weight loss for all groups on the dermal trial is consistent with the results obtained from similar studies with chickens and cats (Abou-Donia and Graham, 1979a; Abou-Donia _et al_., 1979; Abou-Donia _et al_., 1983). Other researchers have found that this loss of body

weight could be associated with loss of appetite or a direct effect of metabolic processes (Abou-Donia et al., 1983c). These weight losses were dose dependent and all the animals had gained weight by the termination of the study. The ferrets on the highest dermal dose exhibited the greatest weight gains. This same relationship was noted in the cat by Abou-Donia et al. (1983b) and has been attributed to the decrease in activity of the highest dose group. The ferrets administered 1000 mg TOTP/kg dermally also had the greatest feed consumption which could account for the greater weight gain in this group. The early weight loss of the animals on the oral trial was not as marked as for the dermal trial and may be attributed to the lack of progressive neurotoxic clinical signs in the oral as compared to the dermal trial.

In this study the low NTE inhibition (as compared to the chicken), 46% in the dermally-treated animals and 37% in the orally-treated animals, suggested that clinical signs of delayed neurotoxicity should not be evident. Johnson (1982) has concluded that 70-75% NTE inhibition is necessary to induce delayed neurotoxic effects in the chicken. However, neurotoxic clinical and morphological changes were observed in the ferrets treated with dermal doses of 500 and 1000 mg/kg TOTP. This can be explained through the findings of other investigators who reported that NTE inhibition was not necessarily correlated with the development of delayed

neurotoxicity. Bobwhites and mallards were found not to exhibit clinical signs of toxicity even with very high inhibition (66%) of NTE, while chickens which also had high inhibition (70%) of NTE did exhibit signs of neurotoxicity (Bursian _et al_., 1983). Thus, high inhibition of NTE does not always imply delayed neurotoxicity, so low inhibition also might not be related to no development delayed neurotoxic effects. Also, the assay used to determine NTE inhibition must be performed after the time of peak inhibition and before the synthesis of new NTE has begun (Johnson, 1977). Subsequent assays (unpublished data) have shown that the 48 hour post-treatment time interval used in this study was not the best estimate of maximum NTE inhibition in the ferret. Further, because NTE inhibition did not exceed 70-75% in the ferret, this target NTE inhibition may not be a pre-requisite for OPIDN.

The severity of the neurological dysfunctions observed in the treated ferrets was directly related to dose and route of exposure. Although the evaluation of the observed clinical signs of delayed neurotoxicity was subjective, the results of such evaluations have been found to be very sensitive in detecting early onset of neurologic deficits (Sprague _et al_., 1980). All animals exhibiting signs of OPIDN followed the same progressive sequence. Initial leg weakness was observed on the average of 16 days post-treatment, with 11

days being the earliest an animal displayed neurotoxic signs. These findings are consistent with the results reported by Abou-Donia et al. (1983c) for cats which indicated that initial delayed neurotoxic signs were present two to three weeks after administration of TOTP. Leg weakness was followed by the progressive onset of other dysfunctions that included ataxia, uncoordinated movements, high stepping gait, sliding walk, and partial paralysis. These progressive clinical signs closely followed the pattern of OPIDN in cats (Abou-Donia et al., 1983b).

The advanced neurological examinations yielded abnormal responses only after the ferrets received an R_3 rating in the clinical observations (Tables 6 and 7). This is consistent with other evidence that postural reactions and spinal cord reflexes remain normal during the early onset of delayed neurotoxicity (Abou-Donia et al., 1983b). During the early ataxia stages (R_3), abnormal extensor thrust reflexes were noted indicating defects in sciatic and tibial nerve functions. Only at the severe ataxia state (R_4) were substantial abnormal postural reactions observed, suggesting lesions in the spinal cord. A clinical examination rating of R_5, indicating partial paresis corresponded with high scores in all neurological tests, except for the wheelborrow test. At this stage, severe deficits in neurologic responses were observed, with no consequential effect on forelimb functions. This dominant

effect on hindlimbs as opposed to frontlimbs is a characteristic of OPIDN (Abou-Donia et al., 1983b; Abou-Donia et al., 1983c). The most characteristic abnormal response of the ferret was demonstrated in the hanging/grasping neurological test. Ferrets at the highest dose level displayed a progressive abnormal response best described as the lack of spreading out of the hindlimbs and complete extraction of the claws when held in a hanging position. This response was also not observed until a general observation rating of R_3 was obtained, implying possible spinal cord lesions.

