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By

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ABSTRACT

NEUROTOXICITY OF TRIPHENYL PHOSPHINE IN THE EUROPEAN FERRET

By

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This study examined the effects of the organophosphorus delayed neurotoxicant triphenyl phosphine (TPPn) in the European ferret (*Mustela putorius furo)*. Adult ferrets were injected subcutaneously with 250 or 500 mg TPPn/kg body weight, or the diethyl ether/peanut oil vehicle. Whole-brain acetylcholinesterase (AChE) and neuropathy target esterase (NTE) activities were measured 24 hours post-dosing. Neither enzyme was inhibited by TPPn. Clinical signs were assessed over a six day observation period and ranged from difficulty in holding the head erect beginning at four days post-treatment to forelimb and hindlimb paralysis which were apparent at six days post-treatment. Neuropathological damage was assessed by the Fink-Heimer silver impregnation method in the brains collected at six days post-dosing. Axonal and terminal degeneration occurred in several forebrain regions including the neocortex, hypothalamus, thalamus, and basal ganglia. Degeneration in the midbrain was present in superior and inferior colliculi, and regions of the pontine gray. Brainstem regions that contained axonal and terminal degeneration were the pons, tegmentum, brainstem nuclei/tracts, and the vestibular nuclear complex. Degeneration was also found throughout the cerebellum. The results of the present study indicate that TPPn is a neurotoxicant that produces clinical signs and pathology consistent with organophosphorus induced delayed neurotoxicity (OPIDN) in the European ferret without inhibition of NTE and AChE.

For Christopher Jay

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FIGURE ABBREVIATIONS

AV	anterior ventral thalamic nucleus
Cb	cerebellum
CM	centrum medianum thalamic nucleus
COn	cochlear nuclei
Cos	coronal sulcus
CRs	cruciate sulcus
CX	external cuneate nucleus
EN	entopeduncular nucleus
ESs	ectosylvian sulcus
FF	fields of Forel
FTG	gigantocellular tegmental field
ICC	central nucleus of the inferior colliculus
IO	inferior olivary complex
LD	lateral dorsal thalamic nucleus
LGN	lateral geniculate nucleus
LL	lateral lemniscus
LP	lateral posterior thalamic nucleus
LRN	lateral reticular nucleus
MAs	marginal sulcus
MD	mediodorsal thalamic nucleus
MGN	medial geniculate nucleus
MMn	medial mammillary nucleus
NC	cuneate nucleus
NG	nucleus gracilis
PAT	parataenial nucleus
PG	pontine gray
PGL	pontine gray, lateral division
PGM	pontine gray, medial division
PSs	presylvian sulcus
RHs	rhinal sulcus
Rn	red nucleus
SCI	superior colliculus, intermediate lamina
SO	superior olivary complex
SOL	lateral nucleus of the superior olive
SOM	medial nucleus of the superior olive
SPs	splenial sulcus
SSs	suprasylvian sulcus
SUB	subthalamic nucleus
TGn	tegmental nuclei

FIGURE ABBREVIATIONS

VA	ventral anterior thalamic nucleus
VB	ventral basal thalamic nucleus
VCx	visual cortex
VL	ventral lateral thalamic nucleus
VLV	lateral vestibular nucleus, ventral division
VM	ventral medial thalamic nucleus
VMN	medial vestibular nucleus
VN	vestibular nuclear complex
VPL	ventral posterior nucleus
VSN	superior vestibular nucleus

INTRODUCTION

Exposure to certain organophosphorus compounds (OP's) in agricultural and industrial settings can cause neurotoxicity in animals including humans. OP-induced neurotoxicity is apparent either immediately (acute effects) and/or several days after (delayed effects) exposure. Reversible acute effects are the result of inhibition of the nervous system enzyme acetylcholinesterase (AChE) (Davis and Richardson, 1980). Delayed effects are non-reversible and are commonly known as organophosphorus-induced delayed neurotoxicity (OPIDN) (Davis and Richardson, 1980).

OPIDN is thought to result from an interaction between a neurotoxic OP compound and the enzyme neuropathy target esterase (NTE) (Johnson, 1987; Peraica et al., 1995). Inhibition of whole-brain NTE exceeding 70% is generally associated with clinical signs and pathology characteristic of OPIDN (Davis and Richardson, 1980; Abou-Donia and Lapadula, 1990; Richardson, 1992). Although recent studies indicate that the threshold of NTE inhibition for OPIDN may be less than 70%, depending upon the species and compound, some degree of NTE inhibition in animals that exhibit OPIDN has always been reported (Larsen et al., 1986; Veronesi and Dvergsten, 1987; Stumpf et al., 1989).

Recently, OPIDN has been divided into two subclasses (Type I and Type II) based on the following classification criteria (Abou-Donia and Lapadula, 1990). Type I OPIDN is produced by organic esters of phosphoric acid such as tri-o-tolyl phosphate (TOTP) and diisopropylfluorophosphate (DFP), while organic esters of phosphorus acid such as triphenyl phosphite (TPP) cause Type II OPIDN. Differences in species sensitivity to Type I and Type II compounds exist. Chickens and ferrets are sensitive to both Type I and Type II OPIDN compounds, but rodents and Japanese quail are only sensitive to Type II compounds (Abou-Donia and Lapadula, 1990; Tanaka et al., 1990a; Tanaka et al., 1992b; Varghese et al., 1995). Sensitive species are also susceptible to Type I and Type II compounds at different ages. The young of sensitive species are typically not sensitive to single doses, but may be sensitive to multiple doses of a Type I compound. In contrast, a single dose of a Type II compound can cause OPIDN in both young and adult animals of sensitive species(Abou-Donia and Lapadula, 1990). Type I compounds are typically associated with a longer delay period before the onset of clinical signs (14-21 days) when compared to Type II compounds (four to seven days) (Abou-Donia and Lapadula, 1990). The characteristic clinical signs produced by delayed neurotoxicants vary by type of compound and species of animal. Clinical signs for Type I OPIDN compounds in the chicken are similar to clinical signs for Type II compounds, but are distinct in species such as the cat, ferret, and rat. Neuropathological damage is usually confined to the peripheral nerves, spinal cord, brainstem, and cerebellum after exposure to a Type I compound, but additional damage occurs in midbrain and forebrain regions of animals exposed to Type II compounds (Veronesi and Padilla, 1985; Abou-Donia and Lapadula, 1990; Tanaka et al., 1992a).

Recently, a third type of OPIDN, distinct from Types I and II, has been proposed by Abou-Donia et al. (1996) after examining the neurotoxic characteristics of triphenyl phosphine (TPPn) in the domestic chicken. It was reported that repeated oral administration of 500, 2,000, or 5,000 mg/kg body weight triphenyl phosphine produced clinical signs similar to those caused by other OPIDN-inducing compounds (ataxia and paralysis), yet whole-brain NTE was not inhibited. Neuropathological examination was confined to the spinal cord where degeneration was found in both ventral and lateral tracts.

Previous work in our laboratory has demonstrated that the European ferret is a sensitive mammalian model for OPIDN (Stumpf et al., 1989). Therefore, it was of interest to examine the effects of this apparently novel OP in this mammalian species to determine if clinical signs and neuropathology consistent with OPIDN could be produced without inhibition of NTE. The specific aims of this study were to:

1. Determine if TPPn inhibits whole-brain NTE in the European ferret
2. Determine clinical signs caused by administration of TPPn and compare them to clinical signs characteristic of Type I and Type II OPIDN
3. Describe the neuropathological effects of TPPn using the Fink-Heimer silver impregnation method and compare them to neuropathology resulting from exposure to Type I and Type II OPIDN compounds

LITERATURE REVIEW

Introduction

Organophosphorus compounds (OP's) have a variety of industrial and agricultural uses such as fire retardants for fabrics, modifiers in plastic processing, petroleum additives, surfactants, chemical intermediates in the manufacture of pharmaceuticals, and insecticides (Davis and Richardson, 1980; Johnson, 1987; Abou-Donia and Lapadula, 1990). These compounds are primarily beneficial as insecticides to protect essential agronomic crops from disease, loss of nutrients, and death. This ultimately leads to higher quality agronomic crops and thus improved human health (Chambers, 1992).

Unfortunately, organophosphorus compounds are also noted for being potent neurotoxic agents, teratogens, and possible reproductive toxins (Proctor and Casida, 1975; Davis and Richardson, 1980). The toxic effects of OP's which have been examined the most extensively are their effects on the nervous system. Organophosphorus compounds cause cellular disruption by altering normal cell function in the central and peripheral nervous systems through phosphorylation of esterases (acetylcholinesterase and/or neuropathy target esterase) (Davis and Richardson, 1980; Abou-Donia and Lapadula, 1990; Peraica et al., 1995)

Chemical Structure

Organophosphorus compounds are organic structures that contain phosphorus-carbon bonds. The phosphorus atom contains two paired and three unpaired electrons in its outer shell and is capable of forming either trivalent (pyramidal configuration), or pentavalent (tetrahedral configuration) compounds (Abou-Donia and Lapadula, 1990). OP phosphorylation reactions are dependent on the reactivity of the central phosphorus atom, which is determined by the electrophilic character of the groups attached to the phosphorus atom.

