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MECHANISMS OF ORGANOPHOSPHORUS ESTER-INDUCED DELAYED NEUROTOXICITY: Type I and Type II

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INTRODUCTION

This review describes a group of organophosphorus compounds with delayed neurotoxic properties (1). Delayed neurotoxicity is a delayed onset of prolonged locomotor ataxia resulting from a single or repeated exposure to an organophosphorus compound (2, 3). For many years, this effect was wrongly termed "demyelinization" or "demyelinating disease" because of the early misinterpretation of pathological lesions as reflecting demyelination instead of being primary axonal degeneration followed by demyelination (4). Since 1978, this effect has been termed organophosphorus ester-induced delayed neurotoxicity or OPIDN (5). Although its major visible consequence is a motor dysfunction resulting from neuropathic lesions, there are other significant changes that preceed or accompany clinical manifestations. These changes are at various levels, i.e. molecular, neurochemical; cellular, neurophysiological; tissue, neuropathological; organism, functional, i.e. neurobehavioral and neurological. The term "neurotoxic" encompasses all these changes and is comprehensive enough to adequately define this effect.

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These compounds are grouped together because they exhibit certain common features (1):

1. They are organophosphorus compounds.
2. They are direct or indirect inhibitors of esterases.
3. They are characterized by a latent interval between the time of their administration and the onset of clinical signs.
4. They affect central and peripheral nervous systems.
5. They produce uniformally characteristic well-defined neuropathologic lesions that appear to have a direct relationship to the neurologic deficit produced.
6. Their effect is species specific.

Soon after the identification of tri-*o*-cresyl phosphate (TOCP; Table 1) as the agent causing delayed neurotoxicity in humans, known then as "Jake paralysis" (1930), it was recognized that this group or organophosphorus compounds produced two distinct delayed neurotoxic actions (6–8). However, only recently have studies in other laboratories (9–11) and ours (12–14) defined these effects. Because all these compounds fit the definition for producing delayed neurotoxicity (1,2), they are termed OPIDN and are designated into two classes: Type I and Type II, based on the following characteristics:

1. chemical structure,
2. species selectivity,
3. age sensitivity,
4. the length of latent period,
5. clinical signs,
6. morphology and distribution of neuropathologic lesions,
7. protection with phenylmethyl sulfonyl fluoride,
8. inhibition of neurotoxic esterase,
9. effect on catecholamine secretion from bovine adrenomedullary chromaffin cells.

CHARACTERISTICS OF OPIDN TYPE I AND TYPE II COMPOUNDS

Chemical Structure

Type 1 compounds have a pentavalent phosphorus atom, whereas Type II compounds have a trivalent phosphorus atom. Type I compounds include derivatives of phosphoric, phosphonic, and phosphoramidic acids, and phos-

Table 1 Chemical designations of organophosphorus esters mentioned in text

Compound	Chemical designation
Chlorpyriphos	*O,O*-Diethyl O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate
Coumaphos	*O,O*-Diethyl *O*-(3-chloro-4-methyl-7coumarinyl) phosphorothionate
DEF	*S,S,S*-Tri-*n*-butyl phosphorotrithioate
DFP	*O,O*-Diisopropyl phosphorofluoridate
Dichlorvos	2,2-Dichloroethenyl *O,O*-dimethyl phosphate
EPN	*O*-Ethyl *O*-4-nitrophenyl phenylphosphonothioate
Fenthion	*O,O*-Dimethyl *O*-4-methylthio-*m*-tolyl phosphorothioate
Leptophos	*O*-4-Bromo-2,5-dichlorophenyl *O*-methyl phenylphosphorothioate
Malathion	*O,O*-Diethyl (dimethoxyphosphinothioyl) thiobutanedithioate
Merphos	*S,S,S*-Tri-*n*-butylphosphorotrithioite
Methamidphos	*O,S*-Dimethyl phosphoramidothioate
Mipafox	*N,N'*-Diisopropylphosphorodiamidic fluoride
Omethoate	*O,O*-Dimethyl *S*-methylcarbamoxylmethyl phosphorothioate
Parathion	*O,O*-Diethyl *O*-4-nitrophenyl phosphate
TMCP	Tri-*meta*-tolyl phosphate
$TMCP_i$	Tri-*meta*-tolyl phosphite
TOCP	Tri-*ortho*-tolyl phosphate
$TOCP_i$	Tri-*ortho*-tolyl phosphite
TPCP	Tri-*para*-tolyl phosphate
$TPCP_i$	Tri-*para*-tolyl phosphite
TPP	Triphenyl phosphate
TPP_i	Triphenyl phosphite
Trichloronate	*O*-Ethyl *O*-2, 4,5-trichlorophenylethyl phosphorothioate
Trichlorphon	2,2,2-Trichloro-1-hydroxyethyl phosphonate

phorofluoridates. They also include sulfur analogs. Type II compounds are phosphorus-acid derivatives, i.e. triphosphites and presumably their sulfur analogs. TOCP and triphenyl phosphite (TPP_i; Figure 1) are representative of Types I and II, respectively.

CHEMISTRY OF ORGANOPHOSPHORUS COMPOUNDS Organic compounds containing phosphorus-carbon (P-C) bonds are termed organophosphorus compounds. The phosphorus atom has two paired (*3S*) and three unpaired (3P) electrons in the outer M shell (15). Phosphorus can be formally trivalent or pentavalent, using only three or five electrons, respectively. The trivalent phosphorus atom has a pyramidal configuration, whereas the pentavalent phosphorus atom has tetrahedral configuration. The trivalent pyramidal compounds can be considered derivatives of phosphine, PH_3. Replacement of hydrogen by hydroxyl group results in phosphinous acid, H_2POH; phosphonous acid, $HP(OH)_2$; and phosphorus acid, $P(OH)_3$. These compounds are, however, unstable pyramidal acids and undergo tauomeric change, via hydrogen transfer, to tetrahedral phosphine oxide, phosphinic

Figure 1 Type I Tri-*o*-cresyl phosphate (TOCP) and type II Triphenyl Phosphite (TPP_i).

acid (hypophosphorus acid), and phosphonic acid (phosphorus acid), respectively. Thus, the free phosphorus acid, monosubstituted ester, and disubstituted ester, that have a P-H bond, but not the stable triester, undergo tautomerization to the more stable corresponding pentavalent phosphorus-containing compounds.

In vivo, metabolic biotransformation of a subcutaneous dose in hens and in vitro stability studies of TPP_i using ^{31}P nuclear magnetic resonance (16) showed that TPP_i was hydrolyzed to diphenyl phosphite, which was rapidly tautomerized into diphenyl phosphonate via hydrogen transfer, as shown in Figure 2. Diphenyl phosphite is a free acid containing three covalent phosphorus that can exist only as a transitory species in concentration of 1 in 10^{12} (15).

CHEMICAL STRUCTURE-NEUROTOXIC EFFECT RELATIONSHIP There are chemical structure-neurotoxic activity differences between Type I and II. Although TPP_i and the three tricesyl phosphite isomers ($TOCP_i$, $TMCP_i$, and $TPCP_i$) are all neurotoxic (7), only TOCP of the corresponding phosphates causes OPIDN (1, 3, 17).

In the Type I class, the presence of the *ortho*-methyl group in the aromatic series seems to be essential for aromatic chemicals to be neurotoxic. This is presumably related to the metabolism of the *ortho*-methyl phenyl derivative to the saligenin *o*-tolyl cyclic phosphate (18–20), which is more potent

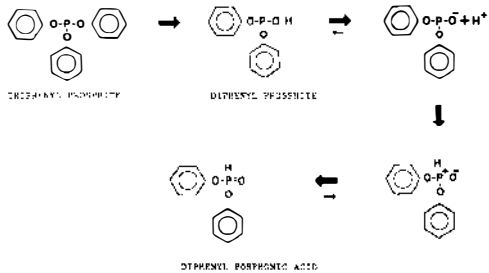


Figure 2 Hydrolysis of triphenyl phosphite to diphenyl phosphite and its tautomerization to diphenyl phosphonic acid.

neurotoxic metabolite (2, 3). Type II aromatic compounds do not need to be metabolized to produce their neurotoxicity. These results suggest that any aryl phosphite may cause Type II OPIDN.

REACTIVITY OF TYPE I AND II COMPOUNDS The chemical reactivity of organophosphorus compounds belonging to Types I and II OPIDN depends on the reactivity of the phosphorus atom. In Type I compounds the phosphorylating ability of the pentavalent phosphorus atom depends on its electrophilic character, which is determined largely by the groups attached to it. In Type II compounds the trivalent phosphorus atom has a pair of electrons available for bonding with other atoms, which make them very reactive irrespective of other substituents. This property is exemplified in the use of TPP_i as antioxidant due to its rapid oxidation to TPP (21).

Species Selectivity

OPIDN compounds are characterized by species selectivity. As early as 1899 (22), it was established that humans are sensitive to delayed neurotoxicity induced by TOCP (Type I). In 1930, however, it was shown that not all animal species are sensitive to the ataxia of OPIDN (3). Farm animals such as cows, lambs, sheep, and water buffaloes are sensitive (1), as are cats and chickens, but rats, mice, rabbits, guinea pigs, hamsters, and gerbils show inconsistent delayed neurotoxicity, in spite of exhibiting a severe acute, i.e. cholinergic, effect (1).

