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THE DELAYED NEUROTOXIC EFFECTS OF TRIPHENYL PHOSPHITE (TPP) IN THE JAPANESE QUAIL

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THE DELAYED NEUROTOXIC EFFECTS OF TRIPHENYL PHOSPHITE (TPP) IN THE JAPANESE QUAIL

By

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ABSTRACT

THE DELAYED NEUROTOXIC EFFECTS OF TRIPHENYL PHOSPHITE (TPP) IN THE JAPANESE QUAIL

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Certain organophosphorus chemicals (OPs) cause a condition called organophosphorus-induced delayed neurotoxicity (OPIDN). Since previous studies indicated Japanese quail were resistant to Type I OPIDN compounds, the purpose of this study was to determine the sensitivity of this species to triphenyl phosphite (TPP), a Type II OPIDN compound. Quail were administered single subcutaneous doses of TPP at concentrations up to 500 mg/kg. At 24 hrs after dosing, half of the birds from each dose group were assessed for whole-brain neurotoxic esterase (NTE) activity. The remaining birds were observed daily for up to 20 days for the development of OPIDN clinical signs. Brains from some of these birds were examined for degeneration. All doses of TPP resulted in NTE inhibition which ranged from 11 % to 87 % and clinical signs as soon as 3 days post-dosing. Widespread degeneration in the brain was also noted.

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INTRODUCTION

Organophosphorus compounds are used extensively in industry and agriculture as pesticides, flame retardants, plasticizers, petroleum additives, and intermediates in the manufacture of pharmaceuticals (Davis and Richardson, 1980; U.S. Environmental Protection Agency, 1985). Exposure to certain of these organophosphorus compounds results in a neurological condition characterized by progressively developing hindlimb ataxia and paralysis, inhibition of the enzyme neurotoxic esterase (NTE) and development of axonal degeneration in both the central (CNS) and peripheral (PNS) nervous systems (Johnson, 1975a,b, 1982; Davis and Richardson, 1980; Abou-Donia, 1981; Baron, 1981; Abou-Donia and Lapadula, 1990; Tanaka *et al.*, 1990a,b, 1991, 1992a). Since 1978, this effect has been termed organophosphorus-induced delayed neurotoxicity or OPIDN (Abou-Donia, 1978). OPIDN's major visible consequence is the motor dysfunction resulting from neuropathic lesions, though there are other significant changes that precede or accompany clinical manifestations. These changes occur at various levels: changes at the molecular level are apparent as neurochemical changes, changes at the cellular level are apparent as neurophysiological changes, changes at the tissue level are apparent as

neuropathological changes, and changes at the organism level are apparent as functional changes, ie neurobehavioral and neurological alterations. The term "neurotoxic" is a general term to encompass all of these changes and is adequate enough to fully define this effect (Abou-Donia, 1981).

The series of poisonings caused by ingestion of tri-o-tolyl phosphate (TOTP) that occurred at intervals during the period from 1930 through the 1970s fully depicts the dangers of human exposure to OP compounds. In most of these cases, poisoning was from the ingestion of beverages or food adulterated with TOTP (Senanayake and Johnson, 1982). The characteristic syndrome was initially called "Ginger Jake paralysis" because of the consumption of illicit alcoholic beverages made from extracts of Jamaican ginger contaminated with TOTP. The victims displayed acute signs of vomiting and diarrhea which were followed by the onset of muscle pain, muscle weakness and parathesias. The chronic cases were characterized by individuals having signs of persistent ataxia and spasticity.

Recently OPIDN has been divided into 2 categories designated as Type I and Type II, with each type possessing distinctive clinical and neurophysiological characteristics (Abou-Donia and Lapadula, 1990). Type I is produced by exposure to organophosphorus compounds such as tri-o-tolyl phosphate or TOTP (also know as tri-ortho-cresyl phosphate or TOCP), bis (1-methylethyl) phosphorofluoridate (DFP), mipafox, and leptophos. Features of Type I OPIDN in animals include

inhibition of the nervous system enzyme neuropathy target esterase (NTE) in excess of 70 % and the presence of a relatively long delay period of 10 - 21 days before the onset of hindlimb ataxia and paralysis. The accompanying neuropathology is localized in peripheral nerves, spinal cord and brainstem. Type II OPIDN may be produced by exposure to triphenyl phosphite or tri-ortho-, tri-meta-, or tri-para-cresyl phosphite. In contrast to Type I OP compounds, delayed neurotoxicity induced by Type II compounds is characterized by NTE inhibition which may be less than 65 - 70 % and by a shortened delay period of 4 - 7 days before the onset of ataxia and hindlimb paralysis. Results of recent work by Tanaka *et al.* (1990b, 1992a,b) indicate that neuropathology resulting from exposure to Type II compounds involves not only the spinal cord and brainstem, but also the midbrain and forebrain. Type I OPs contain a central pentavalent phosphorus atom while Type II OPs contain a central trivalent phosphorus atom.

To determine the suitability of a species as an OPIDN model, the pattern of degeneration in the central nervous system (CNS) is taken into consideration. The chicken is commonly used as a model to study the pathophysiology of this syndrome (Cavanagh, 1954). Several studies using Type I (Tanaka and Bursian, 1989; Tanaka *et al.*, 1990a) and Type II (Carrington *et al.*, 1988a; Konno *et al.*, 1989; Katoh *et al.*, 1990; Tanaka *et al.*, 1991) OPIDN compounds have included a histopathological examination of nervous system tissue in the

chicken. The Fink-Heimer silver impregnation method has been effective in mapping the total extent of neuropathology following exposure to Type I and Type II OPIDN compounds in the chicken (Tanaka and Bursian, 1989; Tanaka *et al.*, 1990a,b, 1992a,b).

Despite the susceptibility of the chicken to Type I and Type II OPIDN, other avian species have not displayed the same sensitivity. For example, the Japanese quail has been shown to be resistant to the effects of the Type I OPIDN compound TOTP based on the lack of clinical signs (Francis *et al.*, 1980; Bursian *et al.*, 1983).

The purpose of the present study was to determine if a species which is apparently resistant to Type I OPIDN will also be resistant to Type II OPIDN. Thus, specific objectives of this study were to: 1) determine if the Japanese quail, a species which is not sensitive to Type I OPIDN, is sensitive to the Type II OPIDN compound triphenyl phosphite (TPP) based on the development of clinical signs, whole-brain NTE inhibition and the presence of characteristic neuropathological lesions, and 2) if the Japanese quail was shown to be sensitive to TPP, to compare the characteristics of clinical signs, NTE inhibition and neuropathological lesions in the Japanese quail with those same parameters in the chicken.

LITERATURE REVIEW

1. ORGANOPHOSPHORUS COMPOUNDS

Organophosphorus (OP) chemicals are used in agriculture as pesticides to kill undesired insects, worms, weeds and fungi, and in industry as plasticizers, antioxidants, stabilizers, plastic extenders, and oil and gasoline additives (Eto, 1979; Davis and Richardson, 1980; US Environmental Protection Agency, 1985; Cherniak, 1988). Cresyl phosphates and other related phosphates also impart flame resistance to fabrics (Fisher and Van Wazer, 1961). While OP compounds have been very beneficial in terms of increased food production and protection of human health (Ecobichon, 1991), they also have adverse effects on both the peripheral and central nervous systems.

The most extensively studied OP effect is the acute inhibition of acetylcholinesterase (AChE), the enzyme which hydrolyses the neurotransmitter acetylcholine (ACh). ACh is released from nerve terminals of postganglionic parasympathetic nerve fibers, somatic motor nerves innervating skeletal muscles, preganglionic fibers of the autonomic nervous system and some neurons in the central nervous system (Murphy, 1975). The OP molecule mimics ACh and phosphorylation of the serine-hydroxyl group at the catalytic center of the enzyme occurs. The potency of the OP compound depends on the degree of enzyme phosphorylation. Inhibition

of AChE causes ACh to accumulate at the synapses and neuromuscular junctions causing excessive stimulation of muscarinic, nicotinic and CNS receptors. Effects of excessive stimulation of muscarinic receptors, which are present on smooth muscles, heart, and exocrine gland, are bronchoconstriction, increased salivation and lacrimation, vomiting, diarrhea and bradychardia. Effects of excessive stimulation of nicotinic receptors, located on skeletal muscles and autonomic ganglia, include fatigue, dyspnea, pallor, increased blood pressure, hyperglycemia and tachycardia. Accumulation of ACh at CNS receptors cause restlessness, anxiety, headaches, confusion and tension (Murphy, 1975; Barrett and Oehme, 1985). OP-induced death may occur 5 minutes to 24 hours post-exposure, depending on the chemical and the dose (Abou-Donia and Lapadula, 1990). In addition to its presence in the nervous system, AChE is also found in red blood cells. Assessment of AChE activity in red blood cells is commonly used as an indication of exposure to and absorption of OP compounds.

In addition to acute inhibition of AChE, some OP compounds have delayed neurotoxic properties (Abou-Donia, 1981). Organophosphorus-induced delayed neurotoxicity involves permanent locomotor ataxia and subsequent paralysis that is apparent 10 - 21 days after exposure (Johnson, 1975a). In addition to the characteristic delay period and reproducible clinical signs, the delayed neurotoxic OP compounds also inhibit the nervous system enzyme neurotoxic

esterase (Johnson, 1982), and induce characteristic neuropathologic lesions in the peripheral and central nervous systems (Cavanagh, 1954; Tanaka *et al.*, 1990a,b, 1991, 1992a). Some animal species are resistant to the effects of typical OPIDN compounds as are the young of sensitive species. An adequate explanation for this apparent lack of sensitivity has yet to be offered.

TWO KINDS OF OPIDN

Soon after tri-o-tolyl phosphate was determined to be the cause of OPIDN in humans during the "Ginger Jake paralysis" incident in 1930, it was apparent that organophosphorus compounds produced 2 distinct types of delayed neurotoxic actions (Lillie and Smith, 1932; Smith *et al.*, 1933; Aird *et al.*, 1940). However, only recently have these effects been defined through various studies (Veronesi *et al.*, 1986a,b; Veronesi and Dvergsten, 1987; Padilla *et al.*, 1987; Carrington and Abou-Donia, 1988a,b; Carrington *et al.*, 1988a; Abou-Donia and Brown, 1990). The OPIDN compounds can be assigned to 2 classes (Type I and Type II) based on the following criteria (Abou-Donia and Lapadula, 1990):

- Chemical structure
- Length of latent period between exposure and appearance of clinical signs and neuropathologic lesions
- Morphology and distribution of neuropathologic

lesions

- Inhibition of NTE
- Age sensitivity
- Species specificity

HISTORY & INCIDENCE - Type I OPIDN

Before the 1930s, cases of delayed neuropathy occurred occasionally in tubercular patients being treated with phosphocreosote, a mixture of esters derived from phosphoric acid and coal tar phenols (Davis and Richardson, 1980). However, the medicant was not realized to be neurotoxic until a massive outbreak of poisoning in the United States occurred in 1930 when approximately 20,000 people were affected. This condition, marked by permanent ataxia and paralysis of the legs and commonly referred to as "Ginger Jake paralysis", was caused by a mixture of cresyl phosphates used to extract ginger for the purpose of flavoring distilled liquors (Smith *et al.*, 1930a,b, 1932; Smith and Lillie, 1931; Kidd and Langworthy, 1933). The symptoms of "Ginger Jake paralysis" started as a slowly developing paralysis in the legs followed by tremors. Even after 6 years, studies conducted on many of these patients showed little recovery with muscle weakness being replaced by spasticity, hyperflexia and abnormal reflexes (Aring, 1942). The historical and epidemiological aspects of this "Ginger Jake" delayed neuropathy have been reviewed extensively (Cavanagh, 1973; Johnson, 1975a,b; Hess

et al., 1978).

Similar cases were reported in European publications shortly after the US outbreak (Barrett and Oehme, 1985). This delayed paralysis, which was found only in women, was linked to "Apiol", an abortifacient containing between 28 and 35 % TOTP. During the next 30 years, studies showed that TOTP was the causative agent in a number of paralytic incidents (Cavanagh, 1964a). Fifty eight cases of delayed neurotoxicity occurred in Durban, South Africa in 1938 after cooking oil was contaminated with TOTP. Another incident involved 11 people who were affected after drinking water from drums which were used to store cresyl phosphates (Susser and Stein, 1957). In 1942, three employees involved in the manufacture of TOTP lubricants in Great Britain developed "polyneuritis" after exposure by inhalation during black-out conditions (Hunter *et al.*, 1944). Susser and Stein (1957) quoted Walthard's (1947) description of the syndrome in 80 men of the Swiss army who also had been poisoned with TOTP-contaminated cooking oil.

