Ann. Rev. Pharmacol. Toxicol. 1981. 21:511–48

ORGANOPHOSPHORUS ESTER–INDUCED DELAYED NEUROTOXICITY

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INTRODUCTION

Most organophosphorus esters are direct inhibitors or are rapidly converted to inhibitors of acetylcholinesterase (AChE, EC 3.1.1.7) (1–3). Some of these compounds produce a more persistent effect: delayed neurotoxicity. In humans, organophosphorus ester–induced delayed neurotoxicity (OPIDN) results in a flaccid paresis which develops distally in the legs and spreads to the hands and thighs. In the later stages, symptoms of spinal cord injury such as spasticity and ataxia become evident as the symptoms of peripheral neuropathy recede. OPIDN has the following features:

1. Most of the delayed neurotoxic organophosphorus esters are AChE inhibitors, but not all anticholinesterase compounds produce delayed neurotoxicity.
2. There is a latent period after the administration of a single dose and before the onset of clinical signs, which ranges between 6 and 14 days.
3. Cellular damage is seen in the sciatic, peroneal, and tibial nerves; spinal cord; and medulla, but not in higher brain.
4. Onset of lesions begins at the distal part of long fibers and of large diameter peripheral nerves.
5. The lesions are characterized by the degeneration of the axons with subsequent secondary degeneration of myelin.
6. Not all animal species are susceptible to OPIDN; humans are believed to be among the most sensitive species.
7. Species sensitivity appears to be related to age; that is, young chicks are insensitive.

0362-1642/81/0415-0511$01.00

The biological and pathological effects have been well reviewed previously (4–10). This review emphasizes the relationship between the chemical structure of organophosphorus esters and their ability to induce delayed neurotoxicity. It also discusses the biochemical target as well as factors affecting the development of OPIDN.

HISTORY

OPIDN was first recognized at the end of the nineteenth century in humans poisoned with TOCP[1] (11, 12). Since then an estimated 40,000 cases of delayed neurotoxicity in humans have been documented. In the 1920s about 20,000 persons in the United States developed "Ginger-Jake" paralysis after the consumption of an extract of ginger called "Jamaica Ginger" that had been adulterated with TOCP (13–21). Later this syndrome was recognized in Europe, South Africa, and India as a result of the deliberate or accidental use of TOCP-containing preparations (22–34). In 1951 three persons were poisoned with a then newly developed insecticide, mipafox, and developed symptoms of delayed neurotoxicity (35). Between 1974 and 1975 the experimental insecticide leptophos was implicated in the poisoning and paralysis of some workers in the Texas factory where it was manufactured and packaged (36, 37). Symptoms associated with TOCP (38–42) and mipafox (35, 43) poisoning in human subjects are well documented. The clinical conditions of persons in the leptophos incident were diagnosed as multiple sclerosis, encephalitis, or psychiatric disorder (35, 37, 44). These cases were further complicated by the possibility that some of the workers were simultaneously exposed to solvents such as toluene and *n*-hexane. Cases of distal neuropathy due to chronic exposure to *n*-hexane have been revealed (45).

THE DEVELOPMENT OF AN EXPERIMENTAL MODEL OF DELAYED NEUROTOXICITY

Species Selectivity

As early as 1930, it was recognized that not all species develop OPIDN after exposure to organophosphorus esters. Some animal species, e.g. cats, cows, (14), lambs (46), sheep (47), and water buffalo (48), are susceptible. Rats, mice (14), rabbits, guinea pigs (15), hamsters, and gerbils[2] did not show consistent delayed response to TOCP, although the acute effects were severe. Dogs could be made ataxic by oral administration only if emulsifying agents were incorporated in the dose, which apparently enhanced gastrointestinal absorption of TOCP (49).

[1]Chemical names are listed in Table 1.
[2]Abou-Donia, M. B., unpublished data.

Table 1 Chemical designations of organophosphorus esters mentioned in text

Compound	Chemical designation
Coumaphos	*O,O*-Diethyl *O*-(3-chloro-4-methyl-7-coumarinyl) phosphorothionate
DEF	*S,S,S*-Tri-*n*-butyl phosphorotrithioate
DFP	Diisopropyl phosphorofluoridate
Dichlorvos	2,2,-Dichloroethenyl dimethyl phosphate
EPN	*O*-Ethyl *O*-4-nitrophenyl phenylphosphonothioate
Leptophos	*O*-4-Bromo-2,5-dichlorophenyl *O*-methyl phenylphosphonothioate
Malathion	Diethyl (dimethoxyphosphinothioyl) thiobutanedioate
Merphos	*S,S,S*-Tri-*n*-butyl phosphorotrithioite
Mipafox	*N,N'*-Diisopropylphosphorodiamidic fluoride
Parathion	*O,O*-Diethyl *O*-4-nitrophenyl phosphate
TOCP	Tri-*o*-tolyl phosphate
Trichlorphon	2,2,2,-Trichloro-1-hydroxyethyl phosphonate

OPIDN could not be produced in early studies in monkeys (15) or baboons (*Papio papio*) despite efforts to improve absorption (50). Delayed neurotoxicity was subsequently observed in baboons and squirrel monkeys following the administration of TOCP orally or intramuscularly (51). Recent reports have shown that the slow loris (*Nycticebus coucang* coucang) is susceptible to TOCP-induced delayed neurotoxicity (52–55).

Most avian species tested, including chickens (14), pheasants (56), and Mallard ducklings (57), were shown to be sensitive to exposure to organophosphorus compounds. Partridge (56) and quail (58) were not sensitive to a single dose of DFP. The adult chicken has been used as the test animal of choice to study OPIDN for a number of reasons, including the following:

1. All organophosphorus esters that have been implicated in delayed neurotoxicity in humans also cause delayed neurotoxicity in adult chickens.
2. The delay period histopathological lesions and clinical signs are similar in humans and chickens.
3. Both sexes are susceptible.
4. Clinical signs of delayed neurotoxicity in chickens, e.g. ataxia and paralysis, are easy to observe and do not require complicated functional tests.
5. Data banks on OPIDN in chickens facilitate testing of the numerous new compounds being made each year.
6. The chicken can be easily treated by dermal or oral administration of the organophosphorus compound.
7. The chicken is readily available, reasonably priced, and easy to handle.