Recent studies demonstrated that chronic exposure to TOCP produced delayed neurotoxicity in mice that was characterized by ataxia and paralysis and accompanied with nervous system degeneration (23). In a more recent study, CD-1 strain mice were acutely exposed to TOCP (B. Veronesi & T. S. Pope, personal communication). Although a highly variable response to both NTE inhibition and structural damage occurred in the treated mice, they developed neuropathic damage in the complete absence of ataxia in response to TOCP.

On the other hand, the effect of TOCP on rats depended on the strain. Early studies reported nerve degeneration in rats repeatedly dosed with the direct-acting delayed neurotoxicant mipafox (24). More recently, Veronesi characterized spinal cord and peripheral nerve degeneration in Sprague-Dawley (25) and Long-Evans (26, 27) rats that had been given a single oral dose of 1,160 mg/kg or daily oral dose of 160 mg/kg for 5 weeks with TOCP. In these studies, later confirmed with mipafox (28), the neuropathic damage was characterized by giant axonal swellings and selective degeneration of the large and long nerve fibers in a dying-back fashion. Despite severe neurological damage to the cervical spinal cord and peripheral nerves, the rats displayed no gross functional disturbances. These findings demonstrated that although

extremely sensitive to the neuropathic or structural damage, rats are highly resistant to the dysfunction seen in the more conventional test model of delayed neurotoxicity, the chicken (1). In contrast, Fischer 344 rats treated with oral administration at doses as large as 100 mg/kg TOCP for 90 days developed neither clinical signs nor degeneration of the nervous system (29).

Differences in the ability of strains to either form or inactivate the active metabolite of TOCP, or variations in the sensitivity of target proteins, may account for the variation observed. Three strains of male rats, Sprague-Dawley (SD), Fischer 344 (F344), and Long-Evans (LE), contained levels of brain NTE varying from 4.87, 5.76, to 7.47 nmol phenylvalerate hydrolyzed/min/mg protein, respectively (30). These strains showed differences in brain NTE inhibition by TOCP. The ED_{50} values for NTE inhibition were 458, 209, and 288 mg/kg for SD, F344, and LE rats, respectively. Although the three strains had comparable levels of brain acetylcholinesterase (AChE), with a range of 68.3–69.3 nmol acetylthiocholine hydrolyzed/min/mg protein, they exhibited different sensitivities to TOCP inhibition. The ED_{50} values for AChE inhibition were 1007, 408, and 420 mg/kg for SD, F344, and LE rats, respectively. Liver microsome content of cytochrome P-450 varied with strains and was 1.50, 1.05, and 1.46 nmol/mg for SD, F344, and LE, respectively.

Although these differences may account for rat-strain differential sensitivity to TOCP-induced delayed neurotoxicity (Type 1), multiple doses of TOCP do not accumulate (M. B. Abou-Donia, unpublished data) or produce cumulative effects that lead to ataxia in some strains of rat, unlike the chicken. Daily oral or dermal application of small doses of Type 1 compounds was more efficient than a single dose in causing OPIDN in treated hens: leptophos (31, 32), EPN (32, 34), DEF (35), merphos (36), coumaphos (37), TOCP (38), dichlrovos, and trichlorophon (M. B. Abou-Donia, unpublished data). Similar results were obtained in the cat following the dermal application of daily doses of EPN and TOCP (39, 40). After subchronic administration of these chemicals, there was little or no damage in the peripheral nerves. It was postulated that the paucity of peripheral nerve lesion may have resulted from its ability to regenerate. It was also suggested that Type I compounds may increase the capacity of peripheral nerves to metabolize these chemicals. In cats treated with TOCP, regeneration of peripheral nerves was accompanied by the disappearance of electromyographic (EMG) abnormalities (40). The repair of peripheral nerves is not surprising given that nerve cells and Schwann cells remain intact (41). It is also possible that small doses of TOCP and other Type I compounds may stimulate peripheral nerve regeneration in hens and cats similar to that found in rats dosed with TOCP (26). In contrast, spinal cord lesions were evident in these animals, which may be explained by the fact that central nervous system does not regenerate.

As early as 1930, it was shown that although the cat, dog, monkey, and chicken were sensitive to Type II compounds such as TPP_i and $TOCP_i$, clinical condition varied with animal species (3).

Age Sensitivity

Early studies showed that the young of sensitive species (i.e. chicken) are not sensitive to a single dose but are sensitive to repeated doses of Type I OPIDN compounds. A single oral dose of DFP did not produce delayed neurotoxicity in young chicks; however, they did develop paralysis after repeated doses of DFP (42). A single oral dose of 750 mg/kg TOCP produced OPIDN in chickens, with severity being age-dependent; older birds developed more severe OPIDN. The age at which chicks became susceptible to TOCP-delayed neurotoxicity was 60 days (43).

Other studies examined the juvenile sensitivity to organophosphorus compounds-induced neuropathic damage and concluded that in the rat, as in chicks (42), OPIDN is an age-dependent phenomenon. Long-Evans rats at various ages (20, 25, 30, 35, 40, 60 days) were dosed with TOCP (2360 mg/kg) and killed for histopathology 2–3 wk later (B. Veronesi, personal communication). Although a high variability occurred in the frequency and severity of cord damage, neuropathic sensitivity gradually increased with age. Definitive neuropathy (damage score ≥ 3) occurred in rats dosed at 30 days or older. This phenomenon does not appear to relate to the maturation of the target enzyme since paraoxon-resistant activity (an index of NTE activity) is not age-dependent and mipafox sensitivity (in vitro) is stable throughout the above time points (S. Padilla, personal communication). However, this age-related susceptibility may result from hepatic microsomal maturation since P-450 activity, as determined by aniline hydroxylase activity, steadily increases throughout these time points, almost doubling in activity from 20–25 days (D. M. Lapadula & M. B. Abou-Donia, unpublished results).

On the other hand, when one-week-old chicks were given one or two s.c. doses of 1000 mg/kg TPP_i, they developed Type II OPIDN. These chicks exhibited ataxia and paralysis accompanied by histopathological lesions of the central and peripheral nervous systems characteristic of Type II OPIDN (14).

Latent Period

Although both Type I and Type II OPIDN are characterized by a delay before the onset of signs of neurologic deficits or neuropathic lesions, the length varies with OPIDN class. Type II seems to have a shorter latent interval than Type I. In the cat (7), clinical signs appear 4–7 days after a single neurotoxic dose of $TOCP_i$ (Type II), whereas there is a delay of 14–21 days (40) before neurologic signs appear following a single dose of TOCP (Type I). Also, in

hens there is a 4–6 day delay after the administration of TPP; (12, 13) compared to 6–14 days following TOCP administration (1). In Long-Evans rats, neurologic signs, e.g. ataxia and pathologic lesions, of Type II OPIDN developed one week after repeated dosing with TPP_i (11), whereas those treated with TOCP (Type I) developed spinal cord damage 2–3 weeks post exposure in the absence of discernible neurologic deficits (27, 28).

Clinical Signs

Signs of neurologic deficits produced by delayed neurotoxic organophosphorus compounds depend on both the type of compound and animal species. Although clinical signs for Types I and II OPIDN were indistinguishable quantitatively in the adult chicken (7, 12, 13), other species exhibited distinct signs characteristic of each syndrome.

CATS Cats treated with Type II compounds, e.g. TPP_i, $TOCP_i$, $TMCP_i$, and $TPCP_i$, developed ataxia after 4–26 days, and 2–16 days thereafter developed extensor rigidity of both fore- and hindlimbs of relatively long duration (9). $TMCP_i$ produced the least acute cholinergic effects, whereas $TPCP_i$ was the most acutely toxic of the four chemicals. Furthermore, aryl phosphite produced a rise in body temperature of cats of 0.9–2.5°C at onset of ataxia or a day sooner (7). In contrast, cats treated with EPN (39) or TOCP (40, 44), both of which are Type I compounds, only exhibited flaccid paralysis.

MONKEYS Type I and Type II compounds produced effects similar to those in the cat. Monkeys treated with a s.c. dose of 1 ml/kg TPP_i in two doses at a 24-day interval, developed ataxia within 12 days of the second dose. Extensor rigidity of the limbs and some retraction of the head developed three days later (7). On the other hand, TPP, a Type I compound, produced pronounced flaccid paralysis of the posterior extremities 8 days after injection of 500 mg/kg (7).

RATS (*a*) *Acute effects:* All four aryl phosphites tested (7) were acutely toxic in the rat after subcutaneous injections: $TPP_i > TPCP_i > TmCP_i > TOCP_i$. Acute effects usually developed within a few hours after dosing in animals that survived. Signs of acute toxicity were reported as generalized tremors involving large muscle groups (7).

Recent studies using Long-Evans rats showed that single or multiple subcutaneous doses of TPP_i produced tremors within 1 hour of injection, although they subsided within 4–6 hours (9, 11). These animals became lethargic for 2–3 days. The site of subcutaneous injection exhibited irritation, edema, congestion, ulceration, and occasional necrosis following multiple injections (9).