The lack of progressive clinical signs in the ferrets on the oral trial is not in complete agreement with the results obtained from other investigators. Although studies have shown the acute toxicity of dermally-applied TOTP to be twice that of oral administration, progressive signs were still apparent in orally-treated animals (Abou-Donia et al., 1983c). However, these clinical signs were not as severe as those found in the animals on the dermal trial and also were not as prominent by the end of the trial (Abou-Donia et al., 1974). The ferrets on the oral trial displayed clinical signs of recovery towards the end of the study, whereas the dermally treated animals did not. The cat also has been found to exhibit signs of recovery (Abou-Donia et al., 1983b).

The lesions present in the lumbar spinal cord of ferrets treated dermally with 1000 mg TOTP/kg are indicative of the

observed clinical signs. The dorsal spinocerebellar tracts have been demonstrated to terminate only in the region of the cerebellum that controls hindlimb functions and these tracts mediate impulses from proprioreceptors (Brodal, 1975). These proprioreceptors convey information to the tracts from muscle spindles, tendon organs, and pressure receptors. Some dorsal spinocerebellar units also convey impulses from flexor reflex afferents which control the flexor reflex in animals. Thus, the clinical dsyfunctions observed in the TOTP-treated ferrets can be histopathologically explained by lesions in these areas, especially the neurological deficits seen in flexor and extensor responses. Although others have demonstrated lesions characteristic of OPIDN in ventral-descending tracts in the lumbar and thoracic regions and also in the dorsal-lateral tracts of the cervical region (Abou-Donia and Graham, 1979; Abou-Donia _et al_., 1983b), these lesions were not noted in the TOTP-treated ferrets. The ventral spinocerebellar tracts are also concerned with the transmission of impulses from the hindlimbs, but the fibers are much thinner than those located in the dorsal region (Brodal, 1975). Conclusions have been drawn suggesting that axon degeneration in OPIDN is most often associated with larger diameter axons (Baron, 1981). This may explain why lesions were not detected in the ventral regions. Further, axon degeneration was more extensive in animal F-13 (Figures

14 and 15) which also exhibited more severe clinical signs, than animal F-2 (Figures 12 and 13) which was also treated dermally with 1000 mg/kg TOTP. This suggests a correlation between severity of clinical signs and histopathologic changes. Abou-Donia et al. (1983b) described histopathological changes as a function of size of the dose, frequency and duration of the administration, severity of clinical signs, length of the period between the onset and termination of ataxia, and the site of tissue sampling.

The evidence obtained from the histopathologic evaluations of the ventral motor neurons also substantiates the clinical results. Although qualitative changes were not easily detectable, area measurements suggested that changes in cell size had occurred in the TOTP-treated ferrets. Differences were only found in neurons in the lateral part of the ventral horn of the cervical spinal cord and in the larger, dorsally-located cells of the lumbar ventral horn. Anatomical investigations have shown that the medial cells supply muscles of the neck and trunk, while the lateral neurons supply the muscles of limbs (Brodal, 1975). Cells associated with functions of the distal extremities are found in the most dorsal regions of the ventral horn. The cells in the cervical spinal cord were significantly larger in the 1000 mg TOTP/kg dermally-treated animals when compared to the controls. The cells in the lumbar region were variable as the cells

of animal F-2 were significantly larger than controls, but the cells from animal F-13 were significantly smaller. Although statistics conducted on a small size sample are not that reliable, these results suggest that the animal, F-13, exhibiting the most severe clinical signs and axon degeneration may also have shown initial stages of neuronal atrophy subsequent to swelling of the cells. Animal F-2 exhibited signs of neuron swelling but had not yet advanced to the atrophy stage. There are conflicting reports concerning on the effect of OPIDN on motor neurons. Some reports indicate that the cell bodies are not involved in the toxic process (Cavanagh and Koller, 1979; Cavanagh, 1973). Ahmed and Glees (1971), however, concluded from their observations in the slow loris that chromalytic neurons were present in the ventral horns of the lumbar regions. These neurons were characterized by an eccentric nucleus, peripheral arrangement of nissl substance, and atrophy or disintegration of neurons.