Age Sensitivity

Age seems to be a significant factor in the development of delayed neurotoxicity (59). A single oral dose of DFP did not cause delayed neurotoxicity

in young chicks. However, repeated doses have caused delayed paralysis in chickens under 12 weeks of age; the age at which the chick usually becomes susceptible to OPIDN is between 55 and 70 days (60). Also, subchronic oral administration of leptophos or TOCP caused delayed neurotoxicity in young Mallard ducklings (57).

Stages of Delayed Neurotoxicity in Chickens

The condition of delayed neurotoxicity in chickens has been well described and can be consistently reproduced. With a single neurotoxic dose of organophosphorus esters the latent period is short—6 to 14 days. At the end of this period ataxia develops. The degree of ataxia prior to paralysis can be graded into four stages (61).

Factors Affecting the Development of Delayed Neurotoxicity

Many earlier reports on delayed neurotoxicity have described OPIDN as an irreversible demyelinating disease. Recent studies, however, have shown that the histopathologic lesion in nerve tissues induced by these chemicals is not a primary demyelination, but rather is characterized by degeneration of axons with subsequent secondary degeneration of myelin. Also, the results indicated that the development and severity of, or recovery from, this syndrome, like the effects of many other toxicants, were dependent on the following factors:

1. The test compound. Not only the delayed neurotoxic activity of a compound, but also its delayed neurotoxic potency, is dependent on its chemical structure. This relationship is discussed in detail below.

2. Dosage. Recent studies on the delayed neurotoxic effect of phenylphosphonothioates and aliphatic organophosphorus esters have shown that this effect is a dosage-dependent response (61–73). The development, intensity of the clinical condition, as well as regression or progression of the clinical condition depended on the dosage used (74).

3. Frequency and duration of exposure. Small, seemingly subneurotoxic doses can build up to cause delayed neurotoxicity (35). This was found to be true when daily oral administration of small doses of TOCP to hens caused paralysis (5, 75, 76). Similar cumulative effects with DFP (75) and tri-*p*-ethylphenyl phosphate and mono-*o*-propyl di-*p*-ethylphenyl phosphate (7) were obtained later.

Recent studies of subchronic oral and dermal administration of leptophos (61, 63, 65), EPN (64), DEF (72), merphos (73), dichlorvos and trichlorphon[2] have shown that the total administered dose needed to produce ataxia was directly proportional to the size of the daily dose.

4. Route of administration. Most animal data available are from studies that used single oral doses, although the skin is an important portal of entry. Dermal absorption of radioactive TOCP has been demonstrated in dogs and

humans (77). When a single topical dose of ^{14}C leptophos was applied on the comb of hens it was more efficiently absorbed, compared to oral administration (78–80). Dermal application of neurotoxic organophosphorus esters has caused delayed neurotoxicity in experimental animals (51–55, 65, 71–73, 82, 83).

5. Metabolism. Some organophosphorus esters that require large doses to cause delayed neurotoxicity undergo metabolic activation to more potent neurotoxic biotransformation products (69). TOCP is metabolized in vivo to the more active neurotoxic agent saligenin cyclic *o*-tolyl phosphate (84–87). The neurotoxic ester tri-*p*-ethylphenyl phosphate (TPEP) was metabolized in vitro to a more potent neurotoxic product, tri-*p*-acetylphenyl phosphate (88). Phenylphosphonothioate esters were metabolized to the more potent neurotoxic phenylphosphonate esters in vivo (78–80) and in vitro (88–90).

6. Toxicokinetics. Recent studies have suggested that species selectivity for delayed neurotoxicity of the neurotoxic phenylphosphonothioate esters leptophos and EPN may be related to the different profiles of the toxicokinetics and metabolism of these insecticides (78–96). When administered orally to nonsusceptible species, e.g. mice or rats, leptophos was rapidly metabolized and excreted as degradation products, mainly in the urine. In hens or cats, however, this insecticide was eliminated at a rate 31 times slower than in mice.

MECHANISMS OF DELAYED NEUROTOXICITY

The common characteristics of organophosphorus compounds capable of causing delayed neurotoxicity are that they are phosphorus esters and they are direct or indirect inhibitors of esterases.

Histopathological Studies in Delayed Neurotoxicity

All organophosphorus esters that have been shown to cause delayed neurotoxicity produce similar histopathologic lesions in the central and peripheral nervous systems: DFP (96, 97–99), TOCP (15, 96–108), mipafox (96, 109), phenylphosphonothioates, and aliphatic esters (64–74).

Histopathologic alterations in peripheral and central nervous tissues in chickens poisoned with these neurotoxic organophosphorus esters generally depend on (*a*) the compound tested, (*b*) the size of the administered dose, (*c*) the severity of the clinical condition, (*d*) the length of the period between onset of clinical signs of delayed neurotoxicity and termination, and (*e*) the site of tissue sampling (64–74). Histopathologic changes in peripheral nerves usually appear earlier than those in the spinal cord (69, 97, 102).

The recovery and clinical improvement of some chickens after developing ataxia or paralysis are interesting. It is unlikely that this improvement represents regeneration within the spinal cord, as the repair phenomenon is not typical of the central nervous system (110). On the other hand, it is possible that the initial peripheral damage had been repaired or regenerated by the end of the experimental period (111). Any acute, reversible spinal cord changes, such as edema, that might have been present initially could have subsided by the time of examination (63). The clinical condition may also improve either as other neurons having the same function meet the added demands and maintain normal activity, or as neurons acquire the needed function. When the spinal cord is severly damaged, neither of these compensatory mechanisms is possible and some loss of function could occur (112). This conclusion is supported by the spasticity seen in human patients, which suggests that there must be damage in the central nervous system (4).