Historical Development

Gerhard Schrader became known as the "father" of organophosphorus esters in the late 1930's and 1940's because of his insight into the potential uses of OP's as industrial chemicals and pesticides (Chambers, 1992). Many OP's were developed throughout the 1930's and 1940's: tabun and sarin in 1937, dimefox and schradan in 1941, diisopropyl phosphorofluoridate (DFP) in 1941, parathion and soman in 1944, and the extensively used insecticide malathion in 1950. (Chambers, 1992). Organophosphorus compounds became the insecticides of choice by the 1970's for fighting pests on a diverse range of crops including cotton, apples, corn, and stored grain (Racke, 1992). They were also applied topically to cattle to control cattle ticks (Racke, 1992).

Inhibition of AChE

OP compounds can affect normal functioning of the central nervous system in animals and humans by interfering with normal transmission of nerve impulses. Specific

neurons in the central and peripheral nervous systems use the neurotransmitter acetylcholine (ACh). ACh is synthesized by choline acetyltransferase from acetyl CoA and choline in the axon terminal, stored in synaptic vesicles, and released into the synaptic cleft after a normal nerve impulse. ACh binds to two types of receptors on the postsynaptic cell (muscarinic and nicotinic) to cause activation of the postsynaptic cell which may be another neuron, a muscle cell, or a glandular cell (Kandel, 1991). After a normal nerve impulse, acetylcholinesterase (AChE) is released from the surface of the postsynaptic membrane into the synaptic cleft to rapidly hydrolyze ACh to acetate and choline thus allowing the postsynaptic cell to return to its resting state. AChE is catalytically active due to the presence of a zone that contains two separate active sites: 1) the anionic site which attracts, binds, and orients the substrate using electrostatic forces and 2) the esteratic site which catalyzes the hydrolysis of the substrate. AChE and ACh combine to form a Michaelis enzyme-substrate complex (Kandel, 1991). The acetyl group from ACh transfers to the esteratic site (serine) of AChE to form an acetylated enzyme which is then rapidly hydrolyzed resulting in a re-activation of the enzyme. Choline is then taken up into the presynaptic terminals for re-synthesis of ACh (Kandel, 1991).

Organophosphorus compounds are effective as insecticides because they target the nervous system of insects, as well as birds and mammals, through phosphorylation of AChE at cholinergic nerve endings. This is considered an acute effect because clinical signs develop shortly (minutes to hours) after exposure. In order to inactivate AChE, organophosphorus compounds mimic ACh by covalently binding to AChE by nucleophilic attack forming an OP-AChE complex. This allows phosphorylation to occur at the esteratic site where the electronegative hydroxyl group of the serine in the esteratic site of

AChE reacts with the central electropositive phosphorus atom of the organophosphorus compound rendering the phosphate acidic (Timbrell, 1991; Chambers, 1992; Katzung and Trevor, 1995). Thus, when an OP is bound to AChE and the enzyme is inactivated, ACh can not be removed effectively from the synaptic cleft after a normal stimulation of the neuron causing over-stimulation of the postsynaptic cell.

Recovery of Enzyme Activity

The reaction between an OP and the active site of AChE results in an inhibited enzyme that undergoes one of two chemical reactions: regeneration or aging. Regeneration is the hydrolytic removal of the phosphoryl moiety that returns the enzyme to its active form (Chambers, 1992). Aging is a stabilization of the OP-AChE complex through a conformational change resulting from the loss of a second group (dealkylation) from the phosphorus atom. The aging process permanently alters the electric charge of the enzyme's active site preventing dephosphorylation (Johnson, 1987; Chambers, 1992). In order for AChE to hydrolyze excess ACh after aging has occurred, new enzyme must be synthesized which takes approximately 20-30 days (Chambers, 1992).

Several chemicals are capable of alleviating symptoms of acute OP poisoning before the process of aging occurs. The chemical pyridine 2-aldoxime (2-PAM) is effective in the regeneration of AChE through dephosphorylation of the phosphorylated enzyme. The nitrogen atom on 2-PAM binds to the anionic site of AChE which facilitates transfer of the substituted phosphate from the esteratic site of AChE to the oxime. This regenerates AChE which allows it to hydrolyze remaining ACh (Wilson et al., 1992). The drug atropine sulfate is also effective in relieving symptoms of acute OP poisoning.

Atropine sulfate competes with ACh for muscarinic binding sites. This reduces excessive stimulation of muscarinic receptor sites by ACh until new AChE is synthesized (Wilson et al., 1992).

Acute Signs of Poisoning

Inhibition of AChE is disruptive at all cholinergic synapses. Cholinergic synapses include synapses of the central nervous system, the neuromuscular junctions of motor nerves, sensory nerve endings, pre-ganglionic synapses of both sympathetic and parasympathetic nerves, postganglionic parasympathetic nerve terminals, and sympathetic nerve terminals on the sweat glands, blood vessels, and adrenal medulla (Timbrell, 1991). Inhibition of AChE greater than 50% at these nerve terminals is associated with classical symptoms of acute OP poisoning (Wilson et al., 1992). Continual stimulation of muscarinic receptors on smooth muscles causes the following symptoms: tightness in the chest and wheezing expiration due to bronchoconstriction and increased bronchial secretions, increased salivation and lacrimation, increased sweating, increased gastrointestinal tone, peristalsis, nausea, vomiting, abdominal cramps, diarrhea, tenesmus and involuntary defecation, bradycardia, frequent and involuntary urination, and constriction of the pupils (Chambers, 1992). Continual stimulation of nicotinic receptors at motor nerve junctions causes fatigue and weakness of innervated muscles, involuntary twitching, scattered fasciculations and cramps, dyspnea, and cyanosis (Chambers, 1992). Continual stimulation of nicotinic receptors at autonomic ganglia causes tachycardia, pallor, elevation of blood pressure, and hyperglycemia (Chambers, 1992). Accumulation of ACh in the central nervous system causes tension, anxiety, restlessness, insomnia, headache, emotional instability, neurosis, apathy, confusion, slurred speech, tremor,

generalized weakness, ataxia, convulsions, depression of respiratory and circulatory centers, and coma. The cause of death in acute OP poisoning usually results from asphyxia due to respiratory failure (Chambers, 1992).

Introduction to OPIDN

Another type of neurotoxicity caused by certain organophosphorus compounds is called organophosphorus-induced delayed neurotoxicity. OPIDN results when there is a delay between exposure to the organophosphorus compound and the appearance of characteristic clinical signs (Abou-Donia and Lapadula, 1990).

OPIDN in Humans

Since the beginning of the 20th century, over 40,000 cases of OPIDN in humans have been reported throughout the world (Abou-Donia and Lapadula, 1990). A number of the early poisonings resulted from a medication for people with tuberculous that was adulterated with phospho-creosote (Davis and Richardson, 1980). As many as 20,000 people were paralyzed in the 1930's in the U.S. by an alcoholic drink made from a Jamaican ginger extract contaminated with tri-ortho-tolyl phosphate (TOTP) (Baron, 1981). Modern concern for potential OP poisoning is related to dangers associated with occupational exposure. Although the toxicology data for OP's in humans are very limited, it is probable that the human may be the most sensitive species since recent studies indicate that exposure to OP's may result in damage to higher order brain functions such as sensorimotor processing and cognitive brain function (Baron, 1981; Tanaka et al., 1990a).

Inhibition of NTE

OPIDN presumably results from an OP's interaction with the enzyme neuropathy target esterase (NTE) (Peraica et al., 1995). NTE is a membrane-bound protein that is proposed to have a role in lipid metabolism (Abou-Donia and Lapadula, 1990; Lotti et al., 1993). OPIDN is thought to be induced by an organophosphorus compound through a two step process: 1) inhibition of NTE by phosphorylation; 2) aging of phosphorylated NTE (Lotti et al., 1993). The interaction between an OP and NTE (occurring within one hour of exposure) at the enzyme's active site creates an NTE-OP complex. The esterase complex is then phosphorylated creating a stable covalent bond between the active site of the enzyme and the phosphate moiety which inhibits the catalytic activity of NTE (Timbrell, 1991; Johnson, 1993; Lotti et al., 1993). Aging involves the loss of an ester functional group (alkyl or aryl group) on the phosphorus atom of the OP-esterase complex by cleavage of an ester or amido bond. This leaves the complex negatively charged. The unbound ester functional group then becomes re-attached to another part of the protein and is not released into the surrounding region (Johnson, 1993). Thus, the important step in the development of OPIDN by NTE could be either the liberation of the ester group or its reattachment to another part of the protein (Johnson, 1993). When NTE is negatively charged in the phosphorus region, a progressive neuropathy of both the peripheral and central nervous systems occurs (Timbrell, 1991; Johnson, 1993; Lotti et al., 1993).

NTE and Organophosphorus Chemical Reactivity

Inhibition of whole-brain NTE greater than 70% is generally associated with the clinical signs and neuropathology characteristic of OPIDN (Johnson, 1977; Abou-Donia

and Lapadula, 1990). All organophosphorus compounds that are known to cause OPIDN are inhibitors of NTE *in vivo* (phosphates, phosphonates, and phosphoramidates), but some organophosphorus compounds are capable of inhibition of NTE without causing OPIDN (sulphonylfluorides and phosphinates). Phosphates, phosphonates, and phosphoramidates are able to induce OPIDN because they are capable of undergoing the aging reaction due to the presence of either oxygen or amine groups that link the R-group to the phosphorus atom through labile ester or amido bonds. Phosphinates are not capable of aging, because they do not contain a labile ester or amido bond and thus do not induce OPIDN.