Since the early studies showed that only the ortho isomer among the symmetrical cresyl phosphates produced a toxic effect (Smith *et al.*, 1930a,b, 1932), it became customary for mixed esters to be prepared from coal-tar stock containing less than a specified low amount of ortho-cresol. In spite of this precaution, further outbreaks of poisoning occurred from time to time. In 1953, three workers who were engaged in the laboratory production of a prospective organophosphate pesticide (mipafox or N, N'-di isopropyl

phosphordiamidofluoridate) were reported to have developed clinical signs typical of OPIDN (Bidstrup *et al.*, 1953). Laboratory studies concluded that there was no essential difference between mipafox-induced neurotoxicity and neurotoxicity caused by some other alkyl phosphoryl esters and by the triaryl phosphates such as TOTP (Barnes and Denz, 1953).

It was determined that a major outbreak of OPIDN in Morocco in 1959 was due to TOTP-contaminated lubricating oil deliberately used to dilute olive oil which was later sold as cooking oil (Smith and Spalding, 1959; Cavanagh, 1964a; Metcalf, 1982). As a result, over 10,000 people were affected with ataxia and paralysis in this outbreak. Dogs also displayed characteristic signs of OPIDN after ingesting the same contaminated substance (Smith and Spalding, 1959). Other examples of TOTP-contaminated food products which resulted in human cases of OPIDN include cooking oil in India (Vora *et al.*, 1962; Anon., 1988), flour in the Fiji Islands (Sorokin, 1969), alcohol in Rumania (Vasilescu and Florescu, 1980) and Morocco (Abou-Donia and Lapadula, 1990), and sesame oil in Sri Lanka (Senanayake, 1981).

Unintentional and intentional exposure to OP insecticides like EPN, omethoate, leptophos, lenthion, trichloronate, trichlorphon, phytosol, tamaron, methamidophos and chlorpyriphos have resulted in clinical signs characteristic of OPIDN (Bidstrup *et al.*, 1953; Fukuhara *et al.*, 1977; Hierons and Johnson, 1978; Xintaras *et al.*, 1978; Jedrzejowska

et al., 1980; De Jager *et al.*, 1981; Senanayake and Johnson, 1982; Vasilescu *et al.*, 1984; Metcalf *et al.*, 1985; Lotti and Morretto, 1986; Abou-Donia and Lapadula, 1990). In addition to human cases of OPIDN, an incident in Egypt resulted in over 1300 water buffaloes developing OPIDN from exposure to leptophos (Abou-Donia *et al.*, 1974).

HISTORY & INCIDENCE - Type II OPIDN

As early as 1930, it was apparent that there were 2 types of OPIDN. Smith and colleagues (Smith *et al.*, 1930a) were the first to study the effects of triphenyl phosphite (TPP), the prototype Type II OPIDN compound, in various animal species. Since the Type II compounds had not resulted in human cases of OPIDN, interest in Type II OPIDN faded. It wasn't until the 1980s that the delayed neurotoxicity effects of TPP were again examined in chickens (Carrington *et al.*, 1988a,b; Konno *et al.*, 1989; Tanaka *et al.*, 1992a,b) and in rats (Veronesi *et al.*, 1986a; Padilla *et al.*, 1987; Veronesi and Dvergsten, 1987). Ironically, TPP has evaded scrutiny, unlike TOTP, and is still being used in industry and agriculture as a rubber and plastic stabilizer, a diluent of epoxy resins, a metal scavenger, an anti-fungal foliar agent and as an insecticidal synergist though it is known to be an OPIDN-causing compound (U.S. Environmental Protection Agency, 1985).

CHEMICAL STRUCTURE & REACTIVITY - Type I OPIDN

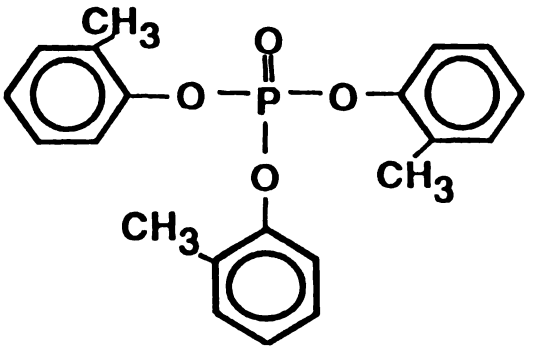
Type I OPIDN compounds have a pentavalent phosphorus atom and are derivatives of phosphoric, phosphonic, and phosphoramidic acids and phosphofluoridates. The pentavalent phosphorus atom has a tetrahedral configuration. Type I OPIDN compounds also include sulfur analogs. TOTP and DFP are examples of Type I compounds (Figure 1) (Stuart and Oehme, 1982).

The presence of the *ortho*-methyl group in the aromatic series may be responsible for the neurotoxic property of these types of OP chemicals (Casida *et al.*, 1961; Nomeir and Abou-Donia, 1984, 1986). TOTP has to be metabolized to tolyl saligenin phosphate (TSP) (Figure I) to be neuroactive (Eto *et al.*, 1962, 1967; Bleiberg and Johnson, 1965; Johnson, 1975b; Davis and Richardson, 1980; Abou-Donia, 1984), while DFP doesn't have to be metabolized to cause OPIDN (Barrett and Oehme, 1985). The chemical reactivity of the Type I OPIDN compounds depends on the phosphorus atom; on its ability to phosphorylate and on its electrophilic character (U.S. Environmental Protection Agency, 1985).

CHEMICAL STRUCTURE & REACTIVITY - Type II OPIDN

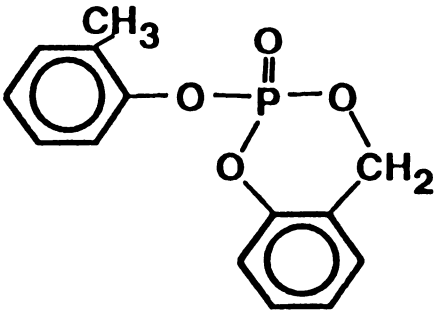
Type II OPIDN compounds have a trivalent phosphorus atom and are phosphorus acid derivatives (ie. triphosphites and presumably their sulfur analogs). Triphenyl phosphite is an

Figure 1. The chemical structures of tri-o-tolyl phosphate (TOTP), o-tolyl saligenin phosphate (TSP), and bis (1-methylethyl) phosphorofluoridate (DFP). These chemicals are Type I OPIDN compounds. TSP is the neurotoxic metabolite of TOTP.



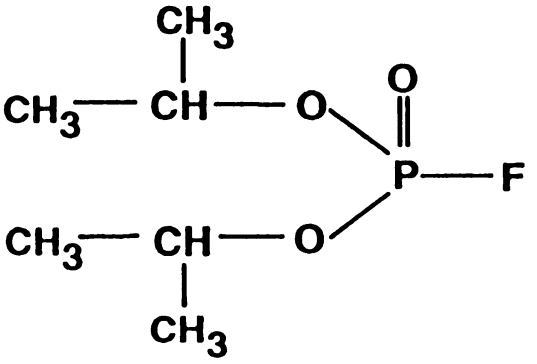
Tri-o-tolyl phosphate

(TOTP)



o-tolyl saligenin phosphate

(TSP)



Bis(1-methylethyl) phosphorofluoridate

(DFP)

example of a Type II compound (Figure 2). The trivalent phosphorus atom has a pyramidal configuration (Abou-Donia and Lapadula, 1990).

Neurotoxicity of Type II OPIDN compounds does not depend on metabolism which in turn means that any aryl phosphite can cause Type II OPIDN. The trivalent phosphorus atom has a pair of electrons which binds with other atoms, making it very reactive regardless of other substituents (U.S. Environmental Protection Agency, 1985).

LATENT PERIOD

Both Type I and Type II OPIDN are characterized by a delay in the onset of clinical signs. However, the period between the time of exposure to the OP compound and the time of onset of clinical signs varies between Type I and Type II compounds in that the latent period is shorter for Type II compounds. It takes only 4 - 6 days for the onset for clinical signs in hens after TPP administration (Carrington and Abou-Donia, 1988a; Carrington *et al.*, 1988a; Katoh *et al.*, 1990; Tanaka *et al.*, 1992a,b) compared to 6 - 14 days for TOTP (Abou-Donia, 1981). Ferrets injected with DFP started showing signs around day 14 post-dosing (Tanaka *et al.*, 1991) and ferrets dosed with TOTP started showing signs by day 50 post-dosing (Stumpf *et al.*, 1989). In contrast, ferrets injected with TPP started showing signs of ataxia by day 4 post-dosing and severe paralysis by day 8 post-dosing (Tanaka *et al.*,

Figure 2. The chemical structure of triphenyl phosphite (TPP), a Type II organophosphorus compound.

1990b).

CLINICAL SIGNS - Type I OPIDN

The clinical signs of organophosphorus-induced delayed neurotoxicity are similar in all susceptible species. Smith and associates (1930a) were the first to report on OPIDN clinical signs in various species of animals. These initial reports were followed by detailed descriptions of clinical signs in humans by Smith and Spalding (1959).

A single injection or oral dose of an OPIDN compound is sufficient to initiate the irreversible clinical response (Abou-Donia and Graham, 1979). Low-level dietary administration (Kinebuchi *et al.*, 1977; Abou-Donia and Graham, 1978a,b; Hussain and Oloffa, 1979) and dermal administration (Abou-Donia and Graham, 1978a,b; Hess *et al.*, 1978) of TOTP have also been shown to induce delayed neurotoxicity. Some of the early studies by Smith and colleagues (Smith *et al.*, 1930a,b; Smith and Lillie, 1931; Smith *et al.*, 1932) indicated that small, individually ineffective doses of TOTP ingested over a period of time can give rise to ataxia and severe paralysis, if the total dose ingested approached the minimum acute dose for paralysis. Smith *et al.* (1930b) reported that 40 mg TOTP/kg body weight was sufficient to produce OPIDN in humans. Hopkins (1975) reported calculations by Staehelin (1941) that suggested as little as 2 mg TOTP/kg body weight (made from feedstock low in ortho cresol) may cause a

neurotoxic response in man.

Ingestion of TOTP by humans may cause some immediate gastrointestinal distress with nausea, vomiting, and diarrhea which may last a few hours to a few days. A latent period of 8 - 18 days generally follows, depending on the size of the dose and length of exposure. This is followed by a sharp, cramp-like pain in the calves and numbness and tingling in the feet and the hands of the patients. This in turn is followed by increasing weakness of the lower limbs. The patient cannot keep his balance and bilateral footdrop has been reported. Depending on the severity, there may be weakness at the knee or hip. Ankle jerks are absent. Knee reflexes may be normal or occasionally depressed. The patient may experience weakness in the hands a week or two after the onset of paralysis in the lower limbs. Wrist-drop and weakness up to the elbow may also be seen in some individuals. Many patients exhibit sensory loss, conforming roughly to the degree of motor loss. Sensory loss in the upper limbs usually appears a few days after it develops in the lower limbs (Susser and Stein, 1957).

The results reported by Smith and co-workers (1930a) on the response of hens to TOTP and other neurotoxic OP esters were remarkably similar to OPIDN clinical signs reported for humans. Birds treated with TOTP appear normal for a period of 10 - 14 days following acute administration. After this period, they spend more time in a sitting position. When exercising, birds begin to display a clumsiness of gait,

accompanied by overt weakness, which is a characteristic sign of ataxia. At 15 - 20 days post-treatment, the birds may become severely paralyzed in the legs and the wings are obviously weakened (Bursian *et al.*, 1983; Calabrese and Bursian, 1984; Tanaka and Bursian, 1989). At low neurotoxic doses, the birds experience a slight initial weight loss which later is reversed. The birds are able to maintain their health, although the proper use of affected limbs may not be fully regained.

In contrast, at higher acute doses, a more severe terminal clinical neurotoxicity is observed. The animal loses its ability (or desire) to eat and subsequently dies following a severe weight loss and complete paralysis of the legs (Smith and Elvove, 1930; Smith *et al.*, 1930a, 1932; Smith and Lillie, 1931; Lillie and Smith, 1932).

In ferrets treated with DFP, the clinical signs at 21 and 28 days post-treatment ranged from slight hind limb weakness to severe ataxia or hindlimb paralysis (Tanaka *et al.*, 1991). Ferrets exposed to TOTP dermally had diarrhea which was the only sign of acute toxicity (Stumpf *et al.*, 1989). The motor defect was confined to the hind limbs. Occasionally, the treated animals exhibited hopping movements rather than normal movement and occasionally animals would fall side to side. Ferrets treated orally with 1000 mg TOTP/kg body weight showed detectable clinical signs of ataxia and high-stepping gait by day 11 post-dosing.

CLINICAL SIGNS - Type II OPIDN

Clinical manifestations of Type II OPIDN have been studied in various species (Smith *et al.*, 1933; Roberts *et al.*, 1982; Veronesi *et al.*, 1986a; Tanaka *et al.*, 1990b, 1992a,b). TPP administration causes ataxia in chickens, rats, cats and ferrets. The pattern of clinical signs produced by TPP are very similar to that produced by neurotoxic phosphoric acid esters like TOTP and DFP (Abou-Donia, 1981). However, ataxia and paralysis tend to develop sooner relative to animals treated with Type I OPIDN compounds (Carrington *et al.*, 1988a; Katoh *et al.*, 1990). Early signs of neurotoxicity can be observed before 7 days post-dosing.