CELL BODIES The nuclear and cytoplasmic changes in nerve cell bodies, which were earlier reported as lipoid granules (4, 15, 19, 100), were later discounted and attributed to artifacts of fixing and staining, or postmortem autolysis (5, 102, 113–115). Subsequently, Cavanagh (102) concluded that the initial injury is at the distal ends of both sensory and motor nerves. Later studies reported changes in the nuclei and suggested that the initial lesion in TOCP poisoning is in the cell body (53, 116) as the result of somatic cell disruption. Recent electron microscopic and electrophysiological studies seem to confirm Cavanagh's theory (98, 99, 105–107).

CENTRAL NERVOUS SYSTEM Degeneration of myelin and axons of the spinocerebellar tracts and the posterior columns showed only in their upper reaches, while the corticospinal tracts in the lateral columns show degeneration below the cervical levels in areas remote from their cell bodies. Degeneration was also present in the lumbar region in tracts lying in the ventral tissue (5, 7, 117). Profound changes were reported in the boutons terminaux in the spinal cord (101, 103–106, 118), consisting of absence of organelles; aggregation and disintegration of degenerated mitochondria, neurofilaments, vesicles, and granular material; an increase in the number of synaptic vesicles; and swelling. Swelling and fragmentation of myelin and axons similar to changes in the spinal cord were present in the lateral portion of the medulla, while sections of cerebrum and optic lobes were unchanged (62).

PERIPHERAL NERVES Electron microscopic studies in chickens and cats treated with TOCP showed that early changes in the axoplasm included

aggregation, accumulation and partial condensation of neurofilaments and neurotubules (103–107, 119). A striking feature was the proliferation of the agranular endoplasmic reticulum. Interestingly, in early stages of ataxia, mitochondria were not involved in this swelling. In the later stages after onset of paralysis, accumulation of mitochondria was evident. These studies confirmed that histopathological changes in OPIDN were of the Wallerian degeneration type (105–120). Recent teased nerve fiber studies showed that DFP induced a focal degeneration in the distal, but not terminal, axons in the cat (98, 99). Focal swellings in internodes seemed to be due to vacuolizations both within and outside the axon. This pattern is distinctly different from the *n*-hexane and related compounds and carbon disulfide neuropathies (10). This chemical transection of the axon then precipitates Wallerian degeneration of the more distal axon. It was suggested that the traditional hypothesis that dying-back neuropathies evolve from retrograde axonal degeneration was not valid for organophosphorus neuropathy (98, 99).

PERIPHERAL SENSORY NERVES Earlier clinical studies of persons poisoned with TOCP suggested damage to sensory fibers (15, 40). Cats treated with TOCP showed severe damage to sensory apparatus in the interosseous and flexor digitorum brevis muscles of the foot (5, 102, 103, 121). Electron microscopic studies of sensory nerve terminations of taste buds in the TOCP-treated slow loris have shown vacuolization, degenerating mitochondria, distended endoplasmic reticulum, and vesicle formation in light cells (55).

NEUROMUSCULAR JUNCTIONS In TOCP-treated cats the neuromuscular junctions showed no histopathological alterations by light microscopy (103). Electron microscopic studies, however, indicated the presence of osmiophilic bodies that contained degenerated mitochondria, synaptic vesicles, and small electron-dense granules in the terminal axoplasmic expansion of the neuromuscular junctions of interosseous muscles. No changes were reported in the pre- or postsynaptic membranes. Similar changes were noted in chicken foot extensor muscles after TOCP treatment, which consisted of the presence of large numbers of enlarged rounded synaptic vesicles (107).

MUSCLE A scattered loss of muscle fibers and replacement by connective tissue and fat have been found in human patients in late stages of TOCP poisoning (40). Spontaneous fibrillation potentials with runs of high frequency discharges were reported in electromyographic studies of muscles of TOCP-poisoned cats (102). Leg muscles from many hens treated with leptophos showed dissolution of mitochondria, sarcoplasmic reticulum, and

other subcellular organelles (62). Wing muscle from a chicken exhibited several foci of small, angulated atrophic fibers.

Electrophysiological Studies

Early findings have led to the conclusion that neither the muscle fiber nor the end plates were affected in animals exhibiting delayed neurotoxicity and that damage must be central to these structures (16). Recent studies have utilized the phenomenon of repetitive electrical activity of posttetanic stimulus in response to high frequency stimulation of soleus motor nerve to quantitate measurement of cat motor nerve terminal function in vivo (122–124). Using this technique, Lowndes et al (125, 126) suggested that an initial functional deficit in cats treated by an intraarterial injection of DFP occurred at the level of motor nerve endings. Injection of DFP into one femoral artery of cats caused functional impairment of motor nerve terminals in the injected hind limbs (127). Recovery from the subacute DFP injury took place by regeneration of the original motor axons, while collateral sprouting reinnervation was insignificant (128). Intraarterially injected DFP caused delayed neuropathy in the treated leg, which was attributed to impairment of both sensory and motor functions, both of which started at the same time (129).

Biochemical Studies

INHIBITION OF ESTERASES Aldridge (130) suggested that the initial event in delayed neurotoxicity was phosphorylation of esterases. A role for brain AChE (131) in the mechanism of delayed neurotoxicity was hypothesized. However, Earl & Thompson (132, 133) showed that while hen brain pseudocholinesterase [butyrylcholinesterase (BuChE, EC 3.1.1.8)] was selectively inhibited by TOCP, AChE was unaffected. They proposed that BuChE was the primary target in delayed neurotoxicity. This hypothesis has subsequently been eliminated by the findings that (*a*) some non-neurotoxic esters also inhibit BuChE in the same way (134) and (*b*) tri-4-ethylphenyl phosphate and other compounds cause delayed neurotoxicity but do not suppress hen brain BuChE (135).

Poulsen & Aldridge (136) pointed out the structural similarity between both phenyl phenylacetate (PPA) and phenyl-3-phenylpropionate (PPP) and saligenin cyclic *o*-tolyl phosphate, the neurotoxic metabolite produced in vivo after a dose of TOCP (84, 86). Their original suggestion that brain esterases which hydrolyze PPA and PPP may be selectively inhibited by delayed neurotoxic organophosphorus esters had to be abandoned, however. Subsequent studies with a range of organophosphorus compounds failed to confirm correlations between OPIDN and inhibitory effect of either of these enzymes in vivo (137).