Prophylaxis

Administration of a non-aging inhibitor of the phosphinate class (such as phenyl methanesulfonyl fluoridate or PMSF) before exposure to a neuropathic organophosphorus compound prevents neuropathy. This occurs because the non-aging compound occupies the active site on the NTE molecule and prevents an ageable OP compound from binding to it. Competition for this active site will continue without causing OPIDN until the NTE critical inhibition level of 70% is reached and aging is occurring (Carrington, 1989). Thus, the determining factor for whether an OP will be neuropathic or protective is its ability to undergo an aging reaction.

Challenge to NTE Threshold

Although few scientists would argue that NTE is not involved in the pathogenesis of OPIDN, many question the enzyme's role as the target enzyme. This position is substantiated by the poor correlation of enzyme inhibition induced by certain OP's with

the appearance of OPIDN clinical signs and pathology. For example, tri-2-ethyl-phenyl phosphate, di-phenyl-2-isopropylphosphate, and the test pesticide Bayer KBR-2822 inhibit whole-brain NTE in excess of 70% and the phosphorylated enzyme does undergo aging, yet clinical signs and pathology characteristic of OPIDN do not occur (Lotti et al.,1993).

Other OP compounds cause OPIDN clinical signs with concurrent central nervous system degeneration even though NTE inhibition is well below 70%. For example, rats exposed to TPP exhibited marked ataxia with spinal cord degeneration despite a whole-brain NTE inhibition of only 33% (Veronesi and Dvergsten, 1987). Larsen et al. (1986) reported clinical signs consistent with OPIDN when whole-brain NTE was inhibited by only 41% in the turkey after exposure to TOTP. Ferrets dosed with TOTP displayed clinical signs indicative of OPIDN when whole-brain NTE was inhibited by only 46% (Stumpf et al., 1989).

Characteristics of OPIDN Type I and Type II Compounds

Differences among clinical and neurophysiological responses to organophosphorus compounds have led to OPIDN being categorized into two distinct classes. These classes differ in terms of molecular structure of the neuropathic OP, species that are sensitive to the compound, age of susceptibility, duration before onset of clinical signs, type of clinical signs, location of affected neural regions, and degree of neuropathy target esterase inhibition (Abou-Donia and Lapadula, 1990).

Chemical Structure

Type I OPIDN compounds are distinguished by a chemical structure that consists of a pentavalent phosphorus atom characteristic of phosphoric, phosphonic, or phosphoramidic acid. Examples of Type I OPIDN compounds are TOTP, which is also known as tri-o-cresyl phosphate (TOCP), and DFP. The presence of an *ortho*-methyl group in the aromatic series seems to be essential for aromatic compounds to be neurotoxic. Type II OPIDN compounds contain a trivalent phosphorus atom and are derivatives of phosphorus acid and their sulfur analogs. Triphenyl phosphite (TPP) is an example of a Type II OPIDN compound.

Species Sensitivity

There are differences in species sensitivity to Type I and Type II compounds. Although chickens are highly sensitive to both Type I and Type II OPIDN compounds, neither rodents nor Japanese quail are sensitive to Type I compounds, but all these species develop OPIDN in response to a single dose of a Type II compound (Abou-Donia and Lapadula, 1990). Cats develop OPIDN only after multiple doses of a Type I compound, but they are sensitive to single doses of a Type II OPIDN compound (Abou-Donia and Lapadula, 1990). Although ferrets develop OPIDN in response to a single exposure to a Type I compound, OPIDN is more severe after a single exposure to a Type II compound (Stumpf et al., 1989; Tanaka, 1990a).

Age Sensitivity

Differences in age sensitivities exist between Type I and Type II compounds. Young animals of sensitive species are not susceptible to single doses, but they may be affected by multiple doses of a Type I compound. A single oral dose of 2 mg/ kg body weight DFP did not produce OPIDN in young chicks, however, they did develop OPIDN after repeated doses of DFP (Johnson and Barnes, 1970). In another study, chicks younger than 60 days of age did not develop OPIDN after receiving a single dose of 1.3 mg/ kg body weight DFP, while administration of the same dosage in chickens 60 days and older produced OPIDN that was more severe with increasing age (Moretto et al, 1991). In contrast, one-week-old chicks developed OPIDN in response to a single dose of 1000 mg/ kg body weight TPP (Abou-Donia and Brown, 1990). Most OPIDN studies involving ferrets have examined susceptibility only in adult ferrets, but a detailed study on ferret age susceptibility was conducted using TPP. Ferrets dosed with 1184 mg/ kg body weight TPP did not develop OPIDN until five weeks of age. Severity of clinical signs and neuropathology then increased with age and reached adult levels at 10 weeks of age (Tanaka et al., 1994).

Latent Period

Both Type I and Type II OPIDN are characterized by a delay between time of exposure and the appearance of clinical signs, but the length of the delay varies by type. Type II OPIDN has a shorter latent interval then Type I OPIDN. The onset of clinical signs in the cat ranges from four to seven days with a Type II compound and 14-21 days with a Type I compound (Smith et al., 1933). Rats exhibit clinical signs characteristic of

OPIDN approximately seven days after exposure to a Type II compound, but the latent interval for Type I compounds is approximately 14-21 days (Veronesi and Padilla, 1985). Hens display clinical signs four to six days after exposure to a Type II compound and six to fourteen days after exposure to a Type I compound (Abou-Donia and Lapadula, 1990). Ferrets display clinical signs four to six days after exposure to a Type II compound and 10-14 days after exposure to a Type I compound (Stumpf et al., 1989; Tanaka et al., 1990a).

Clinical Signs

Clinical signs produced by delayed neurotoxicants vary by type of compound and species of animal. Clinical signs of ataxia and incoordination produced by Type I OPIDN compounds in the chicken are similar to clinical signs produced by Type II compounds, but are distinct in species such as the cat, ferret, and rat. Cats treated dermally with multiple doses of 250 mg/kg body weight TOTP exhibit hind limb ataxia and flaccid paralysis four to seven days after exposure while cats treated dermally with a single dose of 1000 mg/ kg body weight TPP develop ataxia after four to twenty-six days and develop extensor rigidity of both front and hind limbs between eight to thirty days (Smith and Lillie,1931; Abou-Donia et al., 1986). Rats exposed to a single subcutaneous dose of 1000 mg/ kg body weight TOTP do not exhibit any detectable clinical signs, but after subcutaneous exposure to 1000 mg/ kg body weight TPP they exhibit hyperexcitability, spasticity, incoordination, circling behavior, tail-kinking, hind limb ataxia, and partial flaccid paresis of the extremities (Veronesi and Dvergsten, 1987). Ferrets exhibit hind limb ataxia followed by flaccid hind limb paralysis after a single subcutaneous exposure to

500 and 1000 mg TOTP/ kg body weight and single subcutaneous doses of 2 and 4 mg DFP/ kg body weight (Tanaka et al. 1991). Single subcutaneous exposure to 500, 1000, and 2000 mg TPP/ kg body weight in ferrets results in hind limb ataxia leading to extensor rigidity as well as fore limb ataxia which develops into extensor rigidity (Tanaka et al., 1990a).

Neuropathological Changes

Neuropathological changes occur after exposure to an organophosphorus compound that induces OPIDN and they are distinct for Type I and Type II compounds. Type I compounds typically affect only the peripheral nerves, spinal cord, brainstem, and parts of the cerebellum, while Type II compounds affect peripheral nerves, spinal cord, brainstem, cerebellum, midbrain, and forebrain (Abou-Donia and Lapadula, 1990; Tanaka et al. 1992a).

CATS. Type I compounds produce histopathological lesions in the spinal cord of cats (Smith and Lillie, 1931; Cavanagh and Patangia, 1964). Degenerating axons are found in the ascending tracts including the spinocerebellar columns, posterior columns, and gracile tracts. Descending tracts that contain degeneration are the corticospinal tracts of the ventral columns (Smith and Lillie, 1931; Cavanagh and Patangia, 1964).

After a single subcutaneous exposure to 1000 mg TPP/kg body weight, degeneration occurs in many ascending and descending tracts of the spinal cord (Smith et al., 1933). Degenerating ascending tracts include the spinocerebellar and anterolateral spinocerebellar tracts. Degenerating descending tracts include the rubrospinal,

vestibulospinal, tectospinal, lateral corticospinal, and anterolateral tracts. Degeneration also occurs in the medulla and pons (Smith et al., 1933).

RATS. Neuropathological damage caused by Type I OPIDN compounds is far less extensive than damage caused by Type II OPIDN compounds. Rats treated with a single subcutaneous dose of 1000 mg TOTP/kg body weight exhibit degeneration in the ascending and descending tracts of the spinal cord. Degeneration is confined to the ventrolateral and ventral columns of the cervical and lumbar cord (Lehning, 1992; Tanaka et al., 1992a).