Rats treated with TOCP developed cholinergic signs such as tremors, lacrimation, and diarrhea 4 days after dosing (27). Although these signs disappeared with time, the animals appeared slightly hyperactive. (*b*) *Signs of delayed neurotoxicity:* Following treatment with TOCP, neither Long-Evans, Sprague-Dawley, nor Fischer 344 rats exhibited behavioral nor clinical signs of OPIDN (25, 26, 29). However, delayed neurotoxicity signs developed a few days after the injection of aryl phosphites (Type II). These signs were hyperexcitability, some spasticity,, incoordination, and later partial flaccid paresis of the extremities (7). TPP_i and $TOCP_i$ were more effective than $TMCP_i$ and $TPCP_i$ in producing OPIDN. Smith et al suggested that these signs in the rat were a different manifestation than the extensor rigidity seen in the cat (7). Recent studies (9, 11) on Long-Evans rats reported that multiple doses of TPP_i produced tail-kinking in the proximal one-inch of the tail. These rats developed hind-leg ataxia within one week, followed by paralysis. Furthermore, rats developed circling behavior following multiple doses of TPP_i.

CHICKENS (*a*) *Acute effects:* Chickens treated with large oral doses of aryl phosphites exhibited signs of acute effect within 3 hours of dosing (9). These signs were characterized by tremors, somnolence (drowsiness), dyspnea (labored breathing), and leg weakness. Large oral doses of TOCP produced acute cholinergic effect in chickens including diarrhea, salivation, and leg weakness (12, 13). Chickens that survive acute toxicity usually recover within three days of dosing. (*b*) *Delayed neurotoxicity:* Although TPP_i and $TOCP_i$ produced delayed neurotoxicity in chickens, $TMCP_i$ and $TPCP_i$ did not (7). Chickens treated with TPP_i or $TOCP_i$ developed ataxia 4–5 days after dosing. The condition of the chickens progressed to flaccid leg paralysis 10–12 days after dosing (7, 12, 13).

TOCP and other Type I compounds produce ataxia and flaccid paralysis in chickens that are indistinguishable from those produced by Type II compounds (1). The only difference is in the time of onset of clinical signs; Type I compounds produced clinical signs of OPIDN a few (i.e. 6–14) days later than Type II.

Neuropathological Changes

Although all delayed neurotoxic organophosphorus compounds produce histopathological alterations in both the central and peripheral nervous systems, each class of chemicals induces its own characteristic topography of damage. Neuropathological lesions are not only dependent on the chemical but also on the animal species and, in some instances, on the duration of exposure.

CATS Large doses of aryl phosphites (Type II) that produce acute toxicity and death before onset of delayed neurotoxicity signs usually result in a

diffuse degeneration of all tracts of the brain and spinal cord (7). Aryl phosphites produce cellular and axonal changes at doses that produce ataxia and extensor rigidity. However, the most characteristic cellular changes are cellular gliosis and decreased number of motor cells in the anterior horn. Fatty degeneration and cellular necrosis are seen less frequently (7).

TPP_i, a Type II compound, gave rise to a combined degeneration of specific ascending and descending tracts, in addition to a minor degeneration of the lower motor neuron. The ascending (sensory, affector) tracts that are involved are spinocerebellar and anterolateral spinocerebellar tracts. The descending (motor, effector) tracts that are damaged by TPP_i are rubrospinal, vestibulospinal, tectospinal, lateral corticospinal, and anterolateral tracts contributing to the neurotoxic effect on motor cells (3, 6). It was suggested that the extensor rigidity of TPP_i resulted from the removal by decerebration of inhibiting impulses coming through the cortico-pontine-cerebellar tracts.

In Type II OPIDN in the cat the posterior fasciculi are usually not affected. The greatest degree of involvement, however, is seen in the medulla and pons of the median longitudinal bundle, the restiform bodies, and the brachia conjunctive (7). Only slight changes occur in the spinal ganglia in the peripheral nervous system. However, roots and peripheral nerves either were not changed or exhibited moderate to severe fatty degeneration. It was also concluded that although the action of TOCP is more restricted and localized, $TOCP_i$ has a more general and diffuse effect (6, 7).

TOCP produced histopathological lesions in the spinal cord and peripheral nerves of cats in a dose-dependent fashion (40). When a single dose was applied dermally to unprotected shaven skin, the 250 mg/kg dose was the threshold level, whereas 100 mg/kg was the no-observable level. No abnormality was seen in the dorsal-root ganglion or anterior horn cells, although degeneration of the axon and myelin in the cervical spinal cord was present in the ascending tracts, i.e. spinocerebellar and posterior columns especially the gracile tracts (40, 45). In spinal cord, below the cervical levels, degeneration is present in the lateral columns especially the descending tracts, i.e. corticospinal tracts in areas most distal from their cell bodies. In the lumbar region, lesions are seen in the ventral columns, i.e. corticospinal tracts.

In DFP-treated cats, degenerating axons exhibited granular transformation of the axoplasm. These axons lost their neurotubules or neurofilaments and had swollen, degenerating mitochondria. Nondegenerating axons had an excess of axonal smooth endoplasmic reticulum (46).

MONKEYS Neuropathological changes in monkeys given a subcutaneous dose of 1 ml/kg TPP_i showed the involvement of both cell body and the axon (7). The number of nerve cells decreased and cellular gliosis was prominent in

the anterior horns of the spinal cord, as well as in motor nuclei of the midbrain, pons, and medulla, the cerebellar roof nuclei, and Deiter's nuclei. There was also a diminution in the number of the Purkinje cells of the cerebellar cortex in addition to cellular gliosis. Lesions were also seen in the gracilis and cuneaties nuclei. Degeneration was also present in the tracts of the brain and spinal cord: spinocerebellar, vestibulospinal, and cerebrospinal. Minor degeneration was seen in the rubrospinal tracts in contrast to that seen in the cat. One animal showed severe degeneration of the posterior columns, unlike cats or other monkeys.

RATS (*a*) *Lesion morphology:* Giant swollen axons and myelin debris characterized the neuropathologic damage of the spinal cord of TPP_i-treated rats shown in Figure 3 (9, 11). Ultrastructural study of the swollen axons revealed a moderate amount of agranular endoplasmic reticulum (smooth endoplasmic reticulum, SER) or large accumulation of tubulovesicular structure attached to intact mitochondria and neurofilaments.

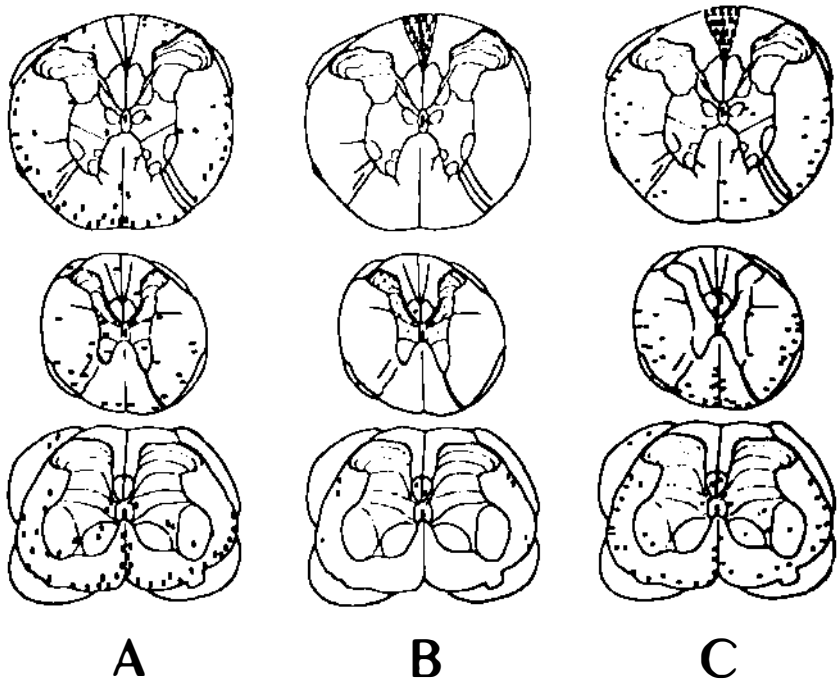


Figure 3 Topography of spinal cord damage (i.e. axonal swelling, myelin ellipsoids) seen Type I and II compounds in Long-Evans rats. (A) Tpp_i, (B) TOCP, and (C) TPP_i and TOCP. Dots represent TPP_i damage, "x's" represent TOCP damage. (From Veronesi & Dvergsten, 1987, reference 11, with permission).

(*b*) *Lesion distribution:* Brain stem degeneration also differentiates between Type I and II OPIDN. In rat spinocerebellar tracts and the medial and lateral reticular formation contained disrupted nerve fibers following treatment with TPP_i (9, 11). Swollen axons and axonal debris were seen in the upper medial longitudinal fasciculus, the medullary reticular formation, and the medial vestibular nuclei. Neuronal necrosis was present in the spinal cord medial and lateral reticular nuclei (9, 11). In the spinal cord of TPP_i-treated rats, the large-diameter ascending tracts of the fasciculus gracilis were preserved. However, occasional degeneration was present in the fasciculus cuneates. Neuropathologic lesions were limited to the ventral and ventrolateral columns of the cervical, lumbar, and sacral spinal cords. Damage in the upper cervical cord was noted in the area that extended dorsolateral fasciculis (Lissauer's zone) through the ventral and ventrolateral columns, and in the prefissurales area. Damage in the grey matter was seen as myelin debris and swollen axons. These lesions were present in Rexed laminae VI, VII, and IX in lumbar cord and laminae VII of the cervical cord. Chromatolysis and necrosis of the anterior horn cells were occasionally seen in the lumbar and sacral spinal cord (9, 11). In Long-Evans rats, exposed to single or multiple doses of TPP_i and sampled 1–3 wk after exposure, neuropathic damage consists of degeneration of the ventrolateral and ventral columns of the spinal cord at all levels and moderate peripheral nerve degeneration. Medullary brain-stem involvement consists of axonal swellings and fragmented axon in the medial longitudinal fasciculus, the reticular formation and the inferior cerebellar peduncles (11).