TPP did not produce severe clinical signs typical of Type II OPIDN in hens when administered orally, but dermal administration and subcutaneous injections did (Carrington and Abou-Donia, 1988a; Carrington *et al.*, 1988a,b; Abou-Donia and Brown, 1990). Hens developed mild ataxia with a spastic and awkward gait within a few days after exposure which progressed to flaccid leg paralysis (Smith *et al.*, 1933; Carrington and Abou-Donia, 1988a; Carrington *et al.*, 1988a, Katoh *et al.*, 1990).

Triphenyl phosphite and other phenolic phosphites produce extensor rigidity in the cat which is not observed with Type I OPIDN (Smith *et al.*, 1932, 1933). Cats treated with Type II compounds developed ataxia and extensor rigidity in both forelimbs and hindlimbs of relatively long duration (Veronesi

et al., 1986a). Monkeys treated with 2 doses of 1 ml TPP/kg body weight over a 24-day interval developed ataxia within 12 days of the second dose. After 3 days, extensor rigidity of the limbs and some retraction of the head developed (Abou-Donia and Lapadula, 1990).

Rats treated with Type II OPIDN compounds showed initial clinical signs of hyper-excitability, some spasticity, and incoordination with partial flaccid paresis of the extremities developing later (Smith *et al.*, 1933). Recent studies by Veronesi and co-workers (Veronesi *et al.*, 1986a; Veronesi and Dvergsten, 1987) on Long-Evans rats have indicated that TPP in multiple doses produced tail-kinking in the proximal 1 inch of the tail. Hindleg ataxia developed within a week, followed by paralysis. In addition, rats developed bidirectional circling behavior following multiple doses of TPP (Veronesi *et al.*, 1986a; Padilla *et al.*, 1987). When placed on their backs, the dosed rats rolled into a ball whereas the control rats rolled over to an upright position.

NEUROPATHOLOGICAL FEATURES

Neuropathological features help in determining the location and severity of somatic and axonal degeneration from exposure to organophosphorus neurotoxicants. OPIDN compounds cause histological alterations in the CNS and PNS, with each class of chemicals inducing characteristic lesions. The pathological lesions depend on the animal species and the

duration of exposure in addition to the chemical.

Type I OPIDN

Degeneration occurs in both the peripheral and the central nervous systems after a single dose of Type I OPIDN compounds (Cavanagh, 1954; Bischoff, 1970; Itoh *et al.*, 1981, 1984; Prentice and Roberts, 1983; Jortner *et al.*, 1989; Tanaka and Bursian, 1989; Tanaka *et al.*, 1992b) and is usually detected at the same time that clinical signs are apparent. However, some degenerative changes have been detected before the development of clinical signs (Wilson *et al.*, 1988; El-Fawal *et al.*, 1990). Histological studies on TOTP-exposed humans showed that lesions were limited to the peripheral nerves and the anterior horn cells of the spinal cord and sometimes to the motor cells in the medulla (Smith and Elvove, 1930; Smith *et al.*, 1930b, 1932; Lillie and Smith, 1932).

Lesions in the CNS of hens exposed to Type I delayed neurotoxicants were restricted to the brain stem, cerebellum, and spinal cord (Tanaka and Bursian, 1989; Tanaka *et al.*, 1990a). Moderate to severe axonal degeneration occurred in the spinal cord of hens exposed to TOTP and DFP (Tanaka *et al.*, 1992b; Pope *et al.*, 1992). Degenerating axons were noted in the cervical parts of the fasciculus gracilis in the spinal cord, dorsal and ventral spinocerebellar tracts, and in the lumbar part of the medial pontine-spinal tract. Moderate terminal degeneration was also noted in the medial part of the

ventral horn at lumbar cord levels.

In the medulla, moderate terminal and preterminal degeneration were noted in the lateral vestibular, gracile-cuneate, external cuneate, and lateral cervical nuclei as a result of exposure to TOTP or DFP. Degeneration was noted in lesser amounts in the solitary, inferior olivary, and raphae nuclei, in the medial and dorsal vestibular nuclei, and in the lateral paragigantocellular, gigantocellular, and lateral reticular nuclei. Fiber degeneration was present in the medullary portions of the dorsal and ventral spinocerebellar tracts and in the spinal lemniscus. Axonal degeneration was also present in nerve fascicles which make up the intermedullary portions of the glossopharyngeal and vagus nerves.

Within the cerebellum, mossy fiber degeneration occurred in the granular layer due to TOTP or DFP exposure. Alternate parasagittal bands of heavy and light degeneration were also seen. Small amounts of coarse fiber degeneration were also noted in the deep cerebellar nuclei (Jortner *et al.*, 1989).

Microscopic lesions were present in the nervous system of hens exposed to mipafox, provided that the dose was high enough to cause greater than 80 % inhibition of NTE (Dyer *et al.*, 1992). The hen had a Wallerian-like degeneration. The fiber breakdown in multiple spinal cord tracts was extensive and varied, with the intensity of degeneration directly proportional to the mipafox dose. The hen develop a slow, consistent and longer lasting neuropathy than the rat (Carboni

et al., 1992), thus being a sensitive species for mipafox-induced OPIDN, and confirming the previous study by Dyer *et al.* (1992).

TOTP produced histopathological lesions in the spinal cord and peripheral nerves of cats in a dose-dependant fashion (Abou-Donia *et al.*, 1986). Cavanagh (1964b) and Cavanagh and Patangia (1965) have studied the TOTP-induced lesions in the cat by light microscopy. In the peripheral nerves, axonal degeneration occurred initially at the distal ends of the longest and largest diameter fibers which spread proximally in a typical "dying-back" process. The areas which were initially and most severely affected were the large diameter fibers from annulo-spinal endings of the muscle spindles. The long pathways in the spinal cord showed a similar "dying-back" lesion. Electron microscopy studies by Prineas (1969) showed that the earliest lesion occurred in the axons and consisted of accumulation of abnormal membrane-bound vesicles and tubules. In DFP-treated cats, granular transformation of the axoplasm was noted in the degenerating axons. All their neurotubules or neurofilaments were lost and the degenerated mitochondria were swollen (Bouldin and Cavanagh, 1979).

In ferrets treated with DFP, dense axonal degeneration was noted in the fasciculus gracilis and dorsal spinocerebellar tract at cervical levels of the spinal cord and in the lateral corticospinal tract at lumbar levels (Tanaka *et al.*, 1992b). Spinal cord laminae VI - VII had degenerating terminals, with lumbosacral levels showing severe

degeneration. Severe degeneration was also noted in the ventral motor nucleus of the cervical enlargement at spinal cord levels. Fasciculus and nucleus gracilis, medial and dorsal accessory nuclei of the inferior olive, inferior nucleus and lateral reticular formation of the medulla had dense axonal and terminal degeneration. The cerebellar mossy fiber degeneration noted in the ferret was similar to that reported for the hen. It consisted of alternate bands of light and dark degeneration.

TOTP-induced lesions in the rat were characterized by giant swellings of myelinated and demyelinated axons, myelin debris, vacuolated myelin sheaths and hyaline bodies (Veronesi, 1984). TOTP-induced degeneration was found in the sensory and motor tracts in a dying-back pattern like in other species. Within the cervical spinal cord, damage was found mainly in the dorsal column. In contrast, multiple doses were needed to produce lesions in the ventrolateral and ventral columns of the cervical and lumbar cord. Neuropathological lesions were noted even in the absence of overt ataxia (Veronesi, 1984). Inui *et al*. (1993) studied TOTP-affected rats and concluded that axonal swelling is due to accumulation of cytoplasmic contents near the node of Ranvier, indicating paranodal degeneration. Topographical examinations of the lesions showed that the fasciculus gracilis in the cervical spinal cord was the major target site (Padilla and Veronesi, 1988).

The nervous system of mipafox-treated rats, like hens,

showed microscopic lesions if the dose was high enough to cause NTE inhibition of 80 % or more. These Wallerian-like lesions were well developed in the highest dosage group and were confined to the rostral level of the fasciculus gracilis in the medulla oblongata (Carboni *et al.*, 1992; Veronesi *et al.*, 1986b). The most prominent lesion in rats was swollen axons with a single vacuole containing flocculent material (Dyer *et al.*, 1992; Carboni *et al.*, 1992). Quantitatively, the percentage of affected fibers in the fasciculus was lower in rats compared to those in hens.

The Type I OPIDN neuropathological studies show that (1) CNS axonal and terminal degeneration apparently is confined to the spinal cord, medulla and cerebellum, (2) it takes 1 to 3 weeks post-exposure for the onset of degeneration in different fiber tracts and nuclei and (3) the delayed onset of OPIDN clinical signs and the delayed onset of degeneration in many of the affected CNS fiber systems are mutually consistent.

Type II OPIDN

Pathological lesions are species-dependent and occur in both the central and peripheral nervous systems (Veronesi and Dvergsten, 1987; Carrington and Abou-Donia, 1988a; Carrington *et al.*, 1988a). Exposure to the Type II neurotoxicant TPP resulted in widespread degeneration in the hen which involved the spinal cord, cerebellum, medulla, midbrain and forebrain (Carrington *et al.*, 1988a; Tanaka *et al.*, 1991; Knoth-Anderson

and Abou-Donia, 1993). Examination showed that the brain stem (mainly the reticular formation and the cerebellar peduncles), the ventral and lateral tracts of the spinal cord, the gray matter of the spinal cord and the sciatic nerve contained swollen and fragmented axons. The damage was more severe in the lower (thoracic and lumbar) portions of the spinal cord. Cellular gliosis and decrease in number of motor cells in the anterior horn were noted in cellular degeneration. These changes were observed in the medulla, pons, brainstem, and cerebellar cortex (Smith *et al.*, 1933). Fatty degeneration, tigrolysis and cellular necrosis were not significantly present.

In cats, Smith and co-workers (Smith *et al.*, 1933) reported a small amount of neuronal somatic degeneration in the thalamus and cerebral cortex similar to the findings in rats. The posterior fasciculi in the cat are not affected by Type II OPIDN. The medulla and pons, the restiform bodies and the brachia conjunctive are the most affected (Smith *et al.*, 1933). The spinal ganglia underwent only slight changes.

Degeneration in the same locations was noted in the studies conducted by Tanaka *et al.* (1990b, 1992b) in the brains of ferrets injected with TPP. The Fink-Heimer method of staining showed widespread axonal and terminal degeneration in the midbrain, thalamus, and cerebral cortex in addition to the spinal cord and brainstem. The degeneration was independent of the individual doses or post-injection survival periods. TPP exposure also affected a number of sensory and

motor systems. Dorsal spinocerebellar tract afferents to the cerebellum were black and fragmented in appearance which is characteristic of degenerating axons. The external cuneate nucleus and the nuclei of the reticular formation also showed neuronal somatic degeneration. Axonal and somatic degeneration were also seen in the nuclei and tracts of several sensory systems. The findings of this study show that CNS damage from TPP exposure is not only in sensorimotor pathways and nuclei of the brainstem and spinal cord, but also may involve other visual, auditory and higher order sensorimotor areas.

Rats, once considered resistant to the delayed neurotoxic effects of OPs based on the absence of clinical signs when dosed with Type I OPs, have been shown to develop lesions in the spinal cord, brainstem, midbrain, and forebrain after dosing with Type II OPIDN compounds (Veronesi, 1984; Veronesi and Padilla, 1985; Padilla and Veronesi, 1985, 1988; Veronesi *et al.*, 1986a; Veronesi and Dvergsten, 1987; Tanaka *et al.*, 1992b). Smith and co-workers (1933) first reported a small amount of neuronal somatic degeneration in the thalamus and cerebral cortex. The CNS of rats exposed to TPP were thoroughly examined using the Fink-Heimer silver impregnation method (Lehning *et al.*, 1990). The studies showed widespread axonal and terminal degeneration not only in the spinal cord and brainstem, as is the case with other OP compounds such as DFP in ferrets (Tanaka *et al.*, 1991), but also in the midbrain, thalamus and cerebral cortex. But there was only

minimal degeneration in the spinal cord of the rat in contrast to the ferret.

ROLE & INHIBITION OF NEUROTOXIC ESTERASE

Over the past 50 years, research efforts have increased in an attempt to develop an understanding of the relationship between chemical structure, biochemical lesion and neurotoxicity. All organophosphate esters that induce delayed neuropathy are either direct esterase inhibitors or are metabolically converted to inhibitors. Thus, it has been generally considered that phosphorylation of an esterase is probably needed for the onset of delayed neurotoxicity, although the phosphorylation doesn't necessarily have to take place in the nervous system.