Rats treated with a single subcutaneous dose of 1000 mg TPP/kg body weight exhibit degeneration in the spinal cord, as well as forebrain, midbrain, and hindbrain areas (Veronesi and Dvergsten, 1987; Lehning, 1992; Tanaka et al., 1992a). Spinal cord degeneration includes tracts affected by exposure to TOTP with additional damage in the small diameter fibers of the gracile fasciculus, and the gray matter in laminae V-IX of the upper cervical cord. Affected forebrain regions include the transitional cortex, the neocortex, the septum and hypothalamus, the hippocampal region, and the thalamus. Midbrain regions affected by a single exposure to TPP in rats include components of the basal ganglia, superior colliculi, inferior colliculi, oculomotor nucleus, and dorsal raphae nucleus. Hindbrain regions affected by a single exposure to TPP are the cerebellum, the vestibular nuclear complex, dorsal cochlear nucleus, and the reticular formation. (Veronesi and Dvergsten, 1987; Lehning,1992; Tanaka et al., 1992a).

CHICKENS. Chickens that are exposed to a single subcutaneous dose of a Type I OPIDN (500 mg TOTP/kg body weight or 1 mg DFP/kg body weight) exhibit degeneration in the spinal cord, medulla, and cerebellum (Tanaka et al., 1990b; Tanaka et

al., 1992a). Degenerating axons are found in the gracile fasciculus at cervical levels, dorsal and ventral spinocerebellar tracts, the medial pontine-spinal tract at lumbar levels, and the medial part of the ventral horn at lumbar cord levels. In the medulla, exposure to a Type I OPIDN results in degeneration in several brainstem nuclei related to the reticular formation. Degeneration also occurs in medullary fiber tracts. Within the cerebellum, degeneration occurs in the granular layer of folia I-Vb. (Tanaka et al., 1990b; Tanaka et al., 1992a).

Chickens that are exposed to a single subcutaneous dose of 1000 mg TPP/kg body weight exhibit degeneration in the same regions affected by Type I OPIDN compounds, as well as in additional regions throughout the spinal cord, medulla, midbrain, and forebrain (Tanaka et al., 1992a). Additional spinal cord damage occurs in the spinocerebellar tract and pontine-spinal tracts at all cord levels, ventral part of the medial longitudinal fasciculus, lateral and ventral funiculi, spinal laminae VII and VIII that extend into the lateral parts of the ventral horn at the cervicothoracic and lumbosacral levels, and laminae V,VI, and IX at all cord levels. (Tanaka et al., 1992a). TPP exposure results in additional degenerated nuclei in the medulla. Degeneration in the cerebellum is more widespread after exposure to TPP than Type I compounds. There is also degeneration in several midbrain and forebrain regions not affected by TOTP (Fioroni et al., 1995).

FERRETS. Pathological damage after single subcutaneous exposure to 2 and 4 mg DFP/kg body weight occurs in the spinal cord, brainstem, and cerebellum of the ferret (Tanaka et al., 1991). Degenerating axons are found in the gracile fasciculus at cervical levels, dorsal spinocerebellar tract at cervical levels (spinoolivary, spinovestibular, and spinoreticular pathways), the lateral corticospinal tract at lumbar levels, spinal cord

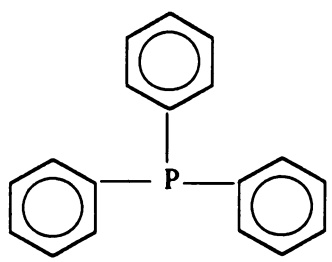
laminae VI-VII, and ventral motor nucleus of the cervical enlargement. In the medulla, degeneration is found in the ventral and lateral portions of the medial and dorsal accessory nuclei of the inferior olive, lateral reticular formation, and inferior vestibular nucleus. In the cerebellum, after exposure to DFP, degeneration occurs in the white matter and granule cell layers of folia I-IV of the anterior lobe of the cerebellum (Tanaka et al., 1991).

Ferrets that are exposed to a single subcutaneous dose of 500, 1000, or 2,000 mg TPP/ kg body weight exhibit degeneration in the same regions affected by Type I OPIDN compounds, as well as in additional regions throughout the spinal cord, medulla, midbrain, and forebrain (Tanaka et al., 1990a). Additional spinal cord damage occurs in the medial and lateral portions of spinal laminae VI-IX at cervical, thoracic, lumbar, and sacral levels, and the lateral and ventral funiculi throughout the length of the cord. In the medulla, degeneration occurs in the external cuneate nucleus, pontine gray, all nuclei of the reticular formation, red nucleus, and lateral vestibular nucleus. Additional regions of degeneration are found throughout the thalamus and hypothalamus, as well as forebrain and brainstem nuclei tracts related to auditory (superior olivary nucleus and inferior colliculus), visual (lateral geniculate nucleus and visual cortex), and sensorimotor systems (ventral posterior thalamic nucleus, ventral lateral nucleus). In the cerebellum, additional degeneration occurs in the granule cell layers of folia I-IX and crura I and II (Tanaka et al., 1990a).

Type III OPIDN

Triphenyl phosphine (TPPn) has been proposed to cause a third type of OPIDN in the domestic chicken with neurotoxic characteristics distinct from Types I and II (Abou-Donia et al., 1996). The chemical properties of TPPn are listed in Table 1. Abou-Donia et al. (1996) reported that repeated oral administration of 500, 2,000, or 5,000 mg/kg body weight TPPn produced OPIDN clinical signs of ataxia, paralysis, and death by four days post-dosing. Neuropathological examination was confined only to the spinal cord where degeneration was found in both ventral and lateral tracts. Chicken whole-brain AChE and NTE activities after a single oral dose of 500 mg TPPn/ kg body weight (Abou-Donia et al., 1996).

Table 1. Identification and physical properties of Triphenyl Phosphine

Chemical Family:	Phosphine compound, aromatic
Component:	Triphenyl phosphorus
Chemical Description:	Clear to off-white, triboluminescent flakes with a characteristic odor
Trade names/Synonyms:	Triphenylphosphane, OHS24360 Triphenylphosphorus
Molecular Formula:	C18-H15-P
Molecular Weight:	262.30
Boiling Point:	711°F (377°C)
Melting Point:	174-178°F (79-81°C)
Solubility in Water:	Insoluble
Solvent Solubility:	Soluble in ether, benzene, chloroform, carbon tetrachloride, acetone, glacial acetic acid, slightly soluble in ethanol
Vapor Pressure:	5 mmHg @ 210°C
Vapor Density:	9.0
Specific Gravity:	1.132
PH:	Slightly basic
Reactivity:	Stable under normal temperatures and pressures
Decomposition:	Thermal decomposition products may include toxic and hazardous oxides of phosphorus and fumes of phosphine
Molecular Structure:	

(Occupational Health Services, Inc., 1995)

MATERIALS AND METHODS

Subjects

Twenty-four adult European ferrets (*Mustela putorius furo)* were used in this study. The ferrets (642-1348 g) were maintained at the Michigan State University Experimental Fur Farm. The ferrets were individually housed in cages (72.2 cm L x 61 cm W x 45.7 cm H) suspended approximately two feet off of the ground in a screen-enclosed pole-type building. The ferrets were exposed to ambient August temperature and humidity with a natural photoperiod. Food and water were available *ad libitum.*

Test Chemicals

Triphenylphosphine $(C_6H_5)_3P$ was purchased in crystalline form (100% pure) from Aldrich Chemical Company Inc., Milwaukee,WI. The vehicle used consisted of a 1:1 ratio of diethyl ether and peanut oil.

Experimental Design

Twenty-four ferrets were randomly assigned to one of three dose groups. Eight ferrets were dosed with single subcutaneous injection of TPPn at 250mg/kg body weight in the dorsum of the neck. Eight ferrets were dosed with single subcutaneous injection of

TPPn at 500mg/kg body weight in the dorsum of the neck. Eight ferrets were injected with the diethyl ether/peanut oil vehicle at a volume of 2 ml/kg body weight in the dorsum of the neck.

Twenty-four hours after dosing, five ferrets from each dose group were killed by cervical dislocation. The brains were rapidly removed, cut in half in the sagittal plane, weighed and frozen on dry ice for subsequent NTE and AChE activity evaluation. NTE activity was determined using the method of Johnson (1977). AChE was assayed according to the method of Reed et al. (1966). Percent inhibition of both NTE and AChE activities was calculated as a function of the control activities. NTE and AChE activities were analyzed statistically using ANOVA. Statements of significance are based on $p<0.05$.

The remaining three ferrets per dose group were observed daily for six days for the onset of clinical signs characteristic of acute toxicity or OPIDN. Evaluation of OPIDN clinical signs was based on the following criteria: difficulty in holding the head erect, the presence and degree of front and hind limb ataxia and front and hind limb rigidity. Each animal was removed from the cage and placed on a flat open surface for approximately 10 minutes each day to qualitatively observe clinical signs.

To assess the extent of central nervous system degeneration, the remaining three ferrets per dose group used for the clinical assessment were perfused for neuropathological analysis six days after injection. Ferrets were administered a lethal dose of sodium phenobarbital (150 mg/ml; 3 ml/animal intraperitoneal) and then perfused transcardially with 10% formalin-saline solution. Just prior to perfusion with fixative, 1 ml of heparin (168 USP units/ml physiological saline) was injected into the left ventricle.