THEORY OF NEUROTOXIC ESTERASE (NTE) In both in vivo and in vitro studies, Johnson (138–140) showed a difference in inhibitory effect on hen brain esterase activity between known delayed neurotoxic and non-neurotoxic compounds. A small proportion (6%) of the total PPA- or phenyl valerate-hydrolyzing activity in hen brain is susceptible to inhibition only by delayed neurotoxic compounds such as mipafox (141). Compounds not capable of producing delayed neurotoxicity, such as paraoxon, do not inhibit this enzymatic activity (142). Johnson called this enzymatic activity *neurotoxic esterase* or *NTE* (15) and proposed that it represented the primary site of action of neurotoxic organophosphorus compounds. The so-called neurotoxic esterase explanation has not been universally accepted as the initial target for delayed neurotoxicity, however, for the following reasons:

1. The esterase activity is restored to greater than 50% of normal before ataxia develops (9) and is completely recovered by the time the animal has become paralyzed.[2]

2. Some carbamates structurally related to PPA (phenyl- and benzyl carbamic acids) have been synthesized and found to inhibit NTE activity (143). However, neither single nor repeated doses of these carbamates produce delayed neurotoxicity in chickens (144). These results were attributed to the instability of the inhibited enzyme (138).

3. Some phosphinates and sulfonyl fluorides produce a stable phosphinylated or sulfonylated enzyme. The rate of return to original activity in vivo is similar to that after injection of the delayed neurotoxic ester DFP (9, 144). Yet compounds belonging to these classes are not capable of causing OPIDN. To explain these findings it was suggested that NTE inhibited with these esters cannot undergo "aging," thus causing no delayed neurotoxicity (144).

4. The strongest support of the NTE hypothesis came from the correlations between the inhibitory effect of many organophosphorus compounds on NTE and their ability to produce delayed neurotoxicity in hens (9). However, these correlations have not been consistently maintained. For example, although several dimethyl phosphates cause high inhibition of the brain NTE, the same doses produce no ataxia in hens (145).

5. The NTE has not been isolated and its physiologic and biochemical functions are not known (146).

6. This hypothesis does not explain species selectivity or age sensitivity, since NTE activity has been found in susceptible and nonsusceptible species and in the young as well as the adult animal (8, 9). Thus, chicks and insensitive species did not develop OPIDN after single doses of delayed neurotoxic agents, even though their neurotoxic esterase was inhibited.

7. This enzymatic activity is present in nonneural as well as neural tissue

of the hen (147). Thus, nonspecific binding is expected to occur in nontarget organs.

8. Except for the TOCP metabolite, saligenin cyclic *o*-tolyl phosphate, there is little structural resemblance between the substrates used to assay NTE activity and other neurotoxic organophosphorus esters, e.g. mipafox. Despite the explanations offered to a number of these points, there is no conclusive proof of the theory as yet.

THE SIGNIFICANCE OF INHIBITION OF NONSPECIFIC ESTERASES

Although the initial event in OPIDN is generally believed to be the phosphorylation of a specific protein in the nervous system, the phosphorylation of nonspecific proteins, including BuChE and NTE, may play an important role in development of this syndrome. Neurotoxic organophosphorus esters seem to have a strong affinity for these nontarget proteins which act as temporary depots or storage for them. Initially, this binding may protect the exposed animals from the acute effect of these chemicals. Later, however, these proteins may contribute to development of OPIDN. Bound esters, slowly released by a reversible process, or in the course of turnover of these proteins, reach the neurotoxicity target. They accumulate at the neurotoxicity target until they reach a minimum amount or threshold that causes OPIDN. A threshold level could be obtained from a single or multiple exposures. The time required for the nonspecific binding, release, and accumulation of the organophosphorus ester at the neurotoxicity target may account at least in part for the typical delay period before onset of ataxia following a single administration.

EFFECT ON ACID PHOSPHATASE ACTIVITY Plasma acid phosphatase activity increased in all hens treated with leptophos or TOCP in a dose-dependent manner (61, 148). The increased acid phosphatase activity in hen plasma indicates possible in vivo lability of lysosomal membranes with the release of this enzyme. Leptophos and TOCP may cause liver damage, which in turn would lead to release of acid phosphatase. This mechanism is in agreement with the finding (149) that malathion released arylsulfatase from rat liver lysosomes.

It is also possible that the source of acid phosphatase is nerve tissue lysosomes. This suggestion is in agreement with the increase in acid phosphatase activity in nerves and neuroglia of TOCP-treated hens (52). These results have led to the suggestion that lysosomal enzymes may play an important role in the development of nerve degeneration (61, 148). This hypothesis is also in harmony with the finding that the membrane permeability of lysosomes of injured cells undergoes changes during Wallerian degeneration (150).

EFFECT OF AXOPLASMIC TRANSPORT Studies on the effect of delayed neurotoxic organophosphorus esters on axoplasmic transport have produced conflicting results. Slow axoplasmic flow was maintained in control and TOCP-treated cats (151). Also, axoplasmic flow was the same in sciatic nerves of both DFP-treated and control chickens (152). Studies of the waves of fast and slow axoplasmic flow in TOCP-treated and control cats showed that the alterations in the amount and rate of axoplasmic transport demonstrated were insufficient to explain the axonal degeneration (153). A recent study to develop a quick screening method reported marked inhibition of fast axoplasmic transport of the rat optic nerve by TOCP and five delayed neurotoxic phenylphosphonothioates (154). The inhibitory effect of these esters on fast axoplasmic transport generally paralleled their potency to elicit delayed neurotoxicity in hens. Although there are conflicting results concerning what the abnormalities of axoplasmic transport are, the evidence overall points to development of some defects in fast axoplasmic transport that may at least play a part in the mechanism of OPIDN.