The lesions of TOCP-induced delayed neurotoxicity (Type I) in the rat were characterized by giant swellings of myelinated and demyelinated axons, myelin debris, vaculated myelin sheaths, and hyaline bodies shown in Figure 3 (26). Ultrastructurally, the giant axonal swellings contain masses of tubulovesicular profiles similar to those seen in TPP_i. TOCP produced degeneration of the large-diameter ascending (sensory) and descending (motor) tracts in a dying-back pattern similar to that seen in other species. In the cervical spinal cord, the damage is most prominent in the dorsal columns. The smaller-diameter fiber dorsolateral columns of the lumbar cord are also affected, but to a lesser extent. On the other hand, repeated administration of TOCP was required to produce neuropathologic lesions in the ventrolateral and ventral columns of the cervical and lumbar cord. TOCP-induced neuropathologic lesions were seen in the absence of overt ataxia (26).

(*c*) *Combined treatment with TPP_i and TOCP:* Rats treated with neuropathic doses of both TPP_i and TOCP showed a composite pattern of degeneration that included damaged sites characteristic of the individual organophosphorus (10). These data indicate that the neuropathic profile of TPP_i differs markedly from the OPIDN Type I associated with exposure to a model compound such as TOCP (Figure 3).

CHICKENS (*a*) *Morphology and distribution:* TPP_i produces neuropathologic lesions in brain, spinal cord, and peripheral nerves in a dose-dependent manner, as shown in Figure 4 (13). In the brain stem, smaller and fragmented axons were primarily seen in the reticular formation and cerebellar peduncles. Similar changes, as well as chromatolysis and neuronal necrosis, were frequently present in the gray matter of the spinal cord, chiefly in the anterior horn. The ventral and lateral tracts of the spinal cord contained swollen and fragmented axons. Thoracic and lumbar spinal cords showed the most severe damage (13).

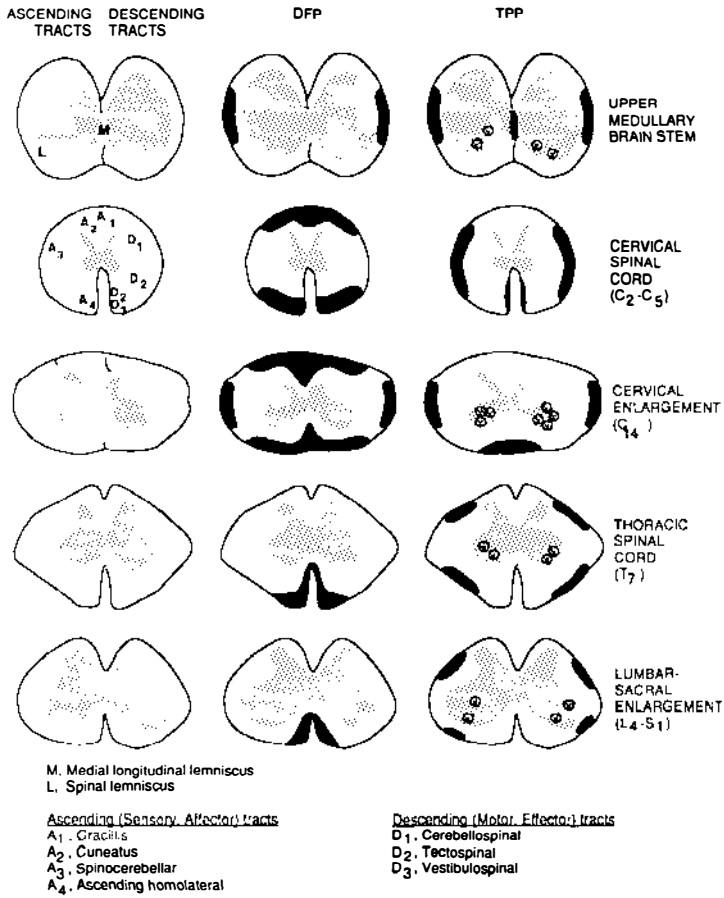


Figure 4 Topography of brain stem and spinal cord damage seen in chickens treated with DFP (Type I) and TPP_i (Type II). Dark areas are damage to white matter and × damage to grey matter (B. Veronesi, M. B. Abou-Donia, unpublished results).

(*b*) *Time course:* Light microscopy examination of nervous tissues from chickens revealed no changes four days after a subcutaneous dose of 1000 mg/kg TPP_i (13). The earliest changes consisted of swollen axons in the grey matter and occasional neuronal necrosis in the anterior horn of the spinal cord seen seven days after dosing. Swollen axons were present in the brain stem at this time. By day 14, all TPP_i-treated animals showed swollen axons in the grey matter, central chromatolysis, or necrotic neurons. This time also coincided with onset of peripheral nerve lesions. From days 17 to 21 swelling was seen in the ventral and lateral columns of the spinal cord. A single ganglion from TPP_i-treated hens examined on day 21 showed severe changes of the cell bodies. This result contrasts with the finding that a single ganglion from a paralyzed TOCP-treated hen was normal. The early onset of neuropathologic lesions induced by TPP_i in the cell body might be associated with the early development of clinical signs of Type II OPIDN.

In TOCP-treated hens, higher brain and grey matter of the spinal cord were spared (13). On the other hand, TOCP produced damage in the large-diameter tracts of the spinal cord, as shown in Figure 4. Axonal swelling and degeneration accompanied by myelin debris were seen in the dorsal and lateral columns of the lumbar and cervical spinal cord, as well as in the lateral and ventral columns of the lumbar and sacral spinal cords 21 days after dosing. Changes were also seen in the distal parts of the sciatic, peroneal, and tibial nerves.

The earliest ultrastructural changes seen in the axoplasm are swelling and proliferation of smooth endoplasmic reticulum concurrently with an aggregation and accumulation of neurofilaments and neurotubules, with partial condensation of these cytoskeletal proteins. Later, these tubular-flamentous organelles are condensed with the proliferated smooth endoplasmic reticulum. Finally, these disordered masses are replaced by a granular and electron-dense webbing (47).

(*c*) *Combined treatment with DFP and TPP_i:* When hens were treated simultaneously with a single neurotoxic dose of DFP and TPP they developed ataxia and paralysis. Neuropathologic lesions characteristic of both Types I and II compounds were observed (12).

Protection with Phenylmethyl Sulfonyl Fluoride

The prophylactic effect of PMSF on TPP_i-induced delayed neurotoxicity is dependent on the size of the TPP_i dose (12). As the TPP_i dose was decreased, the frequency and severity of neurologic dysfunctions and of neuropathologic lesions were decreased by prior treatment with PMSF. Thus, although PMSF fully protected against 250 mg/kg sc dose of TPP_i, it partially afforded protection against 500 and 750 mg/kg in a dose-dependent manner. PMSF enhanced or synergized the delayed neurotoxicity produced by 1000 mg/kg

s.c. dose TPP_i, which resulted in a higher percentage of animals developing ataxia and paralysis than those receiving TPP_i without PMSF treatment.

Prior administration of a 30 mg/kg dose of PMSF (s.c. 30% dimethyl sulfoxide in water) by 24 hours prevented the development of Type I OPIDN produced by DFP. Also, although prophylactic treatment with PMSF totally protected hens treated with small doses of TOCP, i.e. 62.5 and 125 mg/kg, it only partially protected against higher doses, ranging from 250 to 1187 mg/kg. In contrast to TPP_i, however, PMSF did not synergize the neurotoxic action of large doses of TOCP. In these hens, pretreatment with PMSF reduced the incidence of neuropathologic lesions in the spinal cord, but did not protect the sciatic nerve from damage.

The partial protection by PMSF from Types I and II OPIDN produced by TOCP and TPP_i, respectively, might be related to persistence of large doses of these chemicals in the body of treated hens that has been recently demonstrated with ^{31}P NMR spectroscopy (16).

Combined treatment with DFP and TPP_i When a subneurotoxic s.c. dose of 250 mg/kg TPP_i was administered simultaneously with a borderline s.c. dose of 0.25 mg/kg DFP, all hens became paralyzed. These results suggest that both chemicals potentiate the neurotoxic action of each other. Neuropathologic lesions were also characteristic of both chemicals. This action was blocked by PMSF.

Inhibition of Neurotoxic Esterase

RATS The relationship between neurological damage and NTE inhibition was also explored in rats acutely exposed to either TOCP (27) or mipafox (28). Rats exposed to various doses of either organophosphorus compound displayed significant spinal cord damage in instances where the mean NTE inhibition in either brain or spinal cord was depressed to 65–75% of control level, data which suggested that NTE inhibition had predictive value for OPIDN in rats similar to the more conventional test species, the chicken.