The most extensive and successful biochemical investigations were conducted at the Medical Research Council Laboratories in Carshalton, England by Barnes and his co-workers (Barnes and Denz, 1953; Aldridge and Barnes, 1961, 1966) and later by Johnson and his colleagues at the same institution (Aldridge *et al.*, 1969; Johnson, 1969a,b,c, 1975b, 1976a,b, 1977; Johnson and Barnes, 1970; Lotti and Johnson, 1978; Clothier and Johnson, 1979). The studies done there have indicated that delayed neurotoxicity is associated with the inhibition of a specific membrane-bound protein having esterase activity. Johnson and his co-workers named this protein "neurotoxic esterase" (NTE) (Aldridge *et al.*, 1969).

NTE, which is present in various tissues, has no known biochemical or physiological functions (Abou-Donia, 1981). Johnson first suggested, followed by others (Johnson, 1969a,b,c, 1977; Clothier and Johnson, 1979; Abou-Donia, 1981; Williams and Johnson, 1981; Ehrich *et al.*, 1985; Carboni *et al.*, 1992), that an organophosphorus chemical is considered a delayed neurotoxicant when approximately 70 - 80 % of the enzyme is phosphorylated through an irreversible "aging" reaction. Studies have suggested that aging involves the formation of a covalently bound, phosphorylated, and irreversibly inhibited esteratic site on NTE (Johnson, 1982; Richardson, 1984; Davis *et al.*, 1985). Aging leads to a stable, irreversibly phosphorylated enzyme species which when hydrolyzed forms a reactivated protein. The aging reaction may be related to the delayed neurotoxicity induced by certain OPs. The kinetic relationship of anti-esterase OPs with hydrolytic enzymes has been extensively reviewed (Cohen and Osterbaan, 1963; Aldridge and Reiner, 1972).

How the inhibition and aging of NTE leads to neuronal damage is still unknown. Only delayed neurotoxic OP compounds can irreversibly inhibit NTE enzymatic activity while non-OPIDN compounds don't (Johnson, 1982). Some compounds can reversibly inhibit NTE, but not result in OPIDN. However, these compounds can prevent delayed neurotoxicity when given prior to a neuropathic OP. Phenylmethyl sulfonyl fluoride (PMSF) is an example of such a compound. Being a serine/cysteine protease inhibitor, PMSF protects the animal

by a variety of cellular repair and regeneration processes in many tissues (Johnson, 1970; Baker *et al.*, 1980; Caroldi *et al.*, 1984; Veronesi and Padilla, 1985; Bond and Butler, 1987; Pope and Padilla, 1990; Pope *et al.*, 1992). Thus, PMSF pretreatment actually decreases the incidence of neuropathologic lesions.

The role of NTE in OPIDN is still questioned since NTE can be inhibited in non-sensitive species. Rats are less susceptible to OPIDN compounds (Barnes and Denz, 1953; Abou-Donia, 1981; Sokmuti *et al.*, 1988) presumably because of less NTE activity. In addition, inhibited NTE activity in rats is reported to return to normal faster than in hens after an OP-induced suppression of NTE activity (Carrington and Abou-Donia, 1984). But continuous suppression of NTE activity may produce more severe neurologic damage in rats (Majno and Karnovsky, 1961). Despite the equivocal relationship between NTE inhibition and the development of OPIDN, measuring the degree of NTE inhibition is valuable because it helps in assessing potential neurotoxicity of suspect chemicals and ultimately in evaluating the hazards associated with the use of such materials (Johnson, 1977; Lotti and Johnson, 1978).

Type I OPIDN

Type I compounds cause NTE inhibition which can persist for a number of days. A dose of approximately 1200 mg TOTP/kg body weight inhibited brain NTE and sciatic nerve NTE

completely in chickens and this complete inhibition persisted for 21 days (Abou-Donia and Lapadula, 1990). A dose of 1 mg DFP/kg body weight caused 96 % brain NTE inhibition in chickens initially, followed by a progressive increase in activity (Tanaka *et al.*, 1990a). TOTP at a dose of 1000 mg/kg body weight caused significant brain NTE inhibition of over 85 % in pheasants and chickens (Bursian *et al.*, 1983). Significant brain NTE inhibition by TOTP was also reported for turkeys (Larsen *et al.*, 1986).

There are 2 exceptions to the suggestion made by Johnson (1969a,b,c, 1977) that 70 - 80 % of NTE inhibition is needed for Type I OPIDN clinical signs to develop. NTE can be inhibited in excess of 70 % in non-sensitive species, but OPIDN doesn't develop. For example, TOTP at a dose of 1000 mg/kg body weight caused over 85 % brain NTE inhibition in Japanese quail and Bobwhite quail but OPIDN clinical signs did not develop (Bursian *et al.*, 1983). In contrast, the ferret is a sensitive species in terms of Type I OPIDN, but brain NTE in this species is inhibited by considerably less than 70 %. A dermal dose of 1000 mg TOTP/kg body weight in ferrets caused 46 % brain NTE inhibition whereas an oral dose caused 37 % inhibition (Stumpf *et al.*, 1989). Ferrets injected with 4 mg DFP/kg body weight had 42 % brain NTE inhibition (Tanaka *et al.*, 1991). In all these cases, animals subsequently developed clinical signs typical of OPIDN.

Type II OPIDN

Brain NTE must be inhibited by 70 % to produce Type II OPIDN in chickens (Carrington and Abou-Donia, 1988a). Hens injected with 1000 mg TPP/kg body weight showed an initial brain NTE inhibition of 85 % which recovered to 73 % inhibition over a 21 day period (Tanaka *et al.*, 1992a).

Conversely, brain NTE inhibition of 39 % was reported in rats following an injection of 1184 mg TPP/kg body weight (Veronesi *et al.*, 1986a) while rats treated with 1164 mg TPP/kg body weight had brain NTE inhibition of 30 % (Padilla et al., 1987).

AGE SENSITIVITY

Studies have shown that young animals of certain sensitive species are resistant to OPIDN (Barnes and Denz, 1953; Smith and Spalding, 1959; Taylor, 1967; Johnson and Barnes, 1970; Goldstein *et al.*, 1988; Olson and Bursian, 1988; Katoh *et al.*, 1990; Peraica *et al.*, 1993). A single dose of 50 mg TOTP/kg body weight did not cause delayed neurotoxicity in very young chicks and the age at which chicks are sensitive to TOTP was determined to be approximately 60 days (Barnes and Denz, 1953; Aldridge and Barnes, 1966; Abou-Donia *et al.*, 1982). Single doses of DFP ranging from 2 to 5 mg/kg body weight also did not cause delayed neurotoxicity in 7 to 49-day old chicks (Johnson and Barnes, 1970). Studies by Pope *et al.*

(1992) and Peraica *et al.* (1993) showed that 40-day-old chicks treated with 1 mg DFP/kg body weight displayed few motor deficits and the severity of clinical signs increased as the age at dosing increased. The clinical picture in chicks after a single dose of 5 mg 2, 2-dichlorovinyl dibutyl phosphate (DBDCVP)/kg body weight was different from that usually seen in the hen in that spasticity and complete recovery occurred in the chicks, but not in adults (Peraica *et al.*, 1993).

This age susceptibility to OPs and the ability to withstand neurotoxicant insult by young chickens was earlier hypothesized by Bondy *et al.* (1961), Johnson and Barnes (1970), Konno and Kinebuchi (1978), Abou-Donia *et al.* (1982) and Katoh *et al.* (1990). It is believed that this lack of sensitivity could be due to a failure in the absorption of the neurotoxicant (Metcalf, 1984) or it could be due to a faster elimination rate of the neurotoxicant in young animals. Abou-Donia (1981) showed that EPN was eliminated 6 times faster in 1-week-old chicks than in adult hens. EPN was noted in the brain, spinal cord, sciatic nerve, kidneys, and plasma of the adult hens while only polar breakdown products were seen in the tissues of chicks. Similarly, leptophos decayed at a faster rate in 6-month-old chickens than in adult hens. Thus, clinical signs were apparent only in the adult birds (Konno and Kinebuchi, 1978). Histopathological examination of rats dosed with OP compounds showed that young animals had fewer lesions than adults (Johnson and Barnes, 1970).

On the other hand, when TPP was administered to 1-week-

old chicks, they developed clinical signs characteristic of OPIDN such as ataxia and paralysis as well as characteristic lesions in the CNS and PNS (Abou-Donia and Brown, 1990). It is apparent that the development of Type II OPIDN is not dependent on the age of the animal like Type I OPIDN is.

SPECIES SELECTIVITY

It was established at the turn of this century and quoted by Roger and Recordier in 1934, that humans are susceptible to TOTP-induced delayed neurotoxicity (Abou-Donia and Lapadula, 1990). Later it was determined that not all animals are susceptible to OPIDN (Smith *et al.*, 1930a). Sensitive species include the water buffalo (Abou-Donia *et al.*, 1974), sheep, lambs (Draper *et al.*, 1952; Malone, 1964), pigs (Kruckenberg *et al.*, 1973), mallard ducklings (Herin *et al.*, 1978), pheasants (Johnson, 1975b; Bursian *et al.*, 1983), turkeys and chukar partridges (Baron, 1981). Some rat strains, like the Long-Evans, Sprague-Dawley and Fischer 344, were resistant to TOTP-induced OPIDN based on lack of clinical signs (Veronesi and Abou-Donia, 1982; Veronesi, 1984; Somkuti *et al.*, 1988). TOTP does not cause any clinical signs in Japanese quail or Bobwhite quail though NTE inhibition is more than 70 % (Francis *et al.*, 1980; Baron, 1981; Bursian *et al.*, 1983).

The kinetic steps involved in the toxic disposition of the chemical in the animal may account for the difference in species susceptibility. These steps are absorption,

distribution, metabolic transformation and elimination of the OP compound from the body. The atypical response by certain rodents suggests that the difference in species selectivity is not due to absorption, distribution and metabolism. TOTP is rapidly absorbed and metabolized in rats to an OP ester which is neurotoxic in susceptible species (Casida, 1961; Baron *et al.*, 1962).

Differential species sensitivity to Type II OPIDN has not been studied extensively. TPP does cause OPIDN in chickens, rats, ferrets, cats and monkeys (Carrington *et al.*, 1988a,b; Katoh *et al.*, 1990; Veronesi *et al.*, 1986a; Padilla *et al.*, 1987; Tanaka *et al.*, 1992a,b). Rats are not sensitive to Type I compounds, but they are to Type II compounds. This is the rationale for determining if Japanese quail are sensitive to TPP since they are resistant to the effects of Type I OPIDN compounds.

MATERIALS AND METHODS

TEST SPECIES AND HUSBANDRY

Adult Japanese quail *(Coturnix coturnix japonica)* were housed at the Michigan State University Poultry Science Teaching and Research Center in cages measuring 53 cm long X 26.5 cm wide X 26.5 cm high in groups of 5 starting one week prior to the beginning of the trial. Birds were provided with quail breeder ration and water *ad libitum*. Temperature and humidity of the room were ambient and the photoperiod was maintained at 24 hr light : 0 hr dark.

TREATMENT

Seventy birds were allocated into 4 TPP dose groups of 10 birds each, a negative control group (TOTP) of 10 birds, and a control group of 20 birds which received the corn oil vehicle. The TPP solution was prepared by mixing the chemical (97 % pure, Aldrich Chemical Co., Milwaukee, WI) in corn oil to give doses of 62.5, 125, 250 and 500 mg/kg body weight in an injection volume of 1 ml/kg body weight. The solution was prepared just prior to administration. TPP was injected subcutaneously over the breast region of each bird after recording the weight. TOTP (90 % pure, Aldrich Chemical Co., Milwaukee, WI) was prepared in a similar fashion at a dose of 500 mg/kg body weight. TOTP was administered orally at 1

ml/kg body weight. This dose was chosen because it is routinely used as a neurotoxic dose in chicken OPIDN studies. There were no restrictions on the intake of feed or water prior to dosing.

NEUROTOXIC ESTERASE ASSAY

At 24 hr after dosing, half of the birds (5 birds in each of the 4 TPP groups, 5 TOTP-treated birds and 10 control birds) were killed by cervical dislocation. The brains were rapidly removed, weighed and kept frozen at -30°C for subsequent determination of neurotoxic esterase activity (Johnson, 1977).

CLINICAL OBSERVATIONS

The remaining birds in each treatment group were observed daily for development of clinical signs characteristic of OPIDN beginning 24 hours after dosing and continuing for up to 21 days post-treatment. Assessment of clinical signs was based on a 4-point scale where 0 indicates no ataxia, 1 indicates mild ataxia, 2 indicates that the bird has difficulty standing upright and moves primarily on its hocks, 3 indicates that the bird cannot stand, but can sit upright and moves primarily by wing action and 4 indicates that the bird is unable to sit upright and cannot move. Moribund birds were killed to eliminate unnecessary suffering.