Following perfusion, the brains were removed and placed in a 10% formalin-saline solution for two days. Brain blocks were obtained and then cryoprotected by immersion in a 30% sucrose-formalin solution. After cyroprotection, frozen sections were cut from the brains in the parasagittal plane at a thickness of forty μm. Every fifth section was stained using the Fink-Heimer silver method to determine degeneration of cell bodies, axons, and terminals (Tanaka, 1976). Adjacent sections were stained with cresyl violet to delineate nuclear boundaries. All silver-impregnated and cresyl violet stained sections were examined with a light microscope to determine the density and extent of degeneration present. Selected corresponding Fink-Heimer and cresyl violet stained sections were photographed at low power with a Wild M400 Photomacroskop. Degenerated areas were mapped directly onto the photographs using a compound microscope, and line drawings were traced from the photographs. Nuclei and fiber tracts were identified according to the atlases of Adrianov and Mering (1959), Berman (1968), Salazar et al. (1979), and Berman and Jones (1982). The severity of degeneration was classified as light, moderate, or heavy.

RESULTS

Enzyme Activities

The effects of TPPn on whole-brain NTE and AChE activities 24 hours after dosing are presented in Table 2. The administration of 250 mg TPPn/kg body weight and 500 mg TPPn/kg body weight had no significant effect ($p > 0.05$) on whole-brain NTE and AChE activities.

Clinical Signs

Ferrets dosed with the vehicle only did not exhibit any clinical signs characteristic of OPIDN. Clinical signs in dosed ferrets were first apparent four days post-dosing. Both groups of dosed ferrets (250 mg/kg body weight TPPn and 500 mg/kg body weight TPPn) exhibited clinical signs characteristic of OPIDN. These animals had difficulty in holding their head steady and erect and they displayed slight hind limb ataxia. On the fifth day post-dosing, both groups of animals exhibited front limb ataxia, extensor rigidity of the hind limbs and increasing difficulty in maintaining the head erect. The animals moved by use of the front limbs with the hind limbs being extended toward the rear of the animal. Animals would often roll from one side to the other during forward locomotion. On the sixth day post-dosing, animals in both groups were unable to hold their heads erect and

Table 2. Whole-brain neuropathy target esterase (NTE) and acetylcholinesterase (AChE) activities in European ferrets 24hr after exposure to triphenylphosphine (TPPn)

Dose*	NTE Activity	AChE Activity
Control	1841±158.6**	207±10.6**
250 TPPn	1844±83.0	200±13.0
500 TPPn	1932±45.3	212±9.8

*Doses are expressed as mg TPPn/kg body weight

**Data presented as mean ± standard error. NTE activity is expressed as nmoles phenyl valerate hydrolyzed/ hr /gm brain. AChE activity is expressed as umoles ACh hydrolyzed/ hr / gm brain.

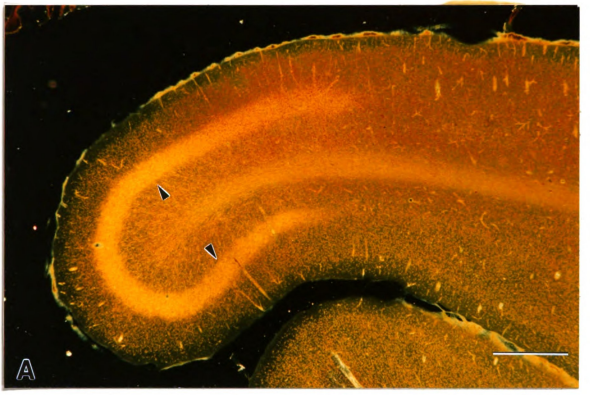
both front and hind limbs were extended and rigid. The observed clinical signs were similar for animals in both the 250 and 500 mg TPPn/kg body weight dose groups.

Neuropathology

A broad pattern of terminal and axonal degeneration was similar for all nine TPPn-exposed ferrets. Degeneration was not present in the brains of control ferrets. Silver-impregnated degeneration appeared as black debris against a light brown or yellow background. Fiber tract axonal degeneration appeared as linearly arranged black fragments. Terminal degeneration in nuclear regions appeared as variably shaped punctate debris. Artifacts present were silver impregnation of reticular fibers in blood vessels, and random silver precipitation. Differentiation between degeneration and artifact was achieved through the recognition of damage within known fiber tracts or nuclei, and the continuity of degeneration in adjacent sagittal sections. Figures 1, 2, and 3 illustrate CNS areas containing degeneration and areas that do not contain degeneration for comparative purposes.

In the forebrain, degenerated areas included the neocortex, the hypothalamus, basal ganglia, and the thalamus (Table 3, Figure 4 A,B,C). In the neocortex, areas of degeneration were located in the visual and motor cortices of all TPPn-exposed ferrets (Table 3, Figure 4 A,B,C). The ferrets had light degeneration in medial portions of the visual and motor cortices, which progressed to moderate degeneration in the lateral portions of these cortices (Figure 4 A,B,C). Two basal ganglia components contained terminal and axonal degeneration. The fields of Forel contained moderate axonal degeneration (Figure 1B). The entopeduncular nucleus contained light terminal

Figure 1. Darkfield photomicrographs illustrating axonal and terminal degeneration in the primary visual cortex and thalamus. Under darkfield illumination, CNS regions containing silver impregnated degenerating elements show up as bright areas against a light brown or dark yellow background. A. Dense band of degeneration (arrowheads) located in lamina IV of the primary visual cortex of a ferret six days after injection with 500mg TPPn/kg body weight. B. Dense terminal degeneration located within the mediodorsal (MD), ventral lateral (VL), and ventromedial (VM) thalamic nuclei of a ferret six days after injection with 250mg TPPn/kg body weight. Scale bars = 1 mm. Figure abbreviations are listed at the beginning of the text.


A

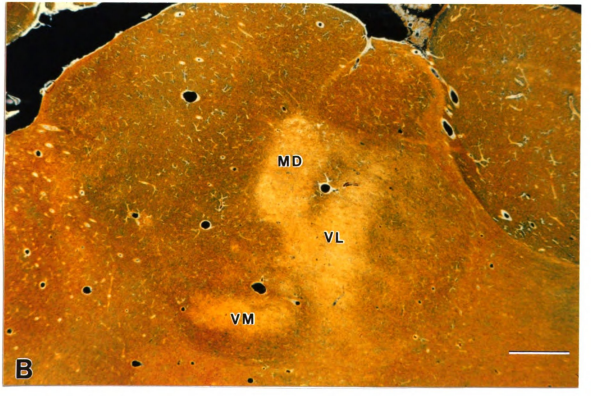

MD
VL
VM
B

Figure 2. Photomicrographs illustrating Fink-Heimer silver impregnated regions showing axonal and terminal degeneration in the thalamus of a ferret six days after injection with 250mg TPPn/kg body weight. Panel A illustrates dense degeneration in the lateral geniculate nucleus (LGN) on the left side of the photomicrograph. Note the sharp demarcation between the degeneration in the LGN and the absence of degeneration in the immediately adjacent area to the right side of the photomicrograph. Panel B is a high power view of the caudal part of the mediodorsal thalamic nucleus. Note the absence of degeneration and the presence of light brown plump cell bodies (arrowheads). Panel C shows a high power view of the ventromedial thalamic nucleus. Note the presence of fine particulate black axonal and terminal degeneration as well as a black silver-impregnated pyknotic degenerating cell body (arrow). Scale bars = 25 um. Scale bar in B applies to C. Figure abbreviations are listed at the beginning of the text.

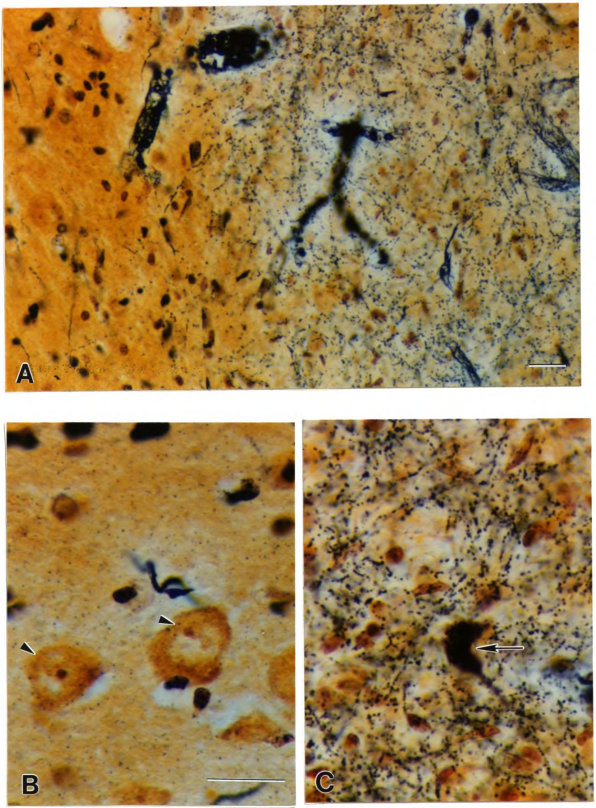

A
B
C

Figure 3. Photomicrographs through three laminae of the primary visual cortex showing axonal and terminal degeneration in a ferret six days after injection with 250mg TPPn/kg body weight. Panel A is from lamina III and contains almost no degeneration. Panel B is from lamina IV and is the site of termination of degenerating axons from the lateral geniculate nucleus. Panel C illustrates degeneration axons passing through lamina VI on their way to terminate in lamina IV. The black ovoid structures in Panels A and C are glial cells. Scale bar = 25 um and applies to all panels. Figure abbreviations are listed at the beginning of the text.