Long-Evans adult rats were treated with two s.c. injections of 1164 mg/kg TPP_i at a one-week interval (10). Both brain AChE and neurotoxic esterase (NTE) showed maximum inhibition at 4 hours after the second dose, with depressed activity of 30% and 39%, respectively, of control. Also, serum cholinesterase was depressed by 33% at the same time. It was concluded that NTE may not play a significant role in the pathogenesis of TPP_i-induced neurotoxicity in the rat (10, 11). These results cast further doubt on NTE as a putative target for OPIDN, since TPP_i contains a reactive phosphorus atom and hydrolyzable ester bond, allowing NTE inhibition and "aging." In the rat TOCP-induced OPIDN (Type I) correlated with 65% inhibition of brain NTE

activity (27), and therefore NTE may not be involved in the pathogenesis of TPP_i-induced OPIDN (Type II).

CHICKENS TPP_i is a potent in vitro inhibitor of hen brain NTE, with a K_i of $2.1 \times 10^5\ M^{-1}\ min^{-1}$ (12). Diphenyl phosphonate (a metabolic product of TPP_i; 16) was 50 times less potent than TPP_i as an inhibitor of NTE. This result suggests that the inhibition of NTE by TPP_i did not result from hydrolysis and tautomerization to diphenylphosphonic acid (16).

A subcutaneous neurotoxic dose of 1000 mg/kg TPP_i produced 80% and 100% inhibition of hen brain and sciatic nerve NTE, respectively, 24 hours after injection. Hen brain NTE activity recovered to 50% control by day 14, but sciatic nerve NTE did not recover. The threshold dose of delayed neurotoxicity of 500 mg/kg TPP_i and subneurotoxic dose of 250 mg/kg TPP_i inhibited hen brain NTE by 70% and 50%, respectively (12). A dose of 1184 mg/kg TOCP produced complete inhibition of both brain and sciatic nerve NTE that persisted for 21 days.

For an organophosphorus compound to have the potential to produce Type I OPIDN in adult chickens, it should inhibit brain NTE by at least 70% of normal (48). A similar rule seems to apply for Type II OPIDN in chickens (12).

Effect on Catecholamine Secretion from Bovine Adrenomedullary Chromaffin Cells

Chromaffin cells develop from the neural crest stem cell and have been considered a truncated sympathetic neuron because they lack axonal-like projections. Therefore, this cell type is well suited for investigating cell body effect in isolation. Because TPP_i (Type II) produces cytotoxic toxicity and DFP (Type I) does not, their differential action on exocytotic secretion of neurotransmitter by chromaffin cells was studied (49). To ascertain whether or not the neurotoxic effects were strictly a receptor-mediated phenomenon, three different secretagogues were used: nicotine, veratridine, and high potassium ions. All three induce secretion of catecholamines via separate mechanisms. Nicotine acts on the receptor, whereas high K^+ depolarizes the membrane, which is a nonreceptor-mediated event. Veratridine acts on Ca^{2+}-sensitive Na^+ channels, an effect that is partially receptor-mediated. Regardless of the secretagogue used, TPP_i inhibited the exocytotic release of endogenous catecholamines in both a time- and dose-dependent manner. These results indicate that TPP_i inhibits nonreceptor- or partial receptor-mediated secretion. The mechanism of this inhibition, therefore, must include some step distal to the receptor, such as interference with a second-messenger system that is common to all three pathways. In contrast, DFP (Type I) and paraoxon (a nondelayed neurotoxicant) had either a minimal inhibitory or a

slightly stimulatory effect. The contrasting effects of TPP_i and DFP on catecholamine secretion from chromaffin cells confirms their differential action on cell body and may be related to the mechanism of their distinct neurotoxic action.

OPIDN IN HUMANS

Type I Compounds

TOCP Tri-*o*-cresyl phosphate first produced OPIDN in humans at the end of the nineteenth century (Table 2). Since then, more than 40,000 episodes of OPIDN have been documented (1, 3). The earliest known cases resulted from the use of creosote oil, a chemotherapeutic treatment for pulmonary tuberculosis (23). In the early 1930s more than 20,000 persons in the eastern and southern states of the United States developed clinical signs of OPIDN, known as "ginger-Jake" paralysis, following the consumption of an extract of ginger called "Jamaica Ginger" that had been adulterated with TOCP (50–53). This episode had a profound social and cultural impact in the South and as a result 12 commercial phonographic recordings were made between 1928 and 1934 by southern rural artists referring to Jake-induced leg weakness (54–56). About the same time, TOCP-containing Apiol, which was used in Europe as an abortifacient, produced OPIDN in women (57). Several cases of OPIDN were reported in Durban, South Africa, and in Europe (58–65) from the use of cooking oil contaminated with TOCP. An estimated 10,000 persons in Meknes, Morocco, developed OPIDN after consuming TOCP-contaminated oil (66). Other TOCP-delayed neurotoxicity resulted from occupational exposure or the consumption of TOCP-contaminated cooking oil in India (67, 68), alcohol in Rumania (69), flour in the Fiji Islands (70) and Morocco (71), and sesame oil in Sri Lanka (72).

OTHER COMPOUNDS During its development, the experimental organophosphorus insecticide mipafox produced OPIDN in a man and a woman. In France (74), a suicide attempt with the insecticide omethoate produced OPIDN in the victim. Leptophos was implicated in the development of neurologic dysfunctions of some workers during its manufacturing and packaging in Bayport, Texas (75). A suicide attempt with the insecticide trichloronate resulted in delayed neurotoxicity (76) in Poland. Intentional or accidental exposure to trichlorphon produced OPIDN in humans in Japan (77), Iran (78), and Rumania (79). The insecticide parathion was implicated in delayed neurotoxicity after a suicide attempt in The Netherlands (80). Methamidophos produced OPIDN in Sri Lanka (81). Three persons who handled (82) fenthion displayed signs of OPIDN. A suicide attempt with chlorpyriphos resulted in delayed neurotoxicity (83).

Table 2 Chronology of organophosphorus compound-induced delayed neurotoxicity (OPIDN) in humans

Chemical[a]	Year	Country	Incidence	Number of cases	References
TOCP	1899	France	Creosote for tuberculosis	59	22
	1930	USA	Ginger extract	10,000–20,000	50–53
	1925–1934	France, Germany, Switzerland	Apiol abortfacient	200–500	57
	1937	South Africa	Contaminated cooking oil	60	58
	1940	Switzerland	Contaminated cooking oil	80	59
	1942	Britain	Manufacturing	3	60
	1945	Britain	Contaminated cottonseed oil	17	61
	1943–1947	Germany	Used as cooking oil	10–20	62
	1947	Switzerland	Contaminated food	73	63
	1952	Switzerland	Contaminated olive oil	80	64
	1955	South Africa	Contaminated water	11	65
	1959	Morocco	Used as cooking oil	10,000	66
	1960	India	Contaminated cooking oil	58	67
	1966	Rumania	Contaminated alcohol	12	69
	1967	Fiji Islands	Contaminated flour		70
	1973	Morocco	Shoe glue exposure	40	71
	1977, 1978	Sri Lanka	Contaminated sesame oil	23	72
	1988	India	Contaminated cooking oil	1,000	68
Mipafox	1952	Britain	Testing	2	73
Omethoate	1972	France	Suicide attempt	1	74
Leptophos	1974, 1975	USA	Manufacturing	12	75
Trichloronate	1975	Poland	Suicide attempt	1	76
Trichlorphon	1975	Japan	Accident	1	77
	1976	Iran	Suicide attempt	1	78
	1984	Rumania	Insecticide exposure	4	79
Parathion	1981	Netherlands	Suicide attempt	1	80
Methamidophos	1982	Sri Lanka	Ingestion/Skin contamination	9	81
Fenthion	1985	USA	During animal dipping	3	82
Chlorpyriphos	1986	Italy	Suicide attempt	1	83

[a]Chemical nomenclatures are listed in Table 1.

MASSIVE EXPOSURE TO ORGANOPHOSPHORUS COMPOUNDS AND OPIDN Some humans exposed to massive doses of organophosphorus insecticides that had not produced OPIDN in experimental animals have developed neurologic deficits associated with delayed neurotoxicity. One example is a farmer in The Netherlands who consumed an estimated 150 g of parathion in 600 ml of methanol (80) and survived the 500- and 10-times estimated human lethal dose for parathion and methanol, respectively, but was in a deep coma for several weeks. Upon recovery from his coma, he had clinical features characteristic of OPIDN, such as flaccid paralysis of both legs and weakness of both hands with muscle atrophy. He partially recovered within one year. Methanol's possible role in synergizing the neurotoxic action of paraoxon is not known. A further complication is the presence of other additive materials in the formulated parathion, whose joint neurotoxic action, if any, is not known. Experimental studies have shown that *n*-hexane, a weak neurotoxicant (84), and methyl *iso*-butyl ketone, a nonneurotoxicant, have potentiated or synergized the action of the delayed neurotoxicant EPN (85).

Methamidophos, another insecticide that has not produced delayed neurotoxicity in experimental animals, was implicated in producing delayed neurotoxicity after massive exposure in 10 humans (81). Following recovery from acute toxicity, they developed OPIDN 2–3 weeks after exposure. These signs were characterized by footdrop, weakness of hand muscles, and absence of ankle jerks. Other signs included spasticity, weakness of hip and knee flexors, and exaggerated knee jerks.