THE SITE OF NEUROTOXIC ACTION

Studies on delayed neurotoxicity have clearly shown that changes in the structure and in the degree of branching and substitution of the hydrocarbon radical of an organophosphorus ester considerably influence both its delayed neurotoxic activity and its potency. Therefore, it is postulated that the ability of organophosphorus esters to interact with the active center of the neurotoxicity protein is not limited to the phosphorylating ability for the serine group. Another factor that may play an important role is the hydrophobic areas in the vicinity of the active center of the target protein. The functional groups at the neurotoxicity active center that react directly with organophosphorus esters are probably similar to those of the two main types of cholinesterases: AChE and BuChE. It is therefore reasonable to assume that the specificity of each of the neurotoxicity protein and cholinesterases is determined not by the differences in the structure of their active centers, but by some differences in the structure of various hydrophobic areas around the active center.

Chemical Structure-Delayed Neurotoxic Activity Relationship

The method of studying the active center of the delayed neurotoxicity target, used in this review, was to compare the neurotoxic potential and potencies of a series of organophosphorus esters differing only in the structure of their hydrocarbon radicals. Many published data pertaining to the delayed neurotoxic effect of organophosphorus esters in chickens have been reviewed. In comparing these data, the following considerations must be

taken into account: (*a*) the purity of chemicals used; (*b*) dosage, and frequency and duration of administration; (*c*) route of administration; and (*d*) the criteria used to define delayed neurotoxicity. There were considerable variations in the reporting of the data. For example, in some studies the lowest dose causing ataxia was reported, while in others the dose necessary to produce paralysis was noted. Furthermore, most studies did not indicate the severity of ataxia. Also, the severity of the clinical condition in adult chickens varies with age as well as with the breed. In some studies chickens were protected by prophylactic treatment with atropine and oxime reactivators, and in other studies no prophylactic treatment was used.

In the subsequent section the data are discussed in accordance with the following conditions: (*a*) organophosphorus esters were classified in relation to their chemical structure and listed in Tables 2 to 7; (*b*) the esters varied in each series only in the structure of the hydrocarbon radical; (*c*) changes in the hydrocarbon radical were gradual and sequential; (*d*) each series was large enough to allow a conclusion to be drawn; and (*e*) the comparison between organophosphorus esters was confined to the chemicals tested in the same study whenever possible.

ALIPHATIC PHOSPHORUS ESTERS The descending order of delayed neurotoxicity of aliphatic phosphorus esters (Table 2) was phosphonates $\simeq$ phosphorofluoridates $\simeq$ phosphonofluoridates $\simeq$ phosphorodiamidofluoridates $\simeq$ phosphoroamidofluoridates $>$ phosphates $>$ phosphorotrithioates $>$ phosphorotrithioites $>$ phosphites.

The following classes did not cause delayed neurotoxicity: phosphorothioates, phosphonothioates, phosphinates, phosphinofluoridates, and phosphorochloridates. It should be pointed out, however, that these data do not rule out the delayed neurotoxicity of all compounds belonging to these classes of organophosphorus esters since only very few compounds of each group were tested.

In the case of dichlorovinyl phosphate series, while dimethyl phosphate ester did not cause delayed neurotoxicity at a single subcutaneous dose of 20 mg/kg (9, 135, 137, 155, 156), it caused delayed neurotoxic effects when applied orally or dermally.[2] The delayed neurotoxic potency of higher radicals in this series increased up to the propyl radical. Once this cutoff point was reached, further lengthening of the alkyl chain reduced the delayed neurotoxic potency. Since all of these compounds possess similar phosphorylating activity and their radicals are not capable of any specific reactions, the increase in the delayed neurotoxic potency can only be attributed to an increase in the adsorption of the organophosphorus esters onto the surface of the neurotoxicity protein. It is believed that this occurred because of the interaction with the hydrophobic areas close to the neurotox-

Table 2 Aliphatic phosphorus esters tested for delayed neurotoxicity in chickens

Substituents				Dose	Route of administration[a]	Delayed neurotoxicity	References
R_1	R_2	R_3	X	(mg/kg)			
Phosphates							
CH_3O	CH_3O	$Cl_2C{=}CHO$	O	20	s.c., (der. p.o.)	–,(+)	9, 135, 137, 155, 156[b]
C_2H_5O	C_2H_5O	$Cl_2C{=}CHO$	O	18	s.c.	+	9
n-C_3H_7O	n-C_3H_7O	$Cl_2C{=}CHO$	O	2	s.c.	+	9
n-$C_5H_{11}O$	n-$C_5H_{11}O$	$Cl_2C{=}CHO$	O	2.5	i.v.	+	9
ClC_2H_4O	ClC_2H_4O	$Cl_2C{=}CHO$	O	25	s.c.	+	135
ClC_2H_4O	CH_3O	$Cl_2C{=}CHO$	O	5	s.c.	+	135
CH_3O	CH_3O	$ClCH{=}CHO$	O	110	i.v.	–	9
C_2H_5O	C_2H_5O	$ClCH{=}CHO$	O	118	s.c.	+	9
CH_3O	CH_3O	ClC_2H_4O	O	50	i.v.	–	9
C_2H_5O	C_2H_5O	ClC_2H_4O	O	30	i.v.	–	9
n-C_4H_9O	n-C_4H_9O	ClC_2H_4O	O	10	i.v.	–	9
CH_3O	CH_3O	$CH{\equiv}C\text{-}CH_2O$	O	60	i.v.	–	9
CH_3O	CH_3O	$2\text{-}CH_2{=}CHO$	O	60	i.v.	–	9
CH_3O	CH_3O	$C_2H_5SC_2H_4O$	O	60	i.v.	–	9
C_2H_5O	C_2H_5O	C_2H_5O	O	10	i.m.	–	9
n-C_4H_9O	n-C_4H_9O	n-C_4H_9O	O	(3.68)[c]	p.o.	–	157
				100	p.o.	+	—[b]
n-C_4H_9O	n-C_4H_9O	OH	O	100 daily[d]	der.	+	—[b]