Many studies have used various staining methods to demonstrate demyelination in OPIDN. In the present study, the stained myelin tissues did not reveal conclusive evidence that demyelination had occurred. Lack of myelin staining was found in both control and treated animals. Therefore, detection of myelin loss due to the administration of TOTP could not be determined. Cavanagh (1973) stated that myelin stains are often unsatisfactory and misleading because only

in cases of advanced degeneration do myelin stains show appreciable differences.

From results obtained in this study, it was concluded that dermal administration of TOTP was more effective than oral administration in producing delayed neurotoxicity. Abou-Donia _et al_. (1983b) have related this difference in toxicity in route of administration in the cat to slow absorption of chemicals from the gastrointestinal tract and variable absorption in individual orally-treated animals. The recovery observed in the ferrets administered TOTP via the oral route may be attributed to peripheral nerve regeneration (Abou-Donia _et al_., 1983c). Peripheral nerve damage occurs earlier in the OPIDN sequence and can be reversible, but spinal cord lesions are irreversible (Abou-Donia and Graham, 1979b). Thus, the neurotoxic effect and recovery may be hypothesized to be dependent upon administered dose and route of exposure.

The ferret's sensitivity to OPIDN differed slightly from both the chicken and the cat. Although the ferret was demonstrated not to be as sensitive to OPIDN as the chicken, similarities exist in that no recovery in the dermally-treated animals was observed. The ferret also had similar responses to the cat in the neurological examinations but no recovery was observed in dermally-treated ferrets as has been reported for cats. The ease of handling and caring for the ferret,

along with the species sensitivity to delayed neurotoxicity, make this species an attractive future alternative for OPIDN testing. However, further research, including long-term chronic trials, sequencial investigations of histopathological changes, and studies in distribution, metabolism, and elimination of delayed neurotoxins should be conducted before an accurate assessment of the use of the ferret as a model species can be made.

CONCLUSIONS

Based upon the results of this study to assess the sensitivity of the ferret to OPIDN, the following conclusions were drawn.

1. Ferrets are a sensitive species to OPIDN when administered dermal doses of TOTP and are less sensitive when administered oral doses.

2. Clinical signs of delayed neurotoxicity were observed 16 days post-treatment and were characterized by ataxia that progressed to partial paralysis of the hindlimbs.

3. Histopathological lesions included axon degeneration in the dorsal spinocerebellar tracts of the lumbar spinal cord and changes in cell size of the ventral horn motor neurons.

4. Onset of delayed neurotoxicity and recovery was dependent upon dose and route of exposure. Recovery was only observed in animals administered TOTP via the oral route.

5. Clinical and histopathological symptoms of OPIDN were present even at minimal levels of NTE inhibition.

6. The ferret was not as sensitive as the chicken to OPIDN but similar in sensitivity to the cat. The ferret appears to be a satisfactory species for testing the delayed neurotoxic potential of chemicals, but further research on OPIDN with the ferret should be conducted.

REFERENCES

Abou-Donia, M.B., 1981. Organophosphorus ester-induced delayed neurotoxicity. Ann. Rev. Pharmacol. Toxicol. 21:511-548.

Abou-Donia, M.B., G. Doyle, H.M. Graham, and K.M. Abdo, 1983a. Effects of subchronic dermal application of 0-ethyl-0-4-nitrophenyl phenyl phosphonothioate on producing delayed neurotoxicity in hens. Neurotoxicol. 4(2):247-260.

Abou-Donia, M.B. and D.G. Graham, 1979a. Delayed neurotoxicity of 0-ethyl-0-4-nitrophenyl phenyl phosphonothioate: toxic effects of a single dose on the nervous system of the hen. Toxicol. Appl. Pharmacol. 48:57-66.

Abou-Donia, M.B. and D.G. Graham, 1979b. Delayed neurotoxicity of a single oral dose of 0-ethyl-0-4-cyanophenyl phenyl phosphonothioate in the hen. Neurotoxicol. 1:449-466.