Where massive exposure to organophosphorus compounds results in deep coma, it is not clear whether the clinical condition of OPIDN that appears later is related to the long period of unconsciousness or is true delayed neurotoxicity.

PROLONGED EXPOSURE TO ORGANOPHOSPHORUS COMPOUNDS AND OPIDN Daily exposure to malathion for 6 weeks resulted in "ascending paralysis" in an 18-month-old child (86). Acute cholinergic toxicity also developed. Flaccid paralysis resulted and continued for several days. This condition was diagnosed as "demyelination" induced by malathion (OPIDN). However, since this condition improved with atropine and rest, more likely it was related to prolonged inhibition of acetylcholinesterase in the nervous system than a true OPIDN.

Exposure to EPN and parathion was implicated in producing signs of mild peripheral neuropathy (87) in a patient who was also concurrently exposed to DDT, dieldrin, and lead arsenate over three seasons. Although EPN is known to produce OPIDN in experimental animals (33, 34, 39), it is difficult to assess the causative agent for this neuropathy since lead arsenate also produces peripheral neuropathy.

The cotton defoliant merphos was implicated in producing OPIDN in an agricultural worker (88). His clinical condition was characterized by influenza-like symptoms and followed with complete recovery within three months. In experimental animals, merphos produces OPIDN after dermal application (36). Merphos and its oxidation product DEF also produce "late acute effect" after oral administration that is related to blood and bone marrow toxicity (35, 36, 89, 90). Spraying of merphos would involve exposure dermally, orally, and by inhalation; therefore, this farmer's condition might well have resulted from a weak OPIDN superimposed on a late acute effect. Weak OPIDN action usually results from effects on peripheral nerves and mild effects on the spinal cord of reversible nature, e.g. swellings or edema (1). Recovery from such a condition is expected.

SCREENING ORGANOPHOSPHORUS COMPOUNDS FOR OPIDN The incidences that result from either massive or prolonged exposure to organophosphorus compounds indicate that using the unprotected median lethal oral dose may not screen them well for the potential to produce OPIDN as required by regulatory agencies (2). Although many organophosphorus compounds can cause OPIDN, only those that are relatively stable and moderately toxic will produce delayed neurotoxicity in this test. This is because there is usually a build-up of a threshold level of the organophosphorus compound at the neurotoxicity target site. Oral administration may not be suitable for testing some organophosphorus compounds. Coumophos has very high oral acute toxicity. Thus, no hens survived an oral dose of coumophos that produced OPIDN. On the other hand, single or multiple dermal applications result in OPIDN (37). Also, oral administration of the cotton defoliant DEF (35, 89, 91) and merphos (36) produced a late acute effect that is related to hematoxicity and resulted in early death of the animals before onset of OPIDN (90). In contrast, each chemical, when applied dermally, produced typical OPIDN. Organophosphorus compounds must be tested for their potential to produce OPIDN using the dermal route. This is of particular importance because the skin is a major port of entry of these chemicals into the human body following exposure during manufacturing, packaging, and spraying.

Type II Compounds

TPP_i Triphenyl phosphite was used as convulsive agent in experimental epilepsy (8), but its use has now been discontinued because it produces spinal cord lesions. Oral administration of this compound failed to produce severe clinical signs of Type II OPIDN in hens, although dermal or s.c. injections did (14).

SIGNS AND SYMPTOMS OF ORGANOPHOSPHORUS COMPOUND POISONING

Some organophosphorus compounds capable of producing OPIDN are direct or indirect inhibitors of AChE. In many cases of organophosphorus compound poisoning, initial toxicity is that of their cholinergic action.

Acute Cholinergic Effects

Inhibition of AChE in the nervous system results in the accumulation of the neurotransmitter acetylcholine (ACh) at the synapses and neuromuscular junctions. Initially, excess ACh results in excitation, then paralysis, of the cholinergic transmission that consists of (*a*) the central nervous system, (*b*) the parasympathetic nerve endings and a few sympathetic nerve endings such as the sweat glands (muscarinic effects), and (*c*) the somatic nerves and the ganglionic synapses of autonomic ganglia (autonomic effects). The resulting signs and symptoms are those produced by excessive and continued stimulation of the muscarinic, nicotinic, and central nervous system receptors. The severity of the clinical manifestations of poisoning depends on the compound and level, frequency, duration, and route of exposure.

MILD POISONING Initial complaints are usually fatigue, giddiness (a whirling, dizzy sensation), and sweating. These symptoms may also be accompanied by anorexia, headache, weakness, anxiety, tremors of tongue and eyelids, miosis (constriction of the pupils), impairment of visual acuity, and tightness in the chest.

MODERATE POISONING Initial symptoms may be followed by nausea, salivation, lacrimation, abdominal cramps, vomiting, sweating, slow pulse, bradycardia, fall in blood pressure, and muscular tremors.

SEVERE POISONING This condition results in diarrhea, pinpoint and nonreactive pupils, muscular twitching, wheezing, increase in bronchial secretion, respiratory difficulty, cough, pulmonary edema, cyanosis, loss of sphinctor and urinary bladder control, tachycardia, elevated blood pressure, convulsions, coma, heart block, and possibly death.

Organophosphorus compound-induced death may take place 5 minutes to 24 hours after a single exposure, depending on the chemical and doses. The cause of death is asphyxia, due to respiratory failure that results from muscarinic bronchoconstriction and laryngospasm; excessive muscarinic tracheobronchial and salivary secretions; nicotinic paralysis of diaphragm and respiratory muscles; and central nervous system depression and paralysis of respiratory centers.

Neurologic Dysfunctions in OPIDN

Symptoms of OPIDN in humans are well-documented from episodes in four continents: America, Africa, Asia, and Europe (Table 2). In the majority of cases, onset of symptoms is insidious. Typically, the patient complains of symmetric leg weakness after a delay of 6 days to 1 month, but usually 2 weeks, after exposure. The course of neurologic deficit of OPIDN may be divided into three distinct phases.

PROGRESSIVE PHASE This phase covers the early stages of neurologic dysfunctions and lasts 3–6 months after onset of symptoms. During this stage, the neurologic deficit is diagnosed as peripheral neuropathy and is characterized by:

1. Early symptoms consisting of symmetric cramping, burning, tightness, and/or stinging pain in the calves of the legs, and less often in the ankles and feet.
2. Concomitantly, or shortly thereafter, numbness and tingling in the feet and legs.
3. Weakness and atrophy of the peroneal muscles resulting in a bilateral dragging of the toes on the floor (foot-drop).
4. A week after onset of leg weakness and atrophy, in moderately severe cases, weakness spreading symmetrically to the hands.
5. Changes, first evident in the legs and feet, known as "glove-and-stocking" hypoethesia. This condition is characterized by a stocking-type decrease in sensitivity to touch, pain, temperature, or tickle (hypoethesia) in the lower extremities and a lesser degree of glove-type hypoethesia in the upper extremities.
6. Steppage gait.
7. Positive Rhomberg.
8. Absent Achilles and ankle jerk reflexes.
9. In some cases, 2–4 weeks after exposure, bilateral and symmetrical flaccid paralysis involving both sides about equally.

STATIONARY PHASE During this phase, which lasts 3–12 months after onset of symptoms, paralysis becomes stationary. Approximately 2–9 weeks after the end of the progressive phase sensory symptoms disappear, but bilateral paraplegia or quadriplegia persist.

IMPROVEMENT PHASE This phase takes place about 6–18 months after onset of neurologic deficits. At this time, there is a definite improvement in the ability to use the hands and arms and in the extensor movement of the feet and toes. Usually, improvement of functions occurs in the reverse order to

that in which the symptoms began; the hands that became involved last, recover first. In mild cases, recovery of function occurs within 15 months. In the moderately severe cases that exhibit impairment of the hands, recovery occurs within 2 years. In the severest cases, even though hands show great improvement, complete paralysis still remains below the knee. Although some of the severe cases may eventually improve greatly, complete recovery is unlikely.

LONG-LASTING EFFECT Later stages of neurologic deficits are diagnosed by spinal cord lesions. The persisting and long-lasting central lesion becomes unmasked as the peripheral neuropathy is diminished and is characterized by spasticity (excessive muscle tone or rigidity) and exaggerated knee jerk. This lasting condition explains the misdiagnosis of OPIDN patients, such as those exposed to leptophos, as having multiple sclerosis or encephalitis (75).

PROGNOSIS The prognosis of OPIDN patients depends on the severity of neurologic deficit, which reflects the extent of nervous system damage. Clinical improvement or even clinical recovery might take place. Mild poisoning with delayed neurotoxic organophosphorus compounds results in damage to the peripheral nervous system. On the other hand, severe toxicity results in neuropathologic lesions produced first in the peripheral nervous system followed by changes in the central nervous system.

Functional improvement might result in mild cases from regeneration of peripheral nerves. However, such improvement is unlikely to be a consequence of regeneration of the spinal cord, since repair process is not typical of the central nervous system (41). On the other hand, it is possible that acute, reversible changes in the spinal cord, such as edema, might subside with time. Clinical improvement may also take place as other neurons with the same functions maintain normal activity by taking over the functions of damaged neurons. Also, other neurons may acquire the needed functions. In patients with severe neurologic dysfunctions, although peripheral nerves regenerate with time, neither recovery of spinal cord damage nor function is possible. In these cases, permanent neurologic deficit occurs.