NEUROPATHOLOGICAL ASSESSMENT

To determine the extent of degeneration in the central nervous system, some birds from each dose group were taken at various times during the 21 day observation period for neuropathological assessment. The birds were anesthetized deeply with sodium phenobarbital (150 ml/kg body weight) and perfused transcardially with 10 % formalin-saline solution. The brains were removed, placed in a 10 % sucrose-formalin solution for a week, frozen, and cut in the coronal or sagittal plane at a thickness of 40 μm. Every tenth section was processed using the Fink-Heimer silver impregnation method for degenerating axons and terminals. Cresyl violet was used to stain adjacent sections to delineate nuclear boundaries.

Selected adjacent Fink-Heimer and cresyl violet stained sections were examined carefully using a standard compound microscope and areas containing axonal and terminal degeneration were noted.

STATISTICAL ANALYSIS

Mean whole-brain NTE activities of each treatment group were compared to the mean control whole-brain NTE activity using Dunnet's test (Gill, 1978). Statements of significance are based on $p < 0.05$.

ANIMAL USE AND CARE

All animal-related aspects of this study were approved by the Michigan State University All University Committee on Animal Use and Care.

RESULTS

CLINICAL SIGNS

The development of clinical signs typical of OPIDN was assessed beginning on day 1 post-treatment as some birds started showing signs within the first 24 hours (Table 1). One of the 5 Japanese quail treated with 62.5 mg TPP/kg body weight appeared ataxic beginning on day 3 post-dosing. One of the affected birds was unable to stand by day 6 and used its wings for movement while the other bird exhibited stiff-legged movement by day 8 post-dosing.

One bird dosed with 125 mg TPP/kg body weight had rigidity of the legs beginning at day 3 post-dosing. By day 5, the bird was moving on its hocks and experienced difficulty in maintaining its balance. The other 2 affected birds in this group began showing signs of mild ataxia on days 5 and 6. One of the these 2 birds later experienced difficulty in standing. The clinical condition of these 3 birds became more severe in subsequent days before they were taken for neuropathological assessment at day 9 post-dosing.

In birds injected with 250 mg TPP/kg body weight, more extensive clinical signs were initially noted on day 1 post-dosing in 3 of the birds. By day 5 post-treatment, 4 of the 5 birds were unable to sit or stand upright and were moving on their abdomen while the 5th bird could move only by using its wings. One of these birds died on day 6 displaying severe

TABLE 1. The effects of TPP and TOTP on the development of clinical signs* characteristic of OPIDN in adult Japanese quail.

		Days Post-Dosing										
ID#	Dose**	1	2	3	4	5	6	7	8	9	---	21
471		0	0	0	0	0	0	0	0	0	---	0
472		0	0	0	0	0	0	0	0	0	---	0
473		0	0	0	0	0	0	0	0	0	---	0
474		0	0	0	0	0	0	0	0	0	---	0
485	Control	0	0	0	0	0	0	0	0	0	---	0
5801		0	0	0	0	0	0	0	0	0	---	0^P
5802		0	0	0	0	0	0	0	0	0	---	0^P
5803		0	0	0	0	0	0	0	0	0	---	0
5804		0	0	0	0	0	0	0	0	0	---	0^P
5805		0	0	0	0	0	0	0	0	0	---	0
556		0	0	1	1	1	2^P					
557		0	0	0	0	1	1	1	1^P			
558	62.5 TPP	0	0	0	0	0	0	0	0	0	---	0
559		0	0	0	0	0	0	0	0	0	---	0
560		0	0	0	0	0	0	0	0	0	---	0
566		0	0	0	0	0	1	2	2	2^P		
567		0	0	1	1	2	2	3	3	3^P		
568	125 TPP	0	0	0	0	0	0	0	0	0^P		
569		0	0	0	0	0	0	0	0	0	---	0
570		0	0	0	0	1	1	2	2	2^P		
571		2	3	4	4	4	4^P					
572		0	0	0	1	2	3^P					
573	250 TPP	1	2	3	3	4	4^P					
574		2	3	4	4	4	D					
575		0	1	2	2	3	4^P					
512		0	0	1	3	4	4	D				
513		3	3	4^P								
547	500 TPP	3	3	4^P								
548		1	1	1^P								
586		1	1	3^P								
581		0	0	0	0	0	0	0	0	0	---	0^P
582		0	0	0	0	0	0	0	0	0	---	0^P
583	500 TOTP	0	0	0	0	0	0	0	0	0	---	0^P
584		0	0	0	0	0	0	0	0	0	---	0^P
585		0	0	0	0	0	0	0	0	0	---	0^P

* Clinical scores were assigned as follows: 0 = no ataxia; 1 = mild ataxia; 2 = has difficulty standing upright, moves on hocks; 3 = cannot stand upright but can sit upright and moves primarily by wing action; 4 = unable to sit upright and cannot move.

** Doses are expressed as mg TPP or TOTP/kg body weight.

P Denotes the bird was perfused on the designated day.

D Denotes the bird died.

paralysis.

Clinical signs in 4 of the 5 birds treated with 500 mg TPP/kg body weight were apparent within 24 hours and became increasingly severe in the subsequent 48 hours. The birds were unable to sit or stand by day 3 and they were perfused. The other bird in this group started showing mild ataxia on day 3 and died on day 6 displaying severe paralysis.

The 5 birds dosed with 500 mg TOTP/kg body weight did not show clinical signs characteristic of OPIDN during the 21 day observation period. They were indistinguishable from the control group in terms of mobility.

NTE ASSAY

The effects of TPP and TOTP on whole-brain neurotoxic esterase activity in adult Japanese quail 24 hours post-dosing are presented in Table 2. TPP-induced NTE inhibition ranged from 11 % (62.5 mg TPP/kg body weight) to 87 % (500 mg TPP/kg body weight). Birds receiving 500 mg TOTP/kg body weight had whole-brain NTE activity inhibited by 90 %.

TABLE 2. The effects of TPP and TOTP on whole-brain neurotoxic esterase (NTE) activity in Japanese quail 24 hours post-administration.

Dose*	NTE Activity**	% Inhibition
Control	1873 +/- 72	----
62.5 TPP	1668 +/- 78	11 %
125 TPP	1146 +/- 102***	39 %
250 TPP	512 +/- 47***	73 %
500 TPP	244 +/- 99***	87 %
500 TOTP	194 +/- 29***	90 %

* Doses are expressed as mg TPP or TOTP/kg body weight.

** NTE activity expressed as nmoles phenyl valerate hydrolyzed/hr/gram brain. Data expressed as mean +/- standard error. Sample size equals 5 in the TPP and TOTP groups and 10 in the control group.

*** Significantly different from control mean at $p < 0.05$

HISTOPATHOLOGICAL ASSESSMENT

Fink-Heimer silver impregnated degeneration appeared as black fragmented fibers against a yellow background. There was no evidence of degeneration in any of the nuclei or fiber tracts of brains from control birds (Figure 4a,c). Light degeneration was noted in the brains of TOTP-treated birds (Figure 7) and significant histopathological changes were noted in all the brains from TPP-treated birds (Figures 3a,b, 4b,d, 5 and 6). Axons were noted to be swollen and fragmented in the brain stem. The brains of Japanese quail perfused at a later stage during the course of clinical observations showed more severe and widespread degeneration than brains of birds perfused at an early stage.

Brainstem

The medulla and cerebellum showed axonal and terminal degeneration in the spinocerebellar tract, spinal lemniscus, reticular formation, ventral part of the medial longitudinal fasciculus and fascicles of cranial nerves IX and X. The gracile-cuneate, lateral cervical, external cuneate and lateral paragigantocellular reticular nuclei contained moderate to heavy terminal degeneration whereas parvocellular and gigantocellular reticular nuclei had light and scattered degeneration. Degenerating axons, degenerating terminals and degenerating cell bodies were noted in the lateral vestibular

Figure 3. Darkfield photomicrographs of parasagittal sections through the forebrain of the Japanese quail illustrating the dense axonal and terminal degeneration present in the lateral forebrain bundle (FPL), caudal neostriatum (NC), and ectostriatum (E) 9 days after a single subcutaneous injection of 125 mg TPP/kg body weight. Plate A illustrates the axonal degeneration passing dorsally and slightly caudally within the lateral forebrain bundle to enter the caudal neostriatum. Plate B illustrates the extremely dense axonal and terminal degeneration present in the ectostriatum. The section in Plate A corresponds to Level L3.5 and the section in Plate B corresponds to Level L5.75 in the pigeon brain atlas of Karten and Hodos (1967). Abbreviations: E, ectostriatum; FPL, lateral forebrain bundle; LMD, lamina medullaris dorsalis; NC, caudal neostriatum; NI, intermediate neostriatum; PA, paleostriatum augmentatum; PP, paleostriatum primitivum

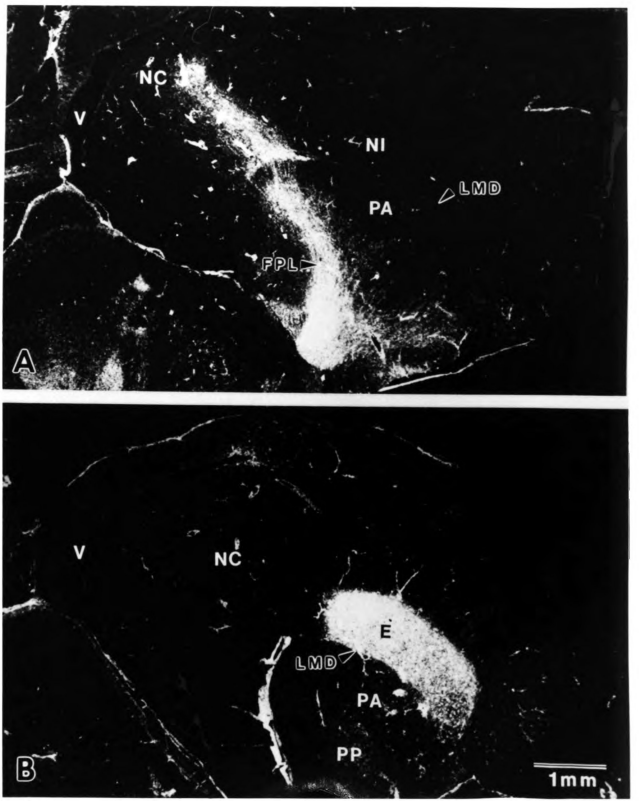
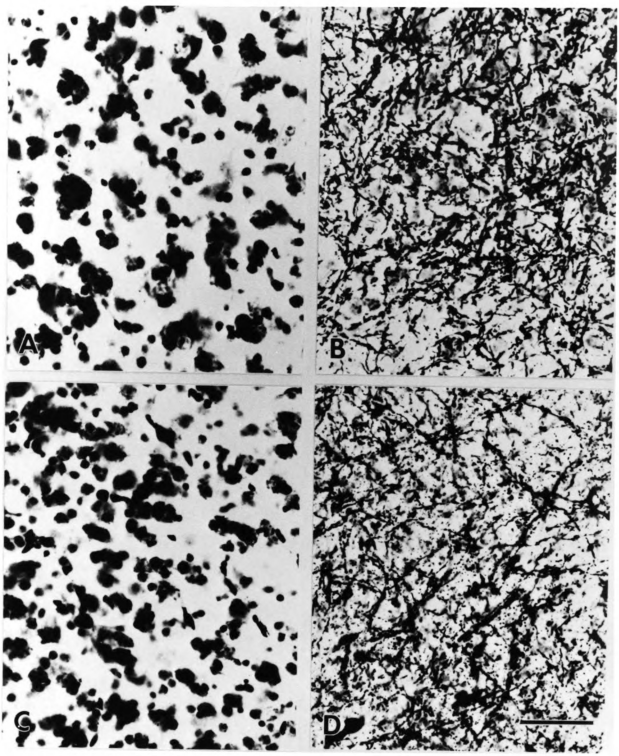

NC
V
NI
LMD
PA
FPL
A
V
NC
E
LMD
PA
PP
B
1mm

Figure 4. Photomicrographs illustrating control and TPP-exposed Fink-Heimer silver impregnated sections through the caudal neostriatum and ectostriatum. Plate A is from a section through the caudal neostriatum of a brain from a bird in the control group. Note the absence of fragmented fibers of particulate debris. Plate B illustrates a section through the neostriatum 7 days after an injection of 250 mg TPP/kg body weight. Noted the dense plexus of degenerating axons and terminals. Plate C is a control section through the ectostriatum. Note the absence of degenerating fibers or terminals. Plate D illustrates a section through the ectostriatum 7 days after an injection of 250 mg TPP/kg body weight. Note the large number of fragmented fibers and punctuate debris. Scale bar = 50 mm