Abou-Donia, M.B., D.G. Graham, and C.G. Kinnes, 1983b. Sensitivity of the cat to delayed neurotoxicity induced by 0-ethyl-0-4-nitrophenyl phosphonothioate. Toxicol. Appl. Pharmacol. 68:54-65.

Abou-Donia, M.B., D.G. Graham, P.R. Timmons, and B.C. Reichert, 1979. Delayed neurotoxic and late effects of S.S.S.-tributyl phosphoronothioate on the hen: Effect of route of administration. Neurotoxicol. 1:425-447.

Abou-Donia, M.B., D.N. Jensen, and D.M. Lapadula, 1983c. Neurologic manifestations of tri-o-cresyl phosphate

delayed neurotoxicity in cats. Neurobehav. Toxicol. Teratol. 5:431-442.

Abou-Donia, M.B., M.A. Othman, G. Tantawy, A.Z. Khalil, and M.F. Shawer, 1974. Neurotoxic effect of leptophos. Experentia 30:63-64.

Ahmed, M.M. and P. Glees, 1971. Neurotoxicity of tricresyl phosphate (TCP) in slow loris (Nycticebus coucany coucany). Acta. Neuropathol. 19:94-98.

Ambrogi, L.P., 1960. Speilmeyer's stain for myelin sheath in frozen sections. IN: **Manual of Histologic and Special Staining Techniques**, 2nd ed. McGraw-Hill Book Company, Inc., New York, NY, 169 pp.

Asdell, S.A., 1946. **Patterns of Mammalian Reproduction.** Comstock Publishing Co., Inc., Ithaca, NY, 670 pp.

Barnes, J.M. and F.A. Denz, 1953. Experimental demyelination with organophosphorus compounds. J. Pathol. Bacteriol. 65:597-605.

Baron, R.L., 1981. Delayed neurotoxicity and other consequences of organophosphate esters. Ann. Rev. Entomol. 26:29-48.

Baron, R.L. and J.E. Casida, 1962. Enzymatic and antidotal studies on the neurotoxic effect of certain organophosphates. Biochem. Pharmacol. 11:1129-1136.

Beck, B.E., C.D. Wood, and G.R. Whenham, 1977. Triaryl poisoning in cattle. Vet. Pathol. 14:128-137.

Bidstrup, P.L., J.A. Bonnell, and A.G. Beckett, 1953. Parlaysis following poisoning by a new organic phosphorus insecticide (Mipafox). Brit. Med. J. 1:1068-1069.

Bleavins, M.R. and R.J. Aulerich, 1981. Feed consumption and food passage time in mink (Mustela vison) and European ferret (Mustela putorius furo). Lab. An. Sci. 31:268-269.

Bleiberg, M.J. and H. Johnson, 1965. Effects of certain metabolically active drugs and oximes on tri-o-cresyl phosphate toxicity. Toxicol. Appl. Pharmacol. 7:227-235.

Bradley, W.A., 1976. The pathology of delayed neurotoxicity due to organophosphates. IN: **Pesticide Induced Delayed Neurotoxicity.** R.L. Baron (ed.). U.S. Environ. Prot. Agency, pp. 84-101.

Brodal, A., 1975. **Neurological Anatomy in Relation to Clinical Medicine.** Oxford University Press, New York, NY, pp. 807.

Bursian, S.J., J.S. Brewster, and R.K. Ringer, 1983. Differential sensitivity to the delayed neurotoxin tri-o-toyl phosphate in several avian species. J. Toxicol. Environ. Health 11:907-916.

Casida, J.E. and R.L. Baron, 1976. Recognition and overview of the organophosphorus induced delayed neurotoxicity problem. IN: **Pesticide Induced Delayed Neurotoxicity,** Proc. Conf. pp. 7-23. U.S. Environ. Prot. Agency, NO. 600/1-76-025.

Casida, J.E., M. Eto, and R.L. Baron, 1961. Biological activity of a tri-ortho cresyl phosphate metabolite. Nature 191:1396.