Treatment of Organophosphorus Poisoning

ACUTE CHOLINERGIC EFFECTS The following treatment measures should be performed for acute cholinergic toxicity of organophosphorus compound poisoning:

1. Carrying out life saving measures, such as clearing airway by removal of secretions, administration of oxygen, and initiation of artificial respiration.

2. Treatment with atropine sulfate, which acts by antagonizing the action of acetylcholine at the muscarinic receptor sites. This treatment may be supplemented with pralidoxime (2-PAM), which acts by hydrolyzing the phosphorylated AChE and reactivating the enzyme. In the event of convulsions, sodium theopental or diazepam (valium) may be used.
3. Decontamination of skin is carried out using alkaline soap and water, while in case of ingestion lavage with 5% sodium bicarbonate is recommended to enhance the hydrolysis of the organophosphorus ester.
4. The following drugs should be avoided: morphine, theophylline, aminophylline, succinylcholine, and tranquilizers of the reserpine or phenothiazine drug groups.

TREATMENT OF OPIDN In most cases, by the time the symptoms of OPIDN occur, the organophosphorus compounds might have been cleared from the body; consequently, there is no antidotal treatment. In some patients, exercise exacerbates the symptoms, whereas in others, the symptoms are more severe at rest, particularly at night. Treatment of OPIDN is symptomatic. Drugs such as diazepam that are used to treat spasticity may be useful for the long-lasting stage of OPIDN.

BIOCHEMICAL MARKERS FOR OPIDN

Acetylcholinesterase (AChE, EC 3.1.1.7)

This enzyme is essential for life and is present in the grey matter of the brain in the nervous system, and in red blood cell. AChE was proposed as the target for OPIDN (92). However, noninsecticide-delayed neurotoxic organophosphorus compounds are weak inhibitors of AChE (1).

Although acute toxicity of organophosphorus compounds results from inhibiting AChE in the nervous system, red blood cell (RBC) AChE activity is an indication of over-exposure to and absorption of these compounds. No symptoms of organophosphorus poisoning occur until RBC AChE activity reaches 25% of the normal preexposure level. A decrease of 40% in RBC AChE is a danger signal and a depression of 60% in RBC AChE from preexposure enzymatic level is an indication for removal from work to prevent overt poisoning. A person should not be allowed to return to work until this value rises to at least 75% of normal. Depression of RBC AChE usually persists for 1–3 months. It regenerates at approximately 1% per day.

AGING OF PHOSPHORYLATED AChE Dephosphorylation of inhibited AChE with some organophosphorus esters occurs very slowly or may not take place at all. An example is the rate of regeneration of DFP-phosphorylated red

blood cell AChE which coincides with the rate of resynthesis of new enzyme. Aging takes place by the hydrolysis of an alkyl group on the phosphorylayed enzyme that results in the negatively charged phosphorylated enzyme (93).

TOLERANCE TO CHOLINERGIC EFFECTS OF SUBLETHAL DOSES OF ORGANOPHOSPHORUS COMPOUNDS Repeated administration of sublethal doses of some organophosphorus compounds initially results in acute cholinergic toxicity. However, with time, the animals recover and no longer exhibit cholinergic signs despite continuing dosing. These animals also show markedly inhibited blood and nervous tissue AChE in brain. Adaptation of ACh receptor has been suggested as a mechanism for the development of tolerance (93).

Non-specific Esterases

NONSPECIFIC CHOLINESTERASE (BuChE, EC 3.1.1.8) This enzyme, also known as pseudocholinesterase or butyrylcholinesterase, is present in myelin in the nervous system, liver, and plasma. BuChE has no known physiologic or biochemical functions. A role for this enzyme in the etiology of OPIDN has been hypothesized (94), but was eliminated because of the inconsistency between the inhibition of the enzyme by organophosphorus compounds and their ability to produce OPIDN (1).

This enzymatic activity may be used to assess exposure and absorption of organophosphorus compounds. Plasma BuChE inhibited by organophosphorus compounds regenerates at a more rapid rate than RBC AChE; approximately 25% in the first 7–10 days. It is completely regenerated in the liver in about 2 weeks.

In humans (73) and experimental animals (91), delayed neurotoxic organophosphorus compounds have more prolonged inhibitory action on plasma BuChE than RBC AChE (95). This prolonged inhibition of plasma BuChE may be attributed to the poor health following development of OPIDN, since BuChE is reduced in malnutrition states. Liver damage by these compounds would result in reduced plasma BuChE activity. It also may reflect the persistence of these chemicals in the body following exposure compared to nondelayed neurotoxic organophosphorus compounds. Nevertheless, plasma BuChE activity is a good index for overexposure to and body absorption of organophosphorus compounds with delayed neurotoxicity.

NEUTOTOXIC ESTERASE OR NEUROPATHY TARGET ESTERASE (NTE) NTE is an enzymatic activity that is preferentially inhibited by delayed neurotoxic organophosphorus compounds, but not by nondelayed compounds (48). It represents approximately 6% of the total phenylvalerate-hydrolyzing activity

in hen brains. It is also present in most tissues assayed. NTE has the following characteristics: (*a*) it is a membrane-bound protein (96), (*b*) it has a molecular mass of 155–178 kDa (97), (*c*) it has a target size, as determined by irradiation inactivation, of 205 kDa (98), (*d*) it is transported in the sciatic nerve of hens at a fast axonal transport rate of about 300 mm/day (99), (*e*) it is reversibly inhibited by paraoxon (100), and (*f*) the higher apparent K_i values that occur with low concentrations of mipafox are attributed to the formation of a Michaelis complex at high concentrations, rather than to the formation of two NTE isoenzymes (101).

A good correlation has been found between the inhibition and "aging" of NTE by organophosphorus compounds and their ability to produce delayed neurotoxicity. For an organophosphorus compound to have the potential to cause OPIDN it must result in 70% inhibition of hen brain NTE activity 24 hours after the administration of the unprotected median lethal dose (LD_{50}) (48).

Although NTE was proposed as the target for OPIDN, the only evidence for that is correlative. There is no hypothesis as to how the inhibition and aging of NTE leads to neuronal damage. NTE, which is present in various tissues, has no biochemical or physiological functions (1).

Acid Phosphatase

In experimental animals, plasma acid phosphatase activity increased in hens treated with leptophos or TOCP in a dose- and time-related manner (5, 102). This increase suggests in vivo lability of lysosomal membranes that results in the leakage of the enzyme. Liver damage by these compounds may result in the release of acid phosphatase from liver lysosomes, and a decrease in the synthesis of plasma BuChE leading to the prolonged inhibition of this enzyme seen in OPIDN. The source of plasma acid phosphatase may also be the nervous system (91, 103). This suggestion is also in harmony with the finding that Wallerian degeneration of the axons is accompanied by changes of the membrane permeability of lysosomes of injured cells and an increase in acid phosphatase (104).

2',3'-Cyclic Nucleotide-3'-Phosphohydrolase (CNPase)

Brain CNPase is a myelin marker (105) whose activity increases in some myelin diseases as the result of myelin breakdown and the release of the enzyme (106). Brain CNPase activity was significantly increased in hens that developed OPIDN following dermal application of TOCP or DEF (107). On the other hand, parathion (a compound incapable of producing OPIDN) produced a lesser increase of CNPase activity.

STUDIES ON KINASE-MEDIATED PROTEIN PHOSPHORYLATION

Hypothesis

Although it has long been assumed that the initial event in the mechanisms of OPIDN is the phosphorylation of a serine hydroxyl group at the target protein, in analogy to their acute cholinergic effect, studics concerning this effect have been limited to esterases. Since reversible kinase-mediated protein phosphorylation plays a vital role in the control of cellular processes essential for neuronal growth and viability, we have been investigating the possibility that delayed neurotoxic organophosphorus compounds may interfere with protein kinases by competing with ATP as phosphoryl group donor and phosphorylating their serine or threonine hydroxyl residues. This action would adversely affect the regulation of normal neuronal processes and result in axonal degeneration.

This hypothesis is based on the pathognomonic feature of OPIDN involving cytoskeletal proteins (47, 108). The earliest ultrastructural neuropathologic changes are aggregation, accumulation with partial condensation of neurofilaments and neurotubules accompanied by proliferation of smooth endoplasmic reticulum (SER). In later changes, these cytoskeletal elements became condensed with proliferated SER to form disordered solid masses that are eventually replaced by a granular electron-dense webbing prior to this destruction.

Studies on the In Vitro Kinase-mediated Protein Phosphorylation

The effect of oral administration of TOCP on endogenous phosphorylation of specific nervous system proteins has been studied in hens, a sensitive species for OPIDN, after development of delayed neurotoxicity. In a preliminary study, the effect of a single 750 mg/kg oral dose of TOCP on the in vitro phosphorylation was examined after the developmnt of OPIDN in chickens (108–113). TOCP treatment increased in vitro kinase-mediated protein phosphorylation that was Ca^{+2}- and calmodulin-dependent. Also, the enhancement of kinase-mediated phosphorylation in brain synaptosomal cytosol fits all criteria for OPIDN, i.e. test compound, dose-dependence, time course of clinical condition, species specificity, sex, and age sensitivity.