A
B
C
D

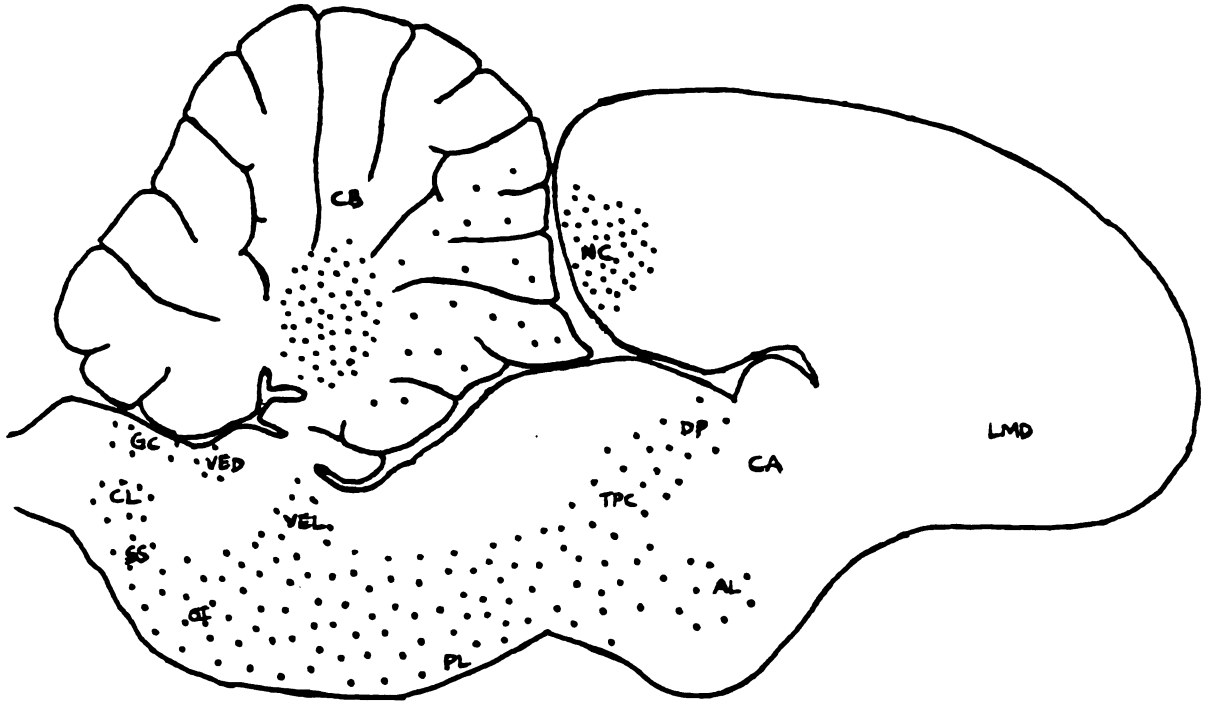


Figure 5. TPP-induced degeneration in the Japanese quail brain (sagittal median view)

AL Ansa Lenticularis
CB Cerebellum
CA Anterior Commissura
CL Lateral Cervical Nucleus
DP Dorso-intermediate Posterior Thalamic Nucleus
GC Gracile-Cuneate Nucleus
LMD Dorsal Medullary Lamina
NC Caudal Neostriatum
OI Inferior Olivary Nucleus
PL Lateral Pontine Nucleus
SS Supraspinal Nucleus
TPC Pedunculo-pontine Tegmental Nucleus, Pars Compacta
VeD Descending Vestibular Nucleus
VeL Lateral Vestibular Nucleus

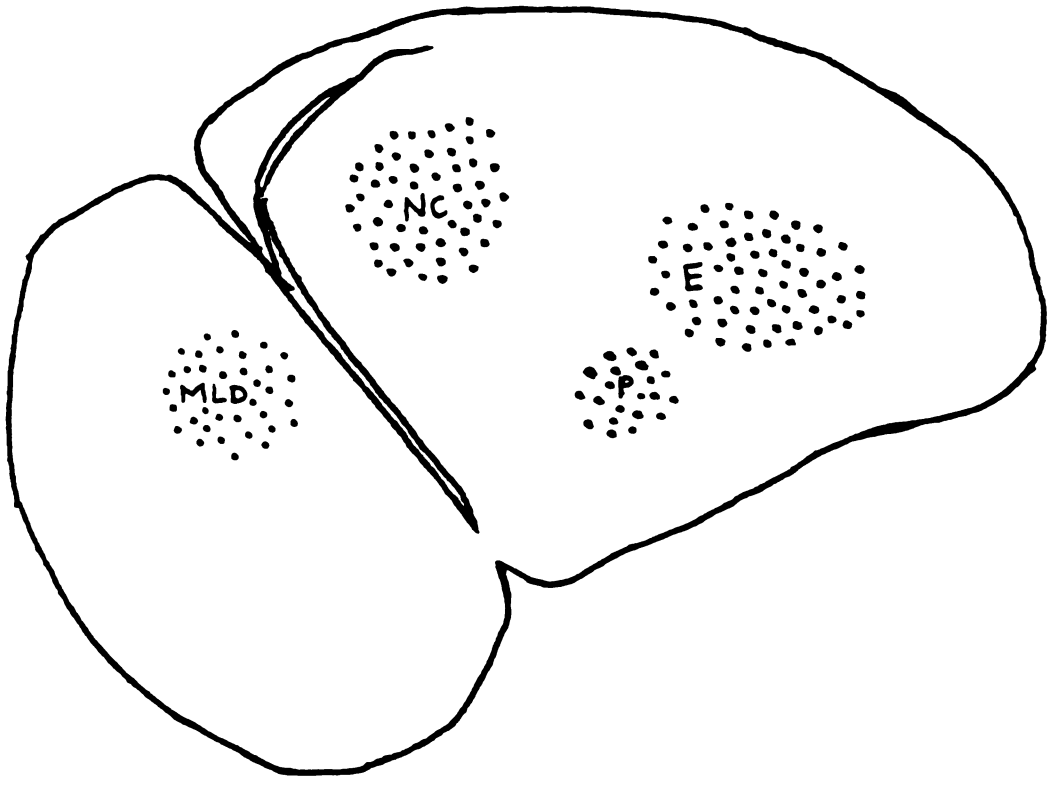


Figure 6. TPP-induced degeneration in the Japanese quail brain (sagittal lateral view)

E Ectostriatum
MLD Lateral Mesencephalic Nucleus, Pars Dorsalis
NC Caudal Neostriatum
P Paleostriatum

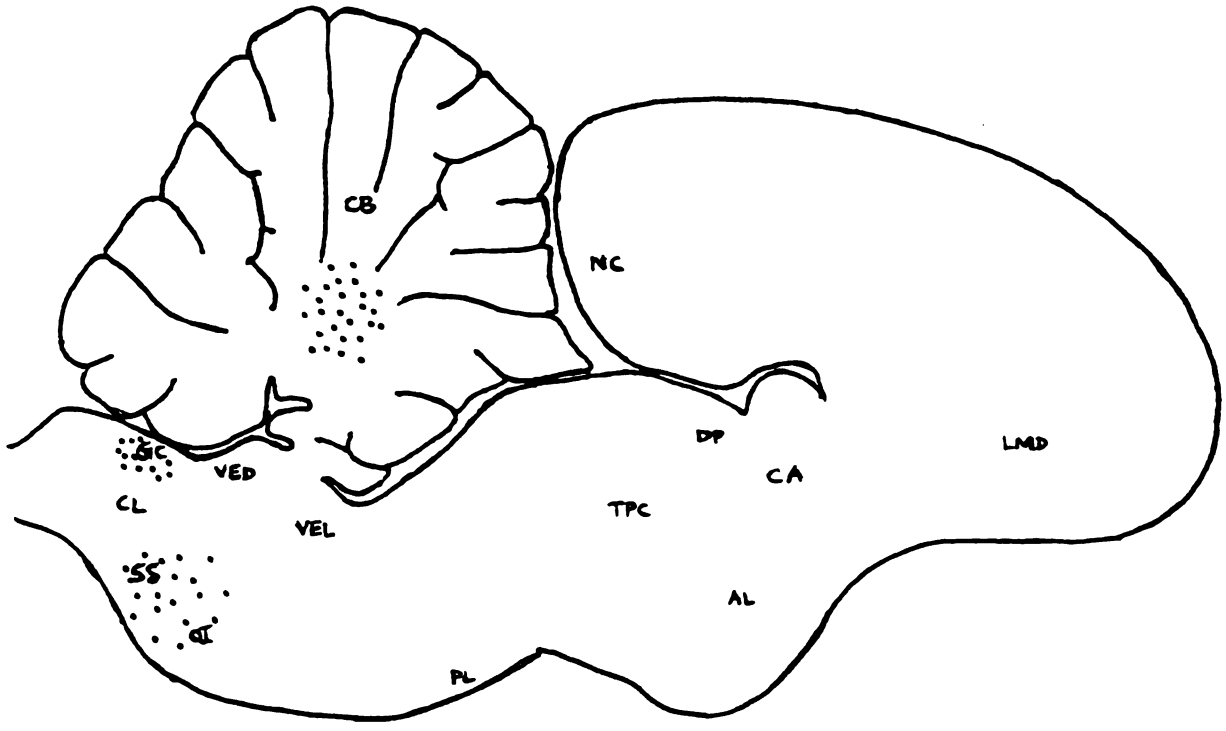


Figure 7. TOTP-induced degeneration in the Japanese quail brain (sagittal median view)

AL Ansa Lenticularis
CB Cerebellum
CA Anterior Commissura
CL Lateral Cervical Nucleus
DP Dorso-intermediate Posterior Thalamic Nucleus
GC Gracile-Cuneate Nucleus
LMD Dorsal Medullary Lamina
NC Caudal Neostriatum
OI Inferior Olivary Nucleus
PL Lateral Pontine Nucleus
SS Supraspinal Nucleus
TPC Pedunculo-pontine Tegmental Nucleus, Pars Compacta
VeD Descending Vestibular Nucleus
VeL Lateral Vestibular Nucleus

nucleus. The cerebellum showed degenerating spinocerebellar fibers. The deep nuclei in the cerebellum had light to moderate degeneration. The granule cell layers of foliae I - VI had heavy mossy fiber degeneration whereas lighter fiber degeneration was noted in the underlying white matter of foliae VII - IX.

Moderate to heavy degeneration was noted in several nuclei of the midbrain and forebrain. Degenerating fibers extended from the paleostriatum primitivum to the lateral forebrain bundle and ansa lenticularis. A group of fibers terminated at the dorso-intermediate posterior thalamic nucleus and pedunculopontine tegmental nucleus. Some fibers extended from the lateral spiriform nucleus into the deeper layers of the optic tectum.

Brain Auditory System :

The 3 principal auditory nuclei in the quail brain which showed degeneration were the lateral mesencephalic nucleus, pars dorsalis, the thalamic nucleus ovoidalis, and the neostriatum caudalis (Table 3 and Figure 6). Seven of the 14 quail examined had degeneration in all of the above 3 areas while 7 birds had degeneration in the thalamic nucleus ovoidalis and neostriatum caudalis. Degenerating fibers originated from the nucleus ovoidalis, and passed through the lateral forebrain bundle and dorsally and caudally into the neostriatum caudalis (Figure 3a). The nucleus ovoidalis

TABLE 3. The distribution of degeneration in the forebrain of the Japanese quail perfused at various times after TPP administration.

ID#	Day Post-dosing*	Dose**	(Auditory) MLD	OV	NC	(Visual) NR	ES	BSC
556	7	62.5	+	+	++	0	0	0
557	8	62.5	0	+	++	0	0	0
566	9	125	0	+	+	0	0	+
567	9	125	++	++	+++	++	+++	+++
568	9	125	0	+	+	0	0	0
570	9	125	+	++	+++	+	+++	0
571	7	250	+++	+++	+++	+++	+++	++
572	7	250	+	+++	+++	++	++	++
573	7	250	+++	+++	+++	++	+++	++
575	7	250	0	++	++	0	0	++
513	3	500	0	+++	++	++	+	0
547	3	500	0	+++	++	++	-	++
548	3	500	++	+++	++	+	+	0
586	3	500	0	+++	++	+++	0	++

* The day post-dosing that the bird was perfused.
** Doses are expressed as mg TPP/kg body weight.
\+ Light degeneration
++ Moderate degeneration
+++ Heavy degeneration
BSC Brachium of superior colliculus
ES Ectostriatum
MLD Lateral mesencephalic nucleus, pars dorsalis
NC Caudal neostriatum
NR Nucleus rotundus
OV nucleus ovoidalis

contained degenerating cell bodies in the form of black-impregnated cell somata in addition to degenerating axons and terminals. The degeneration in the neostriatum caudalis consisted of degenerating fibers primarily, and in some cases, degenerating cell somata. In a few cases, degeneration was noted in the deeper parts of the lateral mesencephalic nucleus. In one case, axonal degeneration was also noted in the nucleus angularis, a nucleus equivalent to the mammalian cochlear nucleus.