Cavanagh, J.B., 1954. The toxic effects of tri-ortho cresyl phosphate on the nervous system. An experimental study in hens. J. Neurol. Neurosurg. Psychiat. 17:163-172.

Cavanagh, J.B., 1973. Peripheral neuropathy caused by chemical agents. Crit. Rev. Toxicol. 2:365-417.

Cavanagh, J.B. and W.C. Koller, 1979. Tri-ortho-cresyl phosphate poisoning. IN: **Handbook of Clinical Neurology.** Vinken, D.J. and G.W. Brwyn (eds). North Holland Publ. Co., Amsterdam, 37(III):471-477.

Clark, G., 1978. Tissue preparation and basic staining techniques. IN: **Neuroanatomical Research Techniques.** Robinson, R.T. (ed). Academic Press, Inc., New York, NY, 312 pp.

Davies, D.R., 1963. Neurotoxicity of organophosphorus compounds. IN: **Handbuch der Experimentellen Pharmacology.** Koelle, G.B. (ed.). Springer Verlag (Berlin) pp. 860-882.

Elizan, T.S., A. Fabiyi, and J.L. Sever, 1964. Experimental teratogenesis in ferrets using rubella virus. J. Mt. Sinai Hospital 36:103-107.

Eto, M., 1974. **Organophosphorus Pesticides: Organic and Biological Chemistry**, CRC Press, Inc., Cleveland, OH, 387 pp.

Farris, E.J., 1950. **The Care and Breeding of Laboratory Animals.** John Wiley and Sons, Inc., New York, NY, 515 pp.

Fassett, D.W., 1967. **Industrial Hygiene and Toxicology,** Vol. II., Interscience, New York, NY, 515 pp.

Federal Register, 1978. **Proposed Guidelines for Registering pesticides in the U.S.; Hazard Evaluations: Humans and Domestic Animals, Sec. 163, 81-7.** Acute and Delayed Neurotoxicity Study. Fed. Regis. 43:37361-37363.

Gill, J.L., 1978. **Design and Analysis of Experiments in the Animal and Medical Sciences,** Vol. 1. The Iowa State Univ. Press, Ames, IA, 409 pp.

Green, R.G., 1945. Zoological and histological modification of the distemper virus by ferret passage. Am. J. Hygiene 41:7-24.

Gordon, J.J., R.H. Inns, M.K. Johnson, L. Leadbeater, M.P. Maidment, D.G. Upshall, G.H. Cooper, and R.L. Pickard, 1983. The delayed neuropathic effects of nerve agents and some other organophosphorus compounds. Arch. Toxicol. 52:71-82.

Haddad, R.K., A. Rabe, and R. Dumas, 1975. CNS defects. Comp. Pathol. Bull. 7:2-4.

Hahn, E.W. and R.C. Wester, 1969. **The Biomedical Use of Ferrets in Research.** Marshall Research Animals, Inc., North Rose, New York, NY, 52 pp.

Hornshaw, T.C., 1984. Development of dietary LC_{50} and reproduction test protocols using mink and ferrets as representative mammalian carnivores. Ph.D. Dissertation, Michigan State University, East Lansing, MI, 212 pp.

Johnson, M.K., 1969. The delayed neurotoxic effect of some organophosphorus compounds; identification of the phosphorylation site as an esterase. Biochem. J. 114:711-717.

Johnson, M.K., 1970. Organophosphorus and other inhibitors of "neurotoxic esterase" and the development of delayed neurotoxicity in hens. Biochem. J. 120:523-531.

Johnson, M.K., 1974. The primary biochemical lesion leading to the delayed neurotoxic effects of some organophosphorus compounds. J. Neurochem. 23:785-789.

Johnson, M.K., 1975. The delayed neuropathy caused by some organophosphorus esters: mechanism and challenge. Crit. Rev. Toxicol. 3:289-316.

Johnson, M.K., 1977. Improved assay of neurotoxic esterase for screening organophosphates for delayed neurotoxicity potential. Arch. Toxicol. 37:113-115.

Johnson, M.K., 1980. The mechanism of delayed neuropathy caused by some organophosphorus esters: Using the understanding to improve safety. J. Environ. Sci. Health B15(6):825-841.