Table 2 *(Continued)*

Substituents				Dose	Route of administration[a]	Delayed neurotoxicity	References
R_1	R_2	R_3	X	(mg/kg)			
Phosphorothioates							
C_2H_5O	C_2H_5O	$C_2H_5SC_2H_4O$	S	20	s.c.	–	155
CH_3O	CH_3O	S-1,2-dicarbethoxy-ethylthio	S	1000	s.c.	–	155, 156
C_2H_5O	C_2H_5O	$S(CH_2)_2N(C_2H_5)_2$	O	20	i.m.	–	158
Phosphonates							
CH_3	CH_3O	tri-ClC–CH(OH)O	O	200 + 100[e]	s.c.	+	159[b]
C_2H_5	ClC_2H_4O	di-CLC=CHO	O	2.5	s.c.	+	9
CH_3	CH_3O	tri-ClC–CH(OH)	O	200 + 100[e]	s.c.	+	159
Phosphonothioates							
C_2H_5	C_2H_5O	C(CN)=NO	S	75	p.o.	–	9
C_2H_5	ClC_2H_4O	ClC_2H_4O	S	40	p.o.	–	135
Phosphinates							
n-C_5H_{11}	n-C_5H_{11}	Cl_2C=CHO	O	5 + 2 × 10[e]	i.v.	–	144
Phosphites							
n-C_4H_9O	n-C_4H_9O	n-C_4H_9O	none	100 daily[d]	der.	+	—[b]
Phosphorotrithioates							
C_2H_5S	C_2H_5S	C_2H_5S	O	10 × 100[e]	i.p.	–	160
n-C_3H_7S	n-C_3H_7S	n-C_3H_7S	O	10 × 5[e]	i.p.	+	160
n-C_4H_9S	n-C_4H_9S	n-C_4H_9S	O	7 × 100[e]	i.p.	+	71, 72, 160–164
n-$C_5H_{11}S$	n-$C_5H_{11}S$	n-$C_5H_{11}S$	O	7 × 200[e]	i.p.	–	73, 160–162
n-$C_6H_{13}S$	n-$C_6H_{13}S$	n-$C_6H_{13}S$	O	10 × 300[e]	i.p.	–	160
n-$C_8H_{17}S$	n-$C_8H_{17}S$	n-$C_8H_{17}S$	O	10 × 300[e]	i.p.	–	160

Phosphorotrithioite							
n-C_4H_9S	*n*-C_4H_9S	*n*-C_4H_9S	none	10×10^e	i.p.	+	73, 160–162
Phosphorofluoridates							
CH_3O	CH_3O	F	O	30	i.m.	+	158
C_2H_5O	C_2H_5O	F	O	0.75	i.m.	+	158
n-C_3H_7O	*n*-C_3H_7O	F	O	0.25	i.m.	+	158
iso-C_3H_7O	*iso*-C_3H_7O	F	O	0.3	i.m.	+	158
n-C_4H_9O	*n*-C_4H_9O	F	O	0.5	i.m.	+	158
iso-C_4H_9O	*iso*-C_4H_9O	F	O	1.5	i.m.	+	158
sec-C_4H_9O	*sec*-C_4H_9O	F	O	1.5	i.m.	+	158
n-$C_5H_{11}O$	*n*-$C_5H_{11}O$	F	O	2.5	i.m.	+	158
C_3H_7-$CH(CH_3)O$	C_3H_7-$CH(CH_3)O$	F	O	2.5	i.m.	+	158
cyclohexyl-O	cyclohexyl-O	F	O	2.5	i.m.	+	158
C_2H_5O	C_3H_7O	F	O	1.0	i.m.	+	158
Phosphonofluoridates							
iso-C_3H_7	CH_3O	F	O	5	i.m.	+	158
CH_3	C_2H_5O	F	O	3	i.m.	+	158
CH_3	*iso*-C_3H_7O	F	O	1	i.m.	+	158
C_2H_5	*iso*-C_3H_7O	F	O	1	i.m.	+	158
C_2H_5	*iso*-C_4H_9O	F	O	1	i.m.	+	158
CH_3	*iso*-C_4H_9O	F	O	3	i.m.	+	158
Phosphinofluoridates							
C_2H_5	C_2H_5	F	O	5	i.m.	–	158
n-C_3H_7	*n*-C_3H_7	F	O	5	i.m.	–	158
iso-C_3H_7	*iso*-C_3H_7	F	O	5	i.m.	–	158
n-C_4H_9	*n*-C_4H_9	F	O	2.5	i.m.	–	158
Phosphoroamidofluoridate							
C_2H_5O	$(CH_3)_2N$	F	O	5	i.m.	+	4

Table 2 *(Continued)*

Substituents				Dose	Route of administration[a]	Delayed neurotoxicity	References
R_1	R_2	R_3	X	(mg/kg)			
Phosphorodiamidofluoridates							
CH_3NH	CH_3NH	F	O	15	i.m.	+	165
C_2H_5NH	C_2H_5NH	F	O	15	i.m.	+	165
n-C_3H_7NH	*n*-C_3H_7NH	F	O	0.25	i.m.	+	165
n-C_4H_9NH	*n*-C_4H_9NH	F	O	0.1	i.m.	+	165
n-$C_5H_{11}NH$	*n*-$C_5H_{11}NH$	F	O	2.5	i.m.	+	165
n-$C_9H_{19}NH$	*n*-$C_9H_{19}NH$	F	O	100	i.m.	+	55, 59, 165
iso-C_3H_7NH	*iso*-C_3H_7NH	F	O	25	i.m.	+	165
iso-C_4H_9NH	*iso*-C_4H_9NH	F	O	1	i.m.	+	165
cyclohexyl NH	cyclohexyl NH	F	O	5	i.m.	+	165
Phosphorochloridates							
C_2H_5O	C_2H_5O	Cl	O	100	i.m.	–	158
n-C_4H_9O	*n*-C_4H_9O	Cl	O	20	i.m.	–	158
iso-C_4H_9O	*iso*-C_4H_9O	Cl	O	20	i.m.	–	158
Miscellaneous							
C_2H_5O	$(CH_3)_2N$	CN	O	5 X 3	i.m.	–	166
C_2H_5O	C_2H_5O	CN	O	50	i.m.	–	158
iso-C_3H_7O	*iso*-C_3H_7O	N	O	5	i.m.	+	165

[a] Abbreviations: s.c., subcutaneous; p.o., oral; i.v., intravenous injection; i.p., intraperitoneal injection; i.m., intramuscular injection.
[b] Abou-Donia, M. B., unpublished data.
[c] Cumulative dose.
[d] 90 daily doses.
[e] Interval between successive doses is one day.

icity active site. The abrupt cutoff point of this increase indicates that the corresponding hydrophobic areas on the neurotoxicity protein probably are of limited size, and can accommodate only three or four methylene groups.