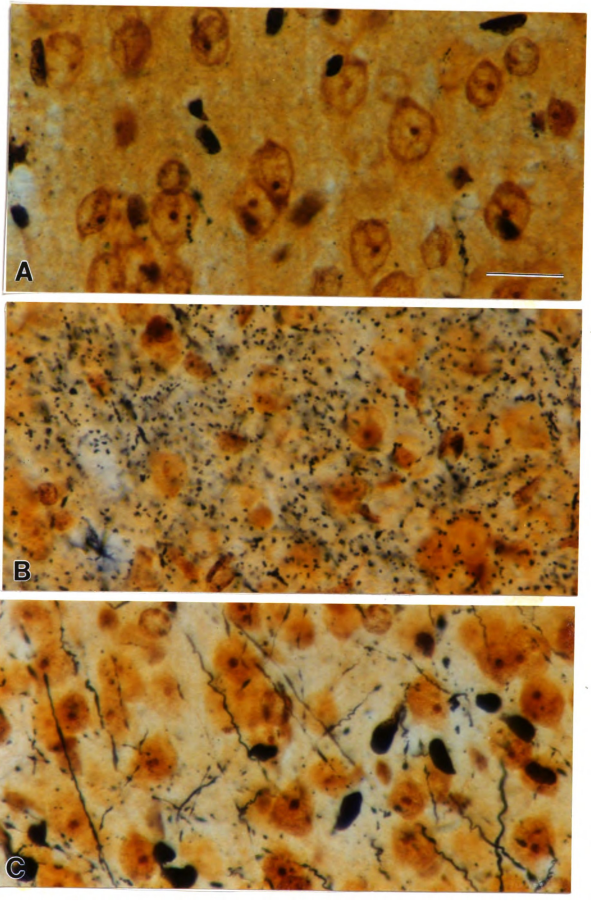

A
B
C

Table 3. Ferret central nervous system regions which contain axonal and/or terminal degeneration after administration of TPPn

Forebrain	
Neocortex	
VCx	++
SMCx	++
Septum and Hypothalamus	
MMn	++
Thalamus	
MD	+++
VM	+++
VA	+++
VL	+++
LGN	+++
MGN	+++
CM	++
PAT	++
VPL	+++
SUB	+++
Basal Ganglia	
EN	+
FF	++
Midbrain	
SCI	++
ICC	+++
PGL	++
PGM	++
Brainstem	
Pons	
LL	++
Tegmentum	
Rn	++
TGn	++
Brainstem Nuclei/Tracts	
FTG	+

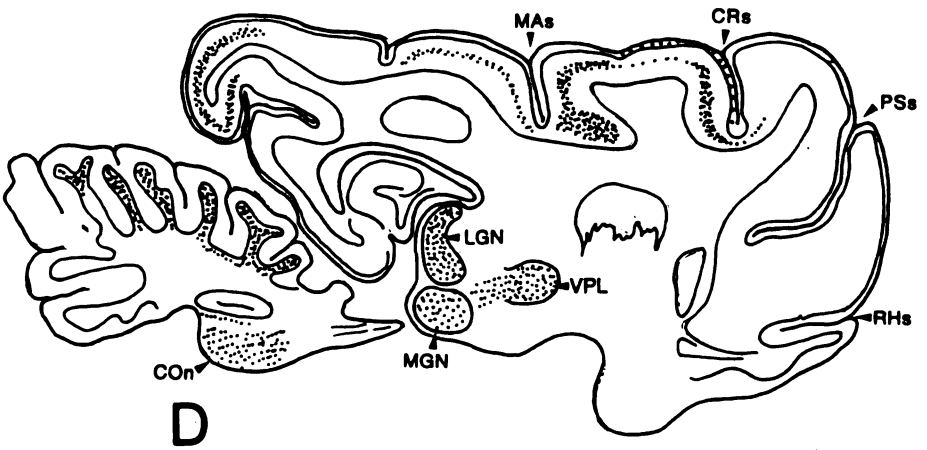
Table 3 Continued

CX	++
SOM	++
SOL	++
COn	++
Vestibular Nuclear Complex	
VMN	++
VSN	++
VLV	++
Cerebellum	
Granular cell layer	+ to +++

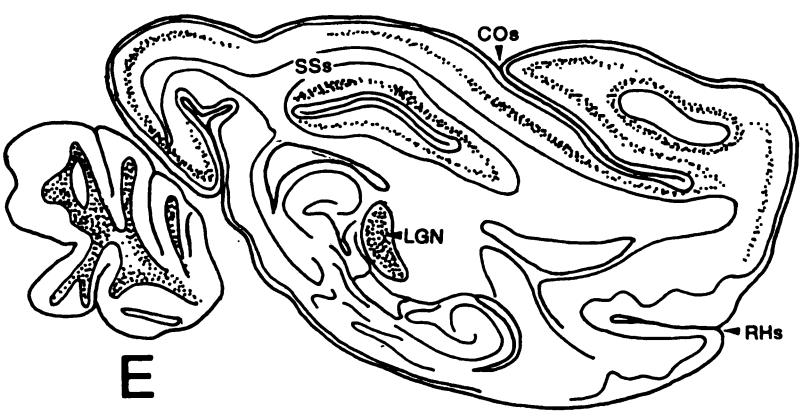
Representation of degeneration density: + = light, ++ = moderate, +++ = dense
Structure abbreviations are found at the beginning of the text

Figure 4. Line drawings of parasagittal sections depicting the locations of axonal and terminal degeneration (stippling) in nuclear regions of the brain in ferrets dosed with 250 or 500 TPPn mg/kg body weight. Drawings are from medial (A) to lateral (C). Structure abbreviations are found at the beginning of the text.

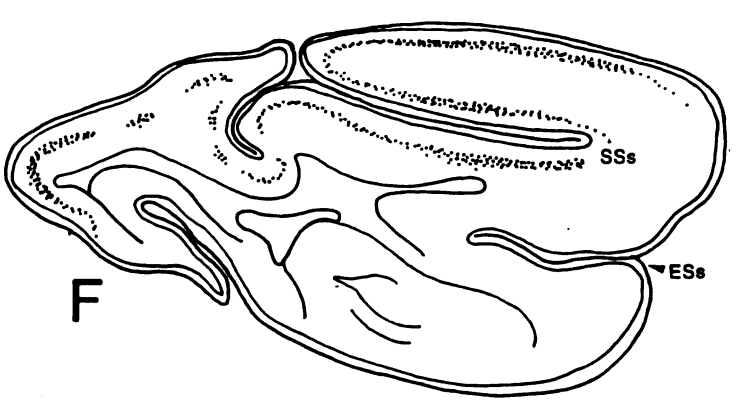
Figure 5. Line drawings of parasagittal sections depicting the locations of axonal and terminal degeneration (stippling) in nuclear regions of the brain in ferrets dosed with 250 or 500 TPPn mg/kg body weight. Drawings are from medial (D) to lateral (F). Structure abbreviations are found at the beginning of the text.



D



E



F

degeneration (Figure 4C). In the hypothalamus, degeneration was detected in the medial, lateral and posterior parts of the medial mamillary nucleus (Table 3, Figure 4 A).

Several thalamic nuclei also contained degeneration (Figures 4 and 5). Moderate terminal degeneration was found in the medial portion of the mediodorsal thalamic nucleus. This progressed to dense degeneration in the lateral portions of the nucleus (Figure 4 A,B). Dense terminal degeneration was found in the ventral anterior, ventral lateral, and ventral medial thalamic nuclei, as well as in the lateral geniculate and medial geniculate nuclei (Figures 4, and 5 C,D,E). The central medial thalamic nucleus also contained moderate terminal degeneration. A band of degenerating axons in the thalamus was found oriented in the rostral-caudal direction. It was bounded by the central medial thalamic nucleus on the dorsal side and fields of Forel on the ventral side (Figure 4 B). The parataenial nucleus contained moderate terminal degeneration. The ventral posterior lateral thalamic nucleus contained dense terminal degeneration. Finally, the subthalamic nucleus contained dense terminal degeneration.