Johnson, M.K., 1982. Initiation of organophosphate-induced delayed neuropathy. Neurobehav. Toxicol. Teratol. 4:759-765.

Koelle, G.B. and A. Gilman, 1946. The chronic toxicity of di-isopropyl fluorophosphate (DFP) in dogs, monkeys, and rats. J. Pharma. Expt. Therap. 87:435-448.

Lillie, R.D. and M.I. Smith, 1932. The histopathology of some neurotoxic phenol esters. National Institute of Health Bulletin 160:54-59.

Metcalf, R.L., 1982. Historical perspective of organophosphorus ester-induced delayed neurotoxicity. Neurotoxicol. 3(4):269-284.

Miller, E.P., 1983. Organophosphate toxicity - 2: delayed effects. Agripractice, May, pp. 771-774.

Modell, W., S. Krop, P. Hitchcock, and W.F. Riker, 1946. General systemic actions of di-isopropyl fluorophosphate (DFP) in cats. J. Pharma. Expt. Therap. 87:400-413.

O'Brien, R.D., 1960. **Toxic Phosphorus Esters; Chemistry, Metabolism, and Biological Effects**. Academic Press, New York, NY, 434 pp.

Perrin, T.B. and J.W. Oliphant, 1940. Pathologic histology of experimental virus influenza in ferrets. Public Health Repts., Washington, D.C., 55:1077-1086.

Pfeiffer, C.J. and J. Weibel, 1973. The gastric mucosal response to acetyl salicyclic acid in the ferret. Am. J. Digest. Dis. 18:834-846.

Rieger, D. and B.D. Murphy, 1977. Episodic fluctuations in plasma testosterone and dihydrotestosterone in male ferrets during the breeding season. J. Reprod. Fert. 51:511-514.

Sax, N.I., 1984. **Dangerous Properties of Industrial Materials,** 6th ed. Von Nostrand Reinhold Company, New York, pp. 3124.

Schaumburg, H.H. and P.S. Spencer, 1979. Toxic neuropathies. Neurol. 29:49-431.

Smith, M.I., E. Elvove, and W.H. Frazier, 1930. The pharmacological action of certain phenol esters, with special reference to the etiology of so-called ginger paralysis. U.S. Public Health Repts. 45:2509-2524.

Smith, H.V. and M.K. Spaulding, 1959. Outbreak of paralysis in Morocco due to ortho-cresyl phosphate poisoning. Lancet 2:1019-1021.

Sprague, G.L., L.L. Sandvik, A.A. Bickford, and T.R. Castles, 1980. Evaluation of a sensitive grading system for assessing acute and subchronic delayed neurotoxicity in hens. Life Sci. 27:25-26.

Stuart, L.D. and F.W. Oehme, 1982. Organophosphate delayed neurotoxicity: a neuromyelopathy of animals and man. Vet. Hum. Toxocol. 24:2.

Takade, D.K., 1982. Delayed neurotoxicity in perspective: Summary and objectives of the workshop. Neurotox. 3:263-268.

Tanaka, D., 1976. Thalamic projections of the dorsomedial prefontal cortex in the Rhesus monkey (Macaca mulatta). Brain Res. 110:21-38.

Taylor, J.D., 1967. A neurotoxic syndrome produced in cats by a cyclic phosphate metabolite of tri-ortho-cresyl phosphate. Toxicol. Appl. Pharmacol. 11:538-545.

Thornton, P.C., P.A. Wright, P.J. Sacra, and T.E. Goodier, 1979. The ferret, Mustela putorius furo, as a new species in toxicology. Lab. An. 13:119-124.

Williams, C.S., 1976. **Practical Guide to Laboratory Animals.** C.V. Mosby Co., St. Louis, MO, 207 pp.

Willis, L.S. and M.V. Barrow, 1971. The ferret (Mustela putorius furo) as a laboratory animal. Lab. An. Sci. 21(5):712-716.

World Health Organization, 1976. Report of the 1975 FAO/WHO joint meeting pesticides residues. WHO Tech. Rep. Ser. No. 592 and FAO Plant Prod. Prot. Ser. No. 1:11-13.

MICHIGAN STATE UNIV. LIBRARIES
31293006886869