The major proteins that are affected were identified as α- and β-tubulin, MAP-2, and neurofilament triplet proteins. Because all these cytoskeletal proteins are phosphorylated by Ca^{2+}/calmodulin kinase II (Ca^{2+}/CaM kinase II), this enzyme is a prime candidate as the initial target for OPIDN (114, 115). Furthermore, studies ruled out ATPase inhibition, phosphatase activation or a change in substrate as an explanation for TOCP-induced endogenous

protein kinase-mediated phosphorylation of cytoskeletal proteins (116). TOCP treatment also increased CaM kinase II activity.

Ca^{2+}/CaM kinase II copurifies with cytoskeletal proteins and has been suggested to be associated either directly with microtubules and neurofilaments or MAP-2. Neurofilaments and microtubules form cytoskeletal assembly by binding to one another, perhaps through cross-binding by MAP-2. Ca^{2+}/CaM kinase II plays a key role in modulating interaction between cytoskeletal elements by phosphorylation of these proteins. This enzyme regulates the phosphorylation state of MAP-2 and the dynamics of microtubule assembly/disassembly. Phosphorylation of MAP-2 by Ca^{2+}/CaM kinase II or by CAMP-dependent protein kinase induces disassembly of microtubules by reducing the interaction between MPA-2 and tubulin. Also, increased Ca^{2+}/calmodulin-dependent protein kinase-phosphorylation of tubulin resulted in twisted filamentous polymers distinct from microtubules (117).

X-ray microprobe analysis was used to investigate the hypothesis that delayed neurotoxicity caused by TOCP involves a perturbation of axonal element homeostasis (118). TOCP caused a marked derangement of intracellular element distribution 14 days after treatment. Sodium concentrations increased 2–3-fold in both mitochondria and axoplasm, whereas potassium levels decreased by one third. In axoplasm, calcium levels increased only slightly, but mitochondrial levels of this element increased substantially. These results suggest that changes in intra-axonal levels of Na, K, and Ca are involved in the pathogenesis of OPIDN.

Recent results indicate that in early axonal changes in OPIDN, prior to the development of clinical signs, there is an acceleration of anterograde transport in sciatic nerve and its branches (119). Such an effect is consistent with early molecular changes in OPIDN that consist of increased phosphorylation of cytoskeletal proteins resulting in disassembly of cytoskeletal elements. Disassembled and modified cytoskeletal proteins are rapidly transported in the axon where they accumulate in the distal portions of the axon and result in distal axonopathy. Also, the neurotoxicant dibutyl dichlorovinylphosphate was found to block the retrograde transport of ^{125}I-tetanus toxic with maximum effect occurring seven days after administration in chickens (120).

Working Hypothesis on the Mechanisms of OPIDN

Based on results of recent studies from other laboratories as well as ours, we propose the following hypothetical cascade of events (Scheme 1) for the pathogenesis of type I OPIDN (121–123).

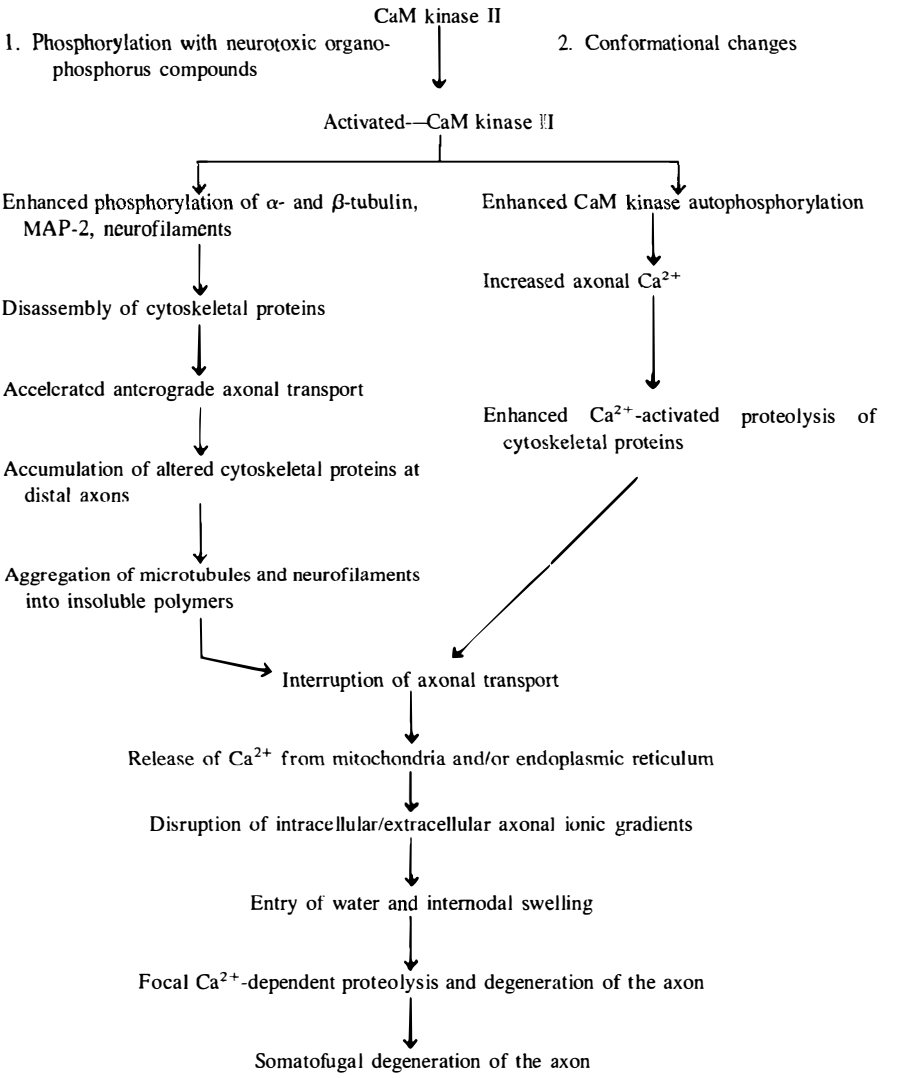
1. Type I compounds phosphorylate CaM kinase II resulting in conformational changes and increased enzymatic activity.
2. CaM kinase II autophosphorylation is increased leading to increased Ca^{2+}

in the axoplasm. This results in enhanced Ca^{2+}-activated proteolysis of cytoskeletal proteins.

3. Increased CaM kinase II activity results in enhanced phosphorylation of α- and β-tubulin, MAP-2, and neurofilaments. Increased phosphorylation of MAP-2 diminishes the polymerization of tubules and promotes the depolymerization of cold-labile microtubule and leads to disassembly of cytoskeletal proteins. Disassembled cytoskeletal elements are transported more rapidly by accelerated anterograde axonal transport, and accumulate at distal axons.
4. Enhanced phosphorylation of accumulated cytoskeletal proteins leads to their inability to assemble into polymers; instead they aggregate into solid masses; also, they may undergo Ca^2-activated proteolysis.
5. Axonal transport is impaired: this results in the accumulation of mitochondria at the distal portion of the axon. Ca^{2+} is released from broken-down mitochondria and/or endoplasmic reticulum. This leads to overloading and disruption of intracellular, extracellular ionic gradients and entry of water into the axon. Focal internodal swelling and Ca^{2+}-activated proteolysis that follow result in focal axonal degeneration that spreads somatofugally to involve the entire axon.

SUMMARY

Some organophosphorus compounds produce neurologic dysfunctions, known as OPIDN, after a delay period that is accompanied by neuropathic damage in the central and peripheral nervous systems. This group of chemicals may be divided into two classes, Type I and II, based on chemical structure, species selectivity, age sensitivity, the length of latent period, clinical signs, morphology and distribution of neuropathologic lesions, protection with phenylmethyl sulfonyl fluoride, inhibition of neurotoxic esterase, and effect on catecholamine secretion from bovine adrenome-dullary chromaffin cells. The importance of this effect is underlined by the fact that incidents involving more than 40,000 cases of OPIDN in humans have been documented from 1899 to 1989. Most of these compounds are direct or indirect inhibitors of AChE, and produce acute cholinergic effects. Neurologic deficits are characterized by three phases: progressive, stationary, and improvement. Prognosis of OPIDN depends on the extent of damage of the nervous system. Improvement or even recovery of functions may follow mild cases, whereas severe toxicity results in long-lasting neurologic dysfunctions reflecting spinal cord damage. Recent studies have shown that delayed neurotoxic organophosphorus compounds interact with Ca^{2+}/calmodulin kinase II (CaM kinase II), an enzyme responsible for the endogenous phosphorylation of cytoskeletal proteins, i.e. microtubules, neurofilaments,



Scheme 1 Proposed mechanism for Type I OPIDN

and MAP-2. This leads to an increased activity of CaM kinase II and enhanced phosphorylation of cytoskeletal elements, and eventually in the disassembly of cytoskeletal proteins. The dissociation of cytoskeletal proteins causes increased fast axonal transport in the treated animals resulting in the accumulation of altered cytoskeletal elements in the distal portions of the

axon. Abnormal tubulin and neurofilaments are transformed into filamentous polymers and undergo condensation and dissolution. Concomitantly, proliferated endoplasmic reticulum and accumulated mitochondria degenerate and release Ca^{2+} ions. This leads to Ca^{2+}-activated proteolysis of the cytoskeleton and interruption of ionic balance across the axonal membrane resulting in the uptake of water and axonal swelling, which subsequently degenerates. A similar mechanism may cause secondary myelin degeneration.

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