Brain Visual System :

Eleven of the 14 quail examined had degeneration in 3 principal nuclei of the visual system, namely, the ectostriatum, the nucleus rotundus, and the brachium of superior colliculus (Table 3 and Figure 6). Degeneration was noted in all the 3 areas in 4 birds while 5 birds had degeneration in only 2 areas. The brachium of superior colliculus was the sole site of degeneration in 1 bird. The majority of cases studied showed the nucleus rotundus with moderate to heavy degeneration. In a few cases, black silver impregnated cell bodies indicating somatic degeneration were also present. Degeneration in the ectostriatum, though inconsistent, encompassed the major portion of the nuclear region (Figures 3b and 6). The brachium of the superior colliculus also showed fiber degeneration which was particularly evident in the portion of the brachium

encompassing the pretectal nucleus before terminating in the nucleus rotundus. A few degenerating fibers were also noted within the pretectal nucleus. The optic tectum did not contain any axonal or somatic degeneration.

DISCUSSION

The data obtained from this study indicate that the Japanese quail, which has been reported to be resistant to the effects of the Type I OPIDN compound TOTP (Francis *et al.*, 1980; Bursian *et al.*, 1983), is susceptible to the Type II delayed neurotoxicant TPP. This was apparent in terms of clinical signs characteristic of OPIDN, whole-brain NTE inhibition, and central nervous system degeneration.

CLINICAL SIGNS

Observation of clinical signs started from day 1 post-treatment as signs were evident immediately at the higher doses (Table 1). There was a positive relationship between the TPP dose and the severity of the clinical signs exhibited by the Japanese quail. Birds dosed with 62.5 mg TPP/kg body weight showed only mild ataxia. Four of the birds were able to stand upright. The birds in the 125 mg TPP/kg body weight group were more ataxic. This was evident by the fact that these birds moved primarily on their hocks. In the 250 mg TPP/kg body weight dose group, the birds displayed an inability to maintain balance. The 500 mg TPP/kg body weight dose group birds showed signs similar to those in the 250 dose group, but clinical signs in the former group were evident earlier.

There was also a positive relationship between the TPP

dose and the time of onset of clinical signs in that clinical signs were evident earlier as the dose increased. The onset of clinical signs occurred at day 3 post-dosing for the 62.5 and 125 TPP birds and as early as day 1 post-dosing for the 250 and 500 TPP birds. The birds in 500 TPP group were unable to stand on day 1. There was also a positive relationship between TPP dose and the number of birds affected. The percentage of birds affected was 40 % in the 62.5 TPP group, 60 % in the 125 TPP group and 100 % in the 250 and 500 TPP groups.

Day 1 through day 9 seems to be the time period in which Japanese quail show clinical signs as no bird started to show signs after that period. This suggests that if an individual quail is susceptible to TPP, it will show signs prior to day 10.

Previous studies reported that the Japanese quail is not sensitive to Type I OPIDN compounds like TOTP (Stuart and Oehme, 1982; Francis *et al.*, 1980; Bursian *et al.*, 1983) based on the absence of clinical signs after doses as high as 1000 mg TOTP/kg body weight. In this study, none of the 5 birds dosed with 500 mg TOTP/kg body weight showed clinical signs typical of OPIDN during the 21 day period. Earlier studies have suggested that differences in absorption, metabolism, and elimination of the neurotoxicant may play an important role in species sensitivity to neurotoxic agents (Smith *et al.*, 1932; Holmstead *et al.*, 1973; Whitacre *et al.*, 1976; Abou-Donia, 1976; Baron, 1981; Chadwick *et al.*, 1982; Abou-Donia, 1983;

Abou-Donia and Nomeir, 1986). Abou-Donia and Nomeir (1986) concluded that the rates of clearance of the delayed neurotoxicants EPN and leptophos in insensitive species were faster than in sensitive species. Lasker *et al.* (1982) studied the *in vitro* metabolism of the Type I OPIDN compound EPN and its oxygen-analog EPNO by rat and chicken microsomal enzymes. The formation of EPNO (active metabolite) and PNP (*p*-nitrophenol) (inactive metabolite) from EPN metabolism was 4.6 and 19.4 times higher in the rat than in the chicken, respectively. In another study with EPN, Abou-Donia *et al.* (1983a,b) concluded that the neurotoxicant remained in neural tissues for a longer time in sensitive species allowing continuous transfer of the compound and its metabolite to the site of action. A possible explanation of why the Japanese quail is resistant to TOTP is that this species may have a faster rate of clearance of TOTP and/or a greater repair capability in the insulted physiological system compared to sensitive species.

Assessment of clinical signs also indicates that the Japanese quail respond with a shorter delay than the usual avian model, the domestic hen. In the present study, Japanese quail in the 2 higher dose groups displayed OPIDN clinical signs within 24 hours of dosing whereas birds in the lower 2 dose groups showed clinical signs by day 3. Previous studies have shown that chickens dosed with TPP first started showing signs between day 5 and day 14 post-dosing (Konno *et al.*, 1989; Katoh *et al.*, 1990; Tanaka *et al.*, 1992). Mammalian

models are similar to the hen in regard to the latent period. Rats treated with 1184 mg TPP/kg body weight developed hindleg ataxia within a week followed by paralysis (Veronesi *et al.*, 1986a; Veronesi and Dvergsten, 1987). Ferrets dosed with 500, 1000 and 2000 mg TPP/kg body weight show clinical signs of ataxia as early as day 4 and paralysis by day 8 (Tanaka *et al.*, 1990).

Not only is the delay period shorter in Japanese quail, but the transition from mild ataxia to severe paralysis occurs more rapidly in Japanese quail than in chickens. Quail treated with 250 and 500 mg TPP/kg body weight deteriorated within 2 days after initial signs were observed. Hens treated with 1000 mg TPP/kg body weight took 7 days to deteriorate after the onset of clinical signs (Konno *et al.*, 1989; Tanaka *et al.*, 1992).

The types of clinical signs noted in the Japanese quail are different from those noted in the hen. Once affected by OPIDN, the hen can stand for a longer period of time whereas the quail tend to sit. Mortality in the hen is usually noted much later than in Japanese quail, if at all. Three hens dosed with 140 mg TPP/kg body weight died at days 16 - 22 post-dosing (Konno *et al.*, 1989), whereas in this study, Japanese quail died as early as day 6 and 7 post-dosing and some probably would have died sooner than day 6 or 7 post-dosing if they weren't taken for neuropathological assessment. Comparison of TPP-induced neuropathological damage in avian species have shown differences in the location of lesions in

the brains of chickens and Japanese quail. The present study has indicated lesions in the neostriatum of the Japanese quail forebrain. Salzen and Parker (1975) have suggested that damage to the neostriatum causes impairment of fine movements such as are involved in feeding and visual discrimination. Thus, TPP-exposed Japanese quail may have trouble locating feed and eating. Since the metabolism of the Japanese quail is relatively high, it is possible that any impairment that affects the bird's ability to eat is going to be more detrimental to the quail than to the chicken. Thus, neostriatal damage in the Japanese quail may be contributing to its early mortality when compared to the chicken. Another possible explanation may be that the higher metabolic rate of the Japanese quail might somehow enhance the rapid distribution of the toxicant to the nervous system.

The development of TPP-induced OPIDN in the Japanese quail occurs at much lower doses when compared to other species. A study by Konno *et al.* (1989) showed that leg paralysis could not be produced in adult chickens by a single dose of 100 or 500 mg TPP/kg body weight which agreed with earlier study by Smith *et al.* (1933). Ferrets were administered 500 mg TPP/kg body weight in order to produce clinical signs (Tanaka *et al.*, 1990). Rats showed clinical signs after a dose of 1184 mg TPP/kg body weight (Veronesi *et al.*, 1986a; Veronesi and Dvergsten, 1987). However, in Japanese quail, a single dose of 62.5 mg TPP/kg body weight caused mild ataxia in 2 of the 5 birds.

NTE ASSAY

TPP doses as low as 125 mg/kg body weight caused significant inhibition of whole-brain NTE activity in the Japanese quail (Table 2). These results agree with previous studies done in the hen and rat (Veronesi *et al.*, 1986a; Padilla *et al.*, 1987; Tanaka *et al.*, 1992; Knoth-Anderson and Abou-Donia, 1993) in that TPP inhibits whole-brain NTE activity. However, the threshold NTE inhibition required to cause clinical signs may vary among species.

The percent inhibition of NTE is related to the dose of TPP administered in that higher doses result in greater inhibition. The percent inhibition is also related to the severity of clinical signs noted. An 11 % NTE inhibition by 62.5 mg TPP/kg body weight resulted in mild ataxia whereas an 87 % NTE inhibition by 500 mg TPP/kg body weight was related to severe paralysis which developed very quickly after dosing.

For Type I compounds, it is generally assumed that NTE must be inhibited by at least 70 % for clinical signs and neuropathy to develop. In this study, OPIDN developed when NTE inhibition was only 11 %. Similar effects in rats have been reported in that TPP caused NTE inhibition of approximately 40 %, yet clinical signs and neuropathy developed (Veronesi *et al.*, 1986a; Padilla *et al.*, 1987). Conversely, TOTP caused threshold inhibition of NTE in Japanese quail yet did not cause OPIDN. The latter agrees with a previous study by Bursian *et al.* (1983). In that

study, Japanese quail were administered TOTP at doses of 125, 250, 500 and 1000 mg/kg body weight, doses sufficient to cause threshold inhibition of whole-brain NTE, yet clinical signs did not develop. The reason why TOTP causes significant NTE inhibition yet doesn't cause clinical signs is unknown.

Johnson (1976) stated that the NTE assay can be used as a tool to determine the neurotoxic potential of OPIDN compounds in that there is a relationship between NTE inhibition in excess of 70 % and the development of clinical signs. The present study indicates that these rules do not apply to Japanese quail.

NEUROPATHOLOGICAL STUDIES

TPP doses as low as 62.5 mg/kg body weight caused histopathological changes in the CNS of Japanese quail. The fact that TPP induces CNS damage in quail agrees with previous studies on the effects of TPP on the CNS of chickens, rats, and ferrets (Veronesi *et al.*, 1986a; Padilla *et al.*, 1987; Veronesi and Dvergsten, 1987; Carrington *et al.*, 1988; Tanaka *et al.*, 1990, 1992), although the minimum dose needed to cause degeneration varied among species.

The present study shows that the Japanese quail is very sensitive to TPP which is indicated by the presence of widespread lesions in the cerebellum, medulla, midbrain and forebrain (Figures 5 and 6). The spinocerebellar tract, spinal lemniscus, reticular formation, ventral part of the

medial longitudinal fasciculus and fascicles of cranial nerves IX and X had axonal and terminal degeneration. Moderate to severe degeneration was noted in the gracile-cuneate, lateral cervical, external cuneate and lateral paragigantocellular reticular nuclei. Parvocellular and gigantocellular reticular nuclei had light and scattered degeneration. The nucleus cerebellaris internus in the cerebellum showed moderate degeneration. Degenerating fibers were noted in the forebrain and midbrain, in the paleostriatum primitivum, lateral forebrain bundle, ansa lenticularis, dorso-intermediate posterior thalamic nucleus, pedunculopontine tegmental nucleus, and lateral spiriform nucleus. The brain auditory and visual systems in the Japanese quail were also affected. Lesions were noted in the lateral mesencephalic nucleus, pars dorsalis, thalamic nucleus ovoidalis, neostriatum caudalis, thalamic nucleus rotundus and ectostriatum.