Moderate numbers of degenerating axons and terminals were found throughout the midbrain in the areas of the intermediate lamina of the superior colliculus, central nucleus of the inferior colliculus, lateral and medial paragigantocellular reticular nuclei, tegmentum, and pons (Figure 4). A small region of the intermediate lamina of the superior colliculus showed moderate degeneration. As more lateral areas of this nucleus were examined, the intermediate lamina of the superior colliculus contained increasing numbers of degenerating terminals. A band of degenerated axons was found in the midbrain region which was on the rostral border by the centrum medianum thalamic and mediodorsal thalamic nuclei and on the caudal border by the superior colliculus (Figure 4 A,B). The

central nucleus of the inferior colliculus contained dense terminal degeneration in medial regions of the nucleus that became less dense in more lateral regions. Both the medial and lateral paragigantocellular reticular nuclei contained moderate terminal degeneration (Figure 4 A,B). The tegmentum of the midbrain contained degeneration in two separate nuclei. The red nucleus contained light terminal degeneration in medial portions of the nucleus and became more degenerated in lateral areas of the nucleus (Figure 4 A,B). The tegmental nucleus contained moderate terminal degeneration (Figure 4 A). A moderately dense band of degenerating axons projected in a rostral-caudal direction at the rostral end by the red nucleus and the caudal end by the tegmental nucleus. The other band of degenerated axons was oriented in the rostral-caudal direction and was bordered by the fields of Forel on the rostral side and the red nucleus on the caudal side. The lateral lemniscus in the pons contained moderate axonal degeneration. This fiber tract formed a band of degenerated axons that was oriented in the dorsal-ventral direction bordered on the dorsal end by the inferior colliculus and the ventral end by the superior olive (Figure 4C).

In the hindbrain, both terminal and axonal degeneration could be found in the vestibular nuclear complex, cerebellum, and several brainstem nuclei and tracts (Figures 4 and 5). Within the vestibular nuclear complex, three nuclei contained degeneration. The medial vestibular nucleus (Figure 4 B), the superior vestibular, and the ventral division of the lateral vestibular nucleus contained moderate terminal degeneration (Figure 4 C). Several nuclei and fiber tracts within the brainstem displayed damage in the form of axonal and terminal degeneration. The gigantocellular tegmental field contained a broad band of light degenerating axons that extended from the rostral end of the tegmental nucleus to the

caudal end of the external cuneate nucleus (Figure 4 A). The cochlear nucleus contained moderate terminal degeneration (Figure 5 D). The external cuneate nucleus, which is located on the caudal side of the medial vestibular nucleus, contained moderate terminal degeneration. The lateral nucleus of the superior olive contained moderate terminal degeneration that projected from the caudal end of the nucleus to form the lateral lemniscus (Figure 4 C). The cerebellum contained varying degrees of degeneration ranging from light in medial sections to heavy in more lateral sections. Within the cerebellum, axonal degeneration was detected in the white matter and granule cell layer underlying folia I-XI (Figures 4 and 5 A,B,C,D,E).

DISCUSSION

AChE Activity

Anticholinesterase activity depends largely on the chemical structure of the OP compound that comes into contact with AChE. Inhibition of AChE is due to the phosphorylation of the esteratic site (serine hydroxyl group) of AChE. OP phosphorylation reactions are dependent on the reactivity of the central phosphorus atom, which is determined by the electrophilic character of the groups attached to the phosphorus atom (Timbrell, 1991; Chambers, 1992; Katzung and Trevor, 1995). The presence of electronegative groups causes the phosphorus atom to become positively charged making it more susceptible to nucleophilic attack (Eto, 1979; Davis and Richardson, 1980). Triphenyl phosphine has no electronegative side groups so it would not be expected that TPPn would inhibit AChE. Inhibition of AChE by an OP compound is also dependent on the lability, stretching frequencies, and hydrolysis rates of the phosphorus-oxygen bond (Eto, 1979). TPPn, however, does not contain any phosphorus-oxygen bonds thus making it an ineffective AChE inhibitor.

NTE Activity

Approximately 70% inhibition of whole-brain NTE activity after administration of a single dose of an OP compound is thought to be required for subsequent expression of OPIDN clinical signs and neuropathy (Richardson, 1992; Johnson, 1993). Several recent studies contradict the 70% inhibition requirement in that OPIDN occurred when NTE activities were inhibited by less than 70%.

Stumpf et al. (1989) reported OPIDN signs in ferrets dosed with various concentrations of TOTP with no more than 47% NTE inhibition, while Larsen et al. (1986) reported OPIDN clinical signs in TOTP-dosed turkeys with NTE inhibition as low as 41%. Varghese et al. (1995) reported OPIDN clinical signs in quail after administration of TPP at doses which resulted in minimal NTE inhibition (11%). Rats exposed to multiple doses of TPP exhibited OPIDN clinical signs with NTE inhibition of only 33% (Veronesi et al., 1986). Recently, Abou-Donia et al. (1996) reported OPIDN-like clinical signs in chickens after exposure to triphenyl phosphine despite the absence of any NTE inhibition.

It was previously discussed by Varghese et al. (1995) that apparent subthreshold inhibition of NTE with subsequent development of OPIDN may be the result of measurements being taken after the threshold value has been reached and the enzyme actively is beginning to recover due to *de novo* synthesis. However, it is unlikely that synthesis of NTE in amounts sufficient to bring activity up to control levels could have taken place in the 24h period between dosing and harvesting of brains for NTE analysis in the present study. Thus, it is apparent that threshold inhibition (in excess of 70%) of NTE is not essential for the development of OPIDN, and the degree of NTE inhibition

after exposure to a neuropathic organophosphate compound does not appear to be an unequivocal indicator of the compound's ability to produce OPIDN. It must also be reasoned, therefore, that NTE may not be the target esterase in the development of OP-induced neurotoxicity but rather an esterase whose loss in activity is initiated by some second messenger system after CNS degeneration has begun. It is also possible that another (yet unknown) function of NTE, and not its esterase activity, is involved in the initiation of axonal degeneration (Carrington, 1989). It would be of value to measure other esterase activities after exposure to neurotoxic OP's to assess their behavior in relation to NTE activity.

These data also emphasize the erroneous classification of neuropathic OP's into Type I and Type II categories based on the degree of NTE inhibition. As previously demonstrated, many neuropathic OP compounds exhibit variability in the degree of NTE inhibition before the presentation of OPIDN in different susceptible species. Although the chicken has consistently been susceptible to OPIDN only when NTE is inhibited by at least 70% with both Type I and Type II OPIDN compounds, exposure to TPPn resulted in OPIDN clinical signs without NTE inhibition (Abou-Donia et al., 1996). TPPn, therefore, is an OP compound that produces OPIDN-like signs by some other mechanism than inhibition of NTE in both the chicken and the ferret.

Clinical Signs

OPIDN clinical signs observed in the ferret after exposure to TPPn were somewhat similar to clinical signs found after exposure to Type I OPIDN compounds, and closely resembled clinical signs observed following exposure to the Type II compound

TPP. For example, subcutaneous injection in ferrets administered 250, 500, and 1000 mg TOTP/kg body weight exhibited ataxia and flaccid paralysis of the hind limbs (Stumpf et al., 1989). DFP, when administered subcutaneously at doses of 2 or 4 mg DFP/kg body weight, produced ataxia and paralysis of only the hind limbs in ferrets (Tanaka et al., 1991). Yet, ferrets subcutaneously exposed to 500, 1000, and 2000 mg TPP/kg body weight exhibited ataxia, rigidity, and extensor rigidity of both fore- and hind limbs (Tanaka et al., 1990a). In the present study, single subcutaneous doses of TPPn resulted in clinical signs of ataxia, paralysis, and extensor rigidity of both fore- and hind limbs in ferrets similar to that observed in ferrets after exposure to TPP.

Differences in neuropathological damage patterns between Type I OPIDN compounds and TPPn in the ferret may be due to differences in species selectivity or dosage of chemical administered. When comparing Type I degeneration patterns with TPPn degeneration patterns in the ferret, Type I OPIDN degeneration is confined to a smaller number of brain regions. Type I OPIDN compounds would, therefore, be expected to result in less motor control difficulties than TPPn, which is responsible for widespread brain degeneration in the ferret.

The mechanism by which TPP causes deterioration of motor coordination in the ferret is the same as that causing lack of motor control after exposure to TPPn in the ferret. The TPPn-induced axonal and somatic degeneration found in several nuclei and regions of the central nervous system involved in motor control (cerebellar cortex, vestibular nuclei, ventral medial thalamic nuclei, primary cortex) effectively accounts for the severe sensory and equilibrium deficits present in the affected animals. However, since pathological examination has not yet involved the spinal cord or peripheral nerves, it is

possible that degeneration in these structures may have also contributed to the rapid onset and severity of observed clinical signs.