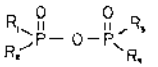
Very interesting results were obtained with fluoride derivatives (158). All of the phosphorofluoridates tested were delayed neurotoxic, but the relative potency of these esters varied and depended on the size of alkyl group. Gradual lengthening of the chain up to the *n*-propyl group led to a marked increase in the delayed neurotoxic effect. All tested alkylphosphonofluoridates produced OPIDN. The effectiveness of these esters to cause delayed neurotoxicity depended upon the alkoxy group rather than the alkyl. The lengthening of the chain increased the potency up to the *iso*-propyl radical, beyond which further lengthening did not influence the delayed neurotoxicity power. Substitution of the ester oxygen of the phosphorofluoridates by an amino group did not abolish the delayed neurotoxicity property, since all phosphorodiamidofluoridates were neurotoxic (55, 59, 165). Their neurotoxic potency, however, depended on the length of the *n*-alkyl carbon chain. Gradual increase in the chain length up to the butyl radical led to a marked increase of the delayed neurotoxicity potency. After this point, further increase of the alkyl chain reduced the delayed neurotoxic effect. These results indicate that the changes in the delayed neurotoxic activity are essentially attributed to changes in the ability of the organophosphorus ester to form a protein-organophosphorus ester complex by means of adsorption of the hydrocarbon radicals of the alkyl group on the hydrophobic areas of neurotoxicity protein. The total length of this area probably corresponds to the *n*-propyl and *n*-butyl groups. It is noteworthy that the suggestion that the fluorine atom plays a direct role in the development of the biochemical lesion was ruled out since it does not explain the delayed neurotoxicity of nonfluorine–containing organophosphorus esters (158).

PYROPHOSPHORUS ESTERS None of the pyrophosphorus esters tested caused delayed neurotoxicity (Table 3). This might be attributed to (*a*) the high acute cholinergic toxicity of these esters, which prevents the use of sufficient dosage to cause delayed neurotoxicity, and/or (*b*) the rapid metabolism of these esters into nonneurotoxic hydrolytic products (59, 134, 158, 159).

ALIPHATIC AROMATIC PHOSPHORUS ESTERS Among the 22 aliphatic aromatic phosphates tested, only five esters were shown to be delayed neurotoxic (Table 4). These chemicals were too scattered to draw conclusions about chemical structure-activity relationship. None of the phosphorothioates tested that contained P–S caused OPIDN.

In contrast, most alkyl phenylphosphonate and phosphonothioate esters

Table 3 *Pyrophosphorus esters tested for delayed neurotoxicity in chickens*



R_1	R_2	R_3	R_4	Dose (mg/kg)	Route of administration[a]	Neurotoxicity	Reference
Phosphates							
C_2H_5O	C_2H_5O	C_2H_5O	C_2H_5O	50	s.c.	–	158
iso-C_3H_7O	*iso*-C_3H_7O	*iso*-C_3H_7O	*iso*-C_3H_7O	3 x 100[b]	s.c.	–	59, 134
Phosphonates							
CH_3	C_2H_5O	*n*-C_3H_7O	CH_3	10	s.c.	–	158
CH_3	*n*-C_4H_9O	*n*-C_4H_9O	CH_3	10	s.c.	–	158
CH_3	C_2H_5O	C_2H_5O	C_2H_5	10	s.c.	–	158
CH_3	*iso*-C_3H_7O	*iso*-C_3H_7O	CH_3	10	s.c.	–	158
CH_3	C_2H_5O	C_2H_5O	CH_3	10	s.c.	–	158
Phosphoramidates							
$(CH_3)_2N$	$(CH_3)_2N$	$(CH_3)_2N$	$(CH_3)_2N$	160	p.o.	–	155
iso-C_3H_7NH	*iso*-C_3H_7NH	*iso*-C_3H_7NH	*iso*-C_3H_7NH	300	p.o.	–	59, 134

[a] See Table 2.
[b] Interval between successive doses is one day.

were delayed neurotoxic. Also, the alkyl phenylphosphonates given in a single administration were always more potent as delayed neurotoxicants than their corresponding alkyl phenylphosphonothioates. Again, in the series of phenylalkylphosphonates the delayed neurotoxic potency varied and depended on the length of the alkyl chain. The increase of the chain length increased the delayed neurotoxic potency up to the compound with two methylene groups. Further lengthening of the chain lessened the delayed neurotoxic potency and abolished it in the compound containing four methylene groups. These results are compatible with those of the aliphatic phosphate compounds, which suggested the presence of a hydrophobic area near the active center of the neurotoxicity protein. It is reasonable to suppose that the hydrocarbon radicals of the aliphatic and aromatic derivatives interact with the same part of the active center surface.