The distribution of neuropathy in hens dosed with TPP (Figures 8 and 9) is similar to that noted in TPP-dosed Japanese quail. Slight to moderate degeneration was noted in hens dosed with 1000 mg TPP/kg body weight (Carrington *et al.*, 1988a; Tanaka *et al.*, 1992). Moderate degeneration was noted in the medulla in the lateral vestibular, gracile-cuneate, external cuneate and lateral cervical nuclei. Less degeneration was noted in the solitary, inferior olivary and raphae nuclei, in the medial and dorsal vestibular nuclei, and in the lateral paragigantocellular, gigantocellular, and lateral reticular nuclei. In the cerebellum, mossy fiber

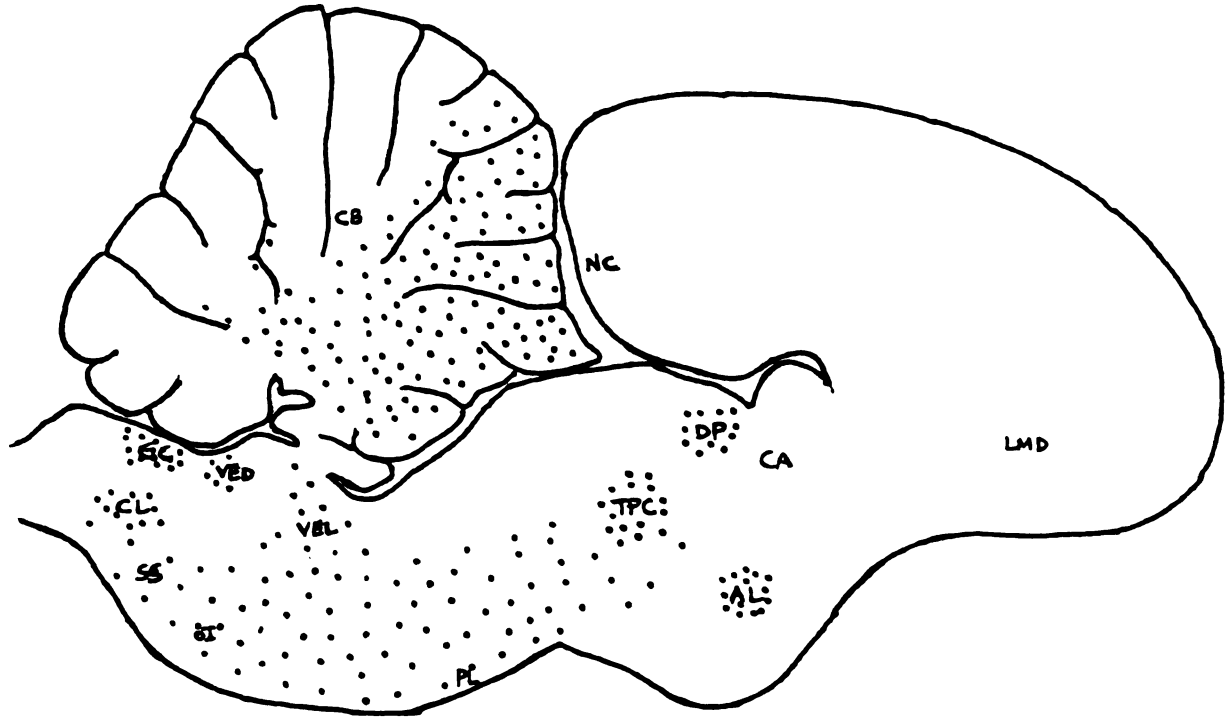


Figure 8. TPP-induced degeneration in the chicken brain (sagittal median view)

AL Ansa Lenticularis
CB Cerebellum
CA Anterior Commissura
CL Lateral Cervical Nucleus
DP Dorso-intermediate Posterior Thalamic Nucleus
GC Gracile-Cuneate Nucleus
LMD Dorsal Medullary Lamina
NC Caudal Neostriatum
OI Inferior Olivary Nucleus
PL Lateral Pontine Nucleus
SS Supraspinal Nucleus
TPC Pedunculo-pontine Tegmental Nucleus, Pars Compacta
VeD Descending Vestibular Nucleus
VeL Lateral Vestibular Nucleus

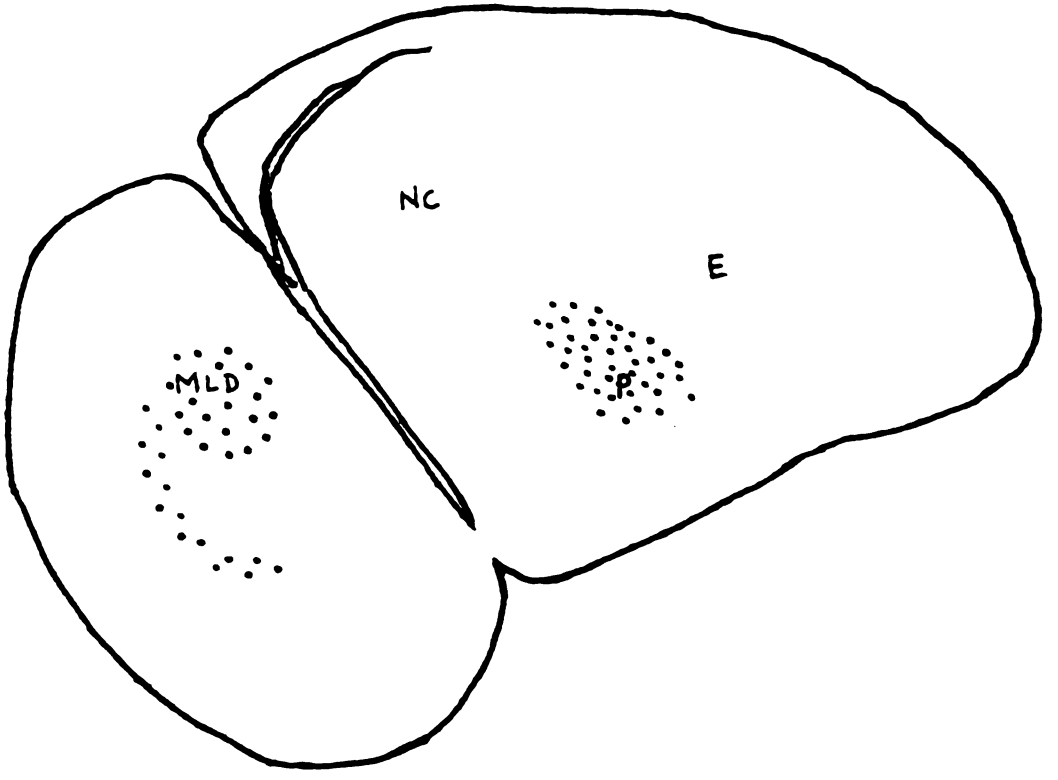


Figure 9. TPP-induced degeneration in the chicken brain (sagittal lateral view)

E Ectostriatum
MLD Lateral Mesencephalic Nucleus, Pars Dorsalis
NC Caudal Neostriatum
P Paleostriatum

degeneration was noted in the granular layer of foliae I - Vb, especially in foliae IV and V. The degenerating fibers were noted as alternating parasagittal bands of heavy and light degeneration. The deep cerebellar nuclei also had small amounts of coarse fiber degeneration. Unlike the Japanese quail, no lesions were found in the auditory and visual systems of the chicken (Figures 8 and 9).

Despite the similarity in neuropathological changes, neuropathy in the Japanese quail was detected earlier and at lower doses than in the hen. Swollen axons are present in the brainstem of hens dosed with 1000 mg TPP/kg body weight at day 7 post-dosing (Carrington *et al.*, 1988) whereas Japanese quail dosed with 250 and 500 mg TPP/kg body weight in the present study showed degeneration on day 3 post-dosing. Some birds dosed with 500 mg TPP/kg body weight showed clinical signs as early as day 1 post-dosing. Although birds were not taken for perfusion until day 3, it can be speculated that degeneration is present in the CNS of the Japanese quail on day 1 post-dosing. The earlier onset of TPP-induced neuropathological lesions in Japanese quail in this study may be due to a more rapid distribution of TPP in the Japanese quail.

Differences in the location of degeneration may contribute to the severity of clinical signs in different species. Studies by Karten (1967, 1968) and Boord (1969) showed that the pathway of the auditory system in birds begins at the nucleus angularis and continues through the lateral mesencephalic nucleus, pars dorsalis and nucleus ovoidalis to

terminate in the caudal neostriatum. Studies on TPP exposure in the chicken by Tanaka *et al*. (1992) showed that TPP caused limited degeneration in the forebrain, which included descending motor pathways originating in the basal ganglia and projecting to several midbrain and medullary nuclei. The midbrain lateral mesencephalic nucleus pars dorsalis contains degeneration whereas neither the nucleus ovoidalis nor the caudal neostriatum show any degeneration (Figures 8 and 9). The results of the present study in Japanese quail document for the first time that TPP exposure results in degeneration of the forebrain auditory and visual systems in an avian species. Degeneration was noted especially in the thalamic nucleus and neostriatum of the auditory pathway (Figure 6). The neostriatum is crucial for simultaneous visual discrimination learning, feeding behavior and bodily movements (Salzen and Parker, 1975).

As mentioned previously, fine adjustments involved in feeding and preening, like neck, limb, and bill movements, are controlled by sensorimotor coordinating circuits in the neostriatum. Damage to the neostriatum may cause difficulty and inaccuracy in pecking. In the present study, lesions were noted in the neostriatum region of the Japanese quail forebrain whereas no such lesions were noted in TPP-exposed chickens (Tanaka *et al*., 1992). It can be suggested that lesions in the neostriatum contributed to mortality of TPP-exposed Japanese quail as the birds face difficulty in locating and consuming feed.

Degeneration has been noted in the forebrain and midbrain motor nuclei and pathways of TPP-exposed mammals. Previous studies in mammals have reported extensive degeneration in the thalamus and cerebral cortex following exposure to TPP (Tanaka *et al.*, 1990, 1992a) and the organophosphate compound soman (McLeod *et al.*, 1984; Churchill *et al.*, 1985; Pazdernik *et al.*, 1985; Lallement *et al.*, 1993). Tanaka *et al.* (1990) reported widespread degeneration in ferrets dosed with 1000 mg TPP/kg body weight. Lesions were noted in the external cuneate nucleus, pontine gray, nucleus of the reticular formation, red nucleus, superior olivary nucleus, inferior colliculus, ventral lateral nucleus and the lateral geniculate nucleus.

Rats, like the Japanese quail, though resistant to TOTP-induced neuropathy, are sensitive to TPP (Lehning *et al.*, 1990; Tanaka *et al.*, 1992b). Large amounts of CNS degeneration were noted in rats administered 1184 mg TPP/kg body weight. Degeneration was noted in the sensorimotor cerebral cortex, auditory cortex, medial geniculate thalamic nucleus, ventral medial thalamic nucleus, substantia nigra, pars compacta and the deeper layers of the superior colliculus.

Though the Japanese quail is sensitive to TPP, the present study confirms the results from previous studies (Francis *et al.*, 1980; Bursian *et al.*, 1982) indicating that this species is resistant to TOTP-induced neuropathy. Widespread degeneration was absent in the brain of the

Japanese quail administered TOTP (Figure 7). Moderate degeneration was noted in nucleus cerebellaris internus in the cerebellum and gracile-cuneate nucleus in the medulla. Lighter amounts of lesions were noted in the medullary nucleus supraspinalis and inferior olivary nucleus.

Unlike the Japanese quail, the domestic chicken is very sensitive to TOTP. Neuropathological lesions were confined to the medulla and cerebellum in hens dosed with 500 mg TOTP/kg body weight (Tanaka and Bursian, 1989). Moderate amounts of degeneration were noted in the lateral vestibular, gracile, external cuneate and lateral cervical nuclei. Lesser amounts of degeneration were noted in the solitary nucleus, inferior olivary nucleus, and raphae nucleus, in the medial, descending and lateral vestibular nuclei, and in the lateral paragigantocellular, gigantocellular, and lateral reticular nuclei. Medullary portions of the dorsal and ventral spinocerebellar tracts and spinal lemniscus had fiber degeneration. Moderate amounts of degeneration were also noted in the deep cerebellar nuclei and granular layers of cerebellar folia I - V in the cerebellum.

Rats appear to be somewhat more resistant to TOTP-induced neuropathy than the Japanese quail. Rats dosed with 1160 mg TOTP/kg body weight had no neuropathological lesions in the brainstem region (Veronesi and Dvergsten, 1987). However, degeneration was noted in the fasciculus gracilis at the cervical level and dorsolateral columns at the lumbar levels in the spinal cord. The rat is an example of a mammalian

species similar to the Japanese quail in terms of resistance to Type I OPIDN.

The present histological examinations are consistent with previous studies which indicate that TPP causes OPIDN. The results indicate that TPP causes degeneration in the auditory and visual areas of the brain in addition to the forebrain, midbrain, cerebellum and medulla. The distribution of neuropathological lesions, however, indicates that the biochemical activity of TPP differs among species.

CONCLUSION

This study demonstrated that TPP is neurotoxic in the Japanese quail based on the development of clinical signs, whole-brain NTE inhibition and the presence of neuropathological lesions. The Japanese quail may serve as an excellent model for the study of TPP neurotoxic effects in avian species. The Japanese quail is perhaps a better model for Type II OPIDN studies than the hen as it appears to be very sensitive to TPP as indicated by the early onset of clinical signs, lower threshold dose for NTE inhibition, and the degeneration of many motor and sensory nuclei and pathways. It should also be noted that the Japanese quail is not a suitable model for Type I OPIDN as it is resistant to neurotoxic effects of TOTP indicated by the absence of clinical signs and widespread degeneration. The Japanese quail is similar to the rat in regard to sensitivity to Type I and Type II OPIDN. The distinctly different clinical, biochemical and neuropathological profiles produced by Type I and Type II OPIDN compounds in Japanese quail suggest that different mechanisms may be responsible for their neuropathological effects. The Japanese quail is an excellent avian model for studying the differences in Type I and Type II OPIDN mechanisms. The neuropathological lesions caused by TPP in Japanese quail are different from those in the hen suggesting further that the neurotoxic effects of the same chemical vary among species.

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