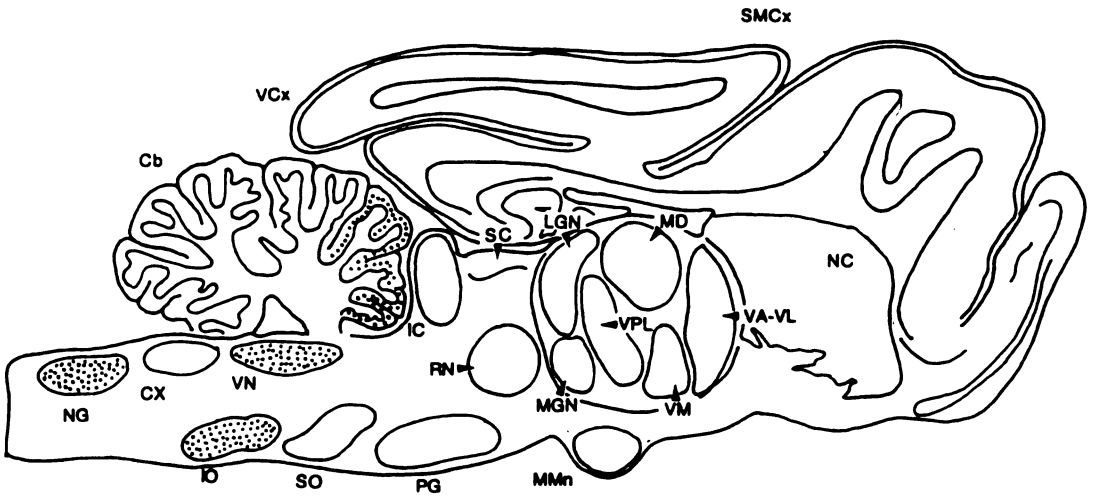
Locus and Extent of CNS Degeneration

The data reported in this investigation show for the first time the distribution of brain degeneration in the ferret after exposure to TPPn. Exposure to this compound results in both axonal and terminal degeneration in many brain regions. The pattern of degeneration caused by TPPn is more extensive than degeneration patterns induced by Type I OPIDN compounds, yet very similar to patterns induced by TPP in the ferret.

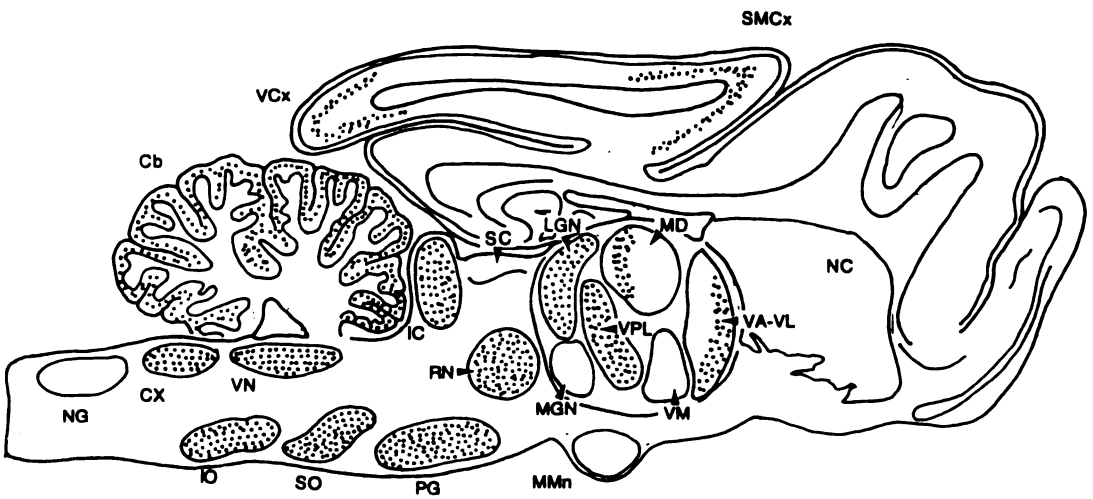
Figure 6 is a comparison of axonal and terminal degeneration in the ferret central nervous system after exposure to three different OPIDN compounds. Degeneration patterns caused by TPP and TPPn are similar, while the degeneration pattern for DFP is distinct. DFP degeneration in the ferret is much less widespread than degeneration patterns for both TPP and TPPn. TPP- and TPPn-induced degeneration is extensive in midbrain, forebrain, thalamic, and cerebral cortical areas involved in processing of visual, auditory, and sensorimotor information. Both TPPn and TPP appear to cause comparable damage to the spinal cord and sciatic nerves (ventral, lateral, and ventral-lateral tracts) in the chicken, although TPPn-induced spinal cord and nerve degeneration has not yet been examined in the ferret (Tanaka et al., 1992b; Abou-Donia et al., 1996). Neuropathological damage caused by DFP in the ferret is restricted to the brainstem and cerebellum (Figure 6).

Exposure to TPP in ferrets results in degeneration patterns characteristic of Type I degeneration in the brainstem and cerebellar regions, as well as additional degenerated

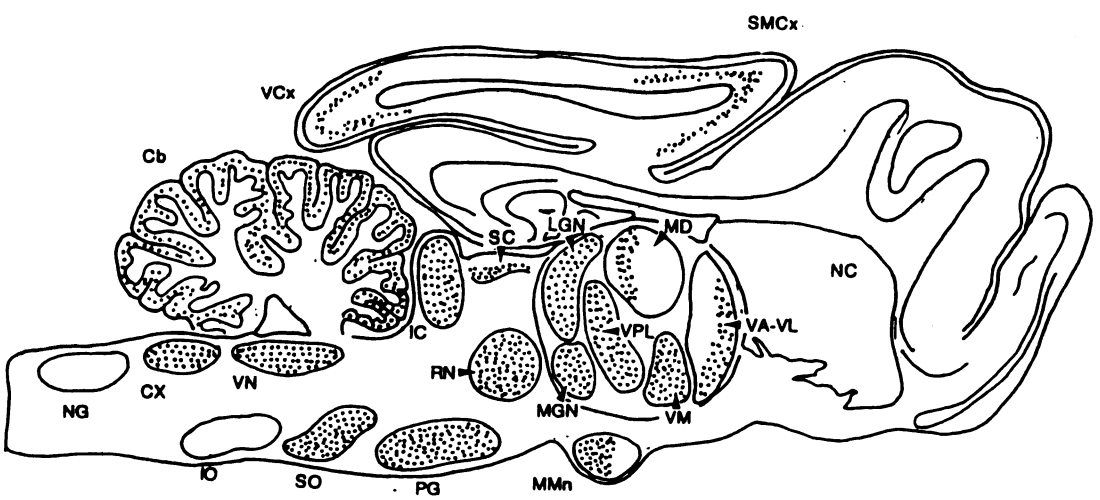
Figure 6. Summary schematics in the sagittal plane which compare qualitatively the extent of degeneration in the brain of the ferret after a single exposure to DFP, TPP, or TPPn. The location of degeneration was assessed using a modified Fink-Heimer silver impregnation technique. Stippling represents areas of degeneration. Figure abbreviations are listed at the beginning of the text.



Diisopropylphosphorofluoridate



Triphenyl Phosphite



Triphenyl Phosphine

areas throughout the medulla, midbrain, and forebrain (Figure 6).

Exposure of ferrets to TPPn results in degeneration patterns similar to TPP-exposed ferret brains with additional damage occurring in the medial mammilary nucleus of the hypothalamus, the ventral medial thalamic nucleus and medial geniculate nucleus of the thalamus, and the superior colliculus of the midbrain. Yet, TPPn- induced degeneration was not detected in the inferior olivary nucleus which does contain axonal degeneration after exposure to TPP in the ferret (Figure 6).

Similar CNS regions are affected by all neurotoxic OP's in the hen, ferret, and rat. Both Type I and Type II OPIDN compounds cause pathogenic damage to tracts of the spinal cord, some brainstem nuclei, and rostral portions of the cerebellum. These CNS regions, therefore, are critical factors in assessing the neuropathic potential of an OP regardless of Type I or Type II classification. The mechanisms underlying resistance and susceptibility to OP's by different neural systems or fiber tracts are not yet understood, although it is clear that certain areas of the CNS seem to be affected preferentially and selectively among susceptible species. This phenomena may be partially resolved by examining the susceptibility of neurons before and after maturation of a neuron cell.

The results of this study indicate that damage in the ferret brain caused by TPPn is much more widespread than damage caused by Type I compounds. The location and extent of axonal and terminal degeneration in the ferret after exposure to Type I OPIDN compounds does not involve midbrain and forebrain regions, and the extent of cerebellar damage is minimal when compared to the effects of TPPn. This strongly suggests that the pathogenic mechanism of Type I degeneration in the ferret are distinct from mechanisms involved in TPPn degeneration. These differences, however, could be more discretely

defined by additionally obtaining patterns of TPPn-degeneration in the hen and rat to compare to Type I OPIDN compounds within the same species.

Although neuropathological damage after exposure to TPPn is more severe and widespread than damage after exposure to a Type I compound (DFP), damage is very similar to that caused by Type II compounds in the ferret. The neural damage caused by both TPP and TPPn is widespread, affecting regions not only associated with basic sensorimotor activities, but those involved in higher order integrative and cognitive functions as well. The absence of significant additional degenerated brain regions by TPPn suggests that the mechanisms by which TPP and TPPn cause neurological damage are similar and distinct from Type I OPIDN compounds. It would be of interest to obtain neuropathological damage patterns at set time intervals after exposure to an OP in order to obtain a chronological map of damage. This would indicate the order that brain regions are affected by an OP and thus, hopefully, reveal its mechanism of action.

In conclusion, results based on clinical signs and neuropathology indicate that TPPn induces OPIDN in the ferret despite the absence of NTE inhibition. The pattern of degeneration, as well as observed clinical signs caused by TPPn, closely resembles those of TPP in the ferret. The clinical signs and neuropathology caused by TPPn in the ferret are not inconsistent with Type II OPIDN despite the lack of NTE involvement and does not necessitate the creation of a Type III OPIDN.

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