A very interesting correlation was obtained between the chemical structure and delayed neurotoxic potency of methyl phenylphosphonothioate esters (48, 69, 91, 157, 172–177). The unsubstituted phenyl ester did not cause delayed neurotoxicity at a single 500 mg/kg oral dose. Among the halogen-substituted phenylphosphonothioates all of the monochloro- and dichlorophenyl analogues caused delayed neurotoxicity. Furthermore, the

Table 4 Aliphatic aromatic phosphorus esters tested for delayed neurotoxicity in chickens

Substituents				Dose (mg/kg)	Route of administration[a]	Delayed neurotoxicity	References
R_1	R_2	R_3	X				
Phosphates							
CH_3O	CH_3O	4-NO_2-C_6H_4O	O	12	s.c.	–	9
C_2H_5O	C_2H_5O	4-NO_2-C_6H_4O	O	15	s.c.	–	139
n-C_3H_7O	*n*-C_3H_7O	4-NO_2-C_6H_4O	O	25	s.c.	–	9
iso-C_3H_7O	*iso*-C_3H_7O	4-NO_2-C_6H_4O	O	15	s.c.	–	134, 139
n-C_4H_9O	*n*-C_4H_9O	4-NO_2-C_6H_4O	O	30	s.c.	–	9
n-$C_5H_{11}O$	*n*-$C_5H_{11}O$	4-NO_2-C_6H_4O	O	22	i.v.	+	9
ClC_2H_4O	Cl-C_2H_4O	4-NO_2-C_6H_4O	O	100	p.o.	+	135, 137, 139
ClC_2H_4O	C_2H_5O	4-NO_2-C_6H_4O	O	20	s.c.	–	135, 137
CH_3O	CH_3O	1-(4-chlorophenyl-thio)-vinyl-O	O	50	s.c.	–	9
CH_3O	CH_3O	2,5-di-Cl-4-iodo-C_6H_2O	O	30	i.v.	–	9
CH_3O	CH_3O	C_6H_5-C_2H_4O	O	60	i.v.	–	9
C_2H_5O	C_2H_5O	2,3,5-tri-ClC_6H_2O	O	500	p.o.	+	134
ClC_2H_4O	ClC_2H_4O	7-(3-Cl-4-CH_3-coumarinyl)O	O	3000	p.o.	+	9, 47, 135
Cl-*n*-C_3H_7O	Cl-*n*-C_3H_7O	7-(3-Cl-4-CH_3-coumarinyl)O	O	400	p.o.	–	135
CH_3-CHCl-CH_2O	CH_3-CHCl-CH_2O	7(3-Cl-4-CH_3-coumarinyl)O	O	2000	p.o.	–	135

Table 4 *(Continued)*

Substituents				Dose	Route of administration[a]	Delayed neurotoxicity	References
R_1	R_2	R_3	X	(mg/kg)			
n-C_3H_7O	*n*-C_3H_7O	2-CH_3-C_6H_4O	O	10	i.m.	–	158
CH_3O	2-CH_3-C_6H_4O	2-CH_3-C_6H_4O	O	50	i.m.	–	158
C_2H_5O	C_2H_5O	2-CH_3-C_6H_4O	O	1000	p.o.	–	167
O-$CH_2CH(C_2H_5)$-C_4H_9	O-$CH_2CH(C_2H_5)$-C_4H_9	2-CH_3-C_6H_4O	O	1000	p.o.	+	167
iso-decyl-O	C_6H_5O	C_6H_5O	O	(120)[b]	p.o.	–	168
ethyl hexyl-O	C_6H_5O	C_6H_5O	O	(120)[b]	p.o.	–	168
n-C_4H_9O	*n*-C_4H_9O	C_6H_5O	O	(2.68)[b]	p.o.	–	168
Phosphorothioates							
CH_3O	CH_3O	4-NO_2-C_6H_4O	S	3 × 10[c]	i.v.	–	59
C_2H_5O	C_2H_5O	4-NO_2-C_6H_4O	S	3 × 20[c]	s.c.	–	59
iso-C_3H_7O	*iso*-C_3H_7O	4-NO_2-C_6H_4O	S	3 × 20[c]	s.c.	–	59
CH_3O	CH_3O	2,5-di-Cl-4-bromo-C_6H_5O	S	400	p.o.	–	139
CH_3O	CH_3O	2,4,5-tri-ClC_6H_2O	S	1600	s.c.	–	156
C_2H_5O	C_2H_5O	7-(3-Cl-4-CH_3-coumarinyl)O	S	100	der.	+	—[d]
CH_3O	CH_3O	3-Cl-4-NO_2-C_6H_3-O	S	1200	s.c.	–	155
CH_3O	CH_3O	S-1,2-dicarbethoxy-ethyl-thio	S	1000	s.c.	–	155, 156
CH_3O	CH_3O	3-CH_3-4-CH_3CO_2-C_6H_3O	S	1000	p.o.	–	169
C_2H_5O	C_2H_5O	$C_2H_5SC_6H_4O$	S	20	s.c.	–	155
C_2H_5O	C_2H_5O	4-$ClC_6H_4SCH_2S$	S	2 × 500[c]	s.c.	–	170

C_2H_5O	C_2H_5O	2-*iso*-C_3H_7-4-CH_3-pyrimidyl 6-0	S	10	s.c.	–	155
Phosphonates							
C_2H_5O	C_6H_5	4-NO_2-C_6H_4O	O	10	s.c.	+	64, 135
C_2H_5O	C_6H_5-CH_2	4-NO_2-C_6H_4O	O	10	s.c.	+	135
C_2H_5O	$C_6H_5(CH_2)_2$	4-NO_2-C_6H_4O	O	8	s.c.	+	135
C_2H_5O	$C_6H_5(CH_2)_3$	4-NO_2-C_6H_4O	O	8 × 4[c]	i.p.	+	135
C_2H_5O	$C_6H_5(CH_2)_4$	4-NO_2-C_6H_4O	O	8 × 4[c]	i.p.	–	135
CH_3O	C_6H_5	2,5-di-Cl-4-BrC_6H_2O	O	50	p.o.	+	171[d]
CH_3O	C_6H_5	2,5-di-ClC_6H_3O	O	10	p.o.	+	—[d]
C_2H_5O	C_6H_5	2,4-di-ClC_6H_3O	O	100	p.o.	+	—[d]
C_2H_5O	C_6H_5	4-CN-C_6H_4O	O	5	p.o.	+	—[d]
CH_3O	C_6H_5	CH_3-C_6H_4O	O	500	p.o.	–	157
2,5-di-Cl-4-BrC_6H_2O	C_6H_5	2,5-di-Cl-4-BrC_6H_2O	O	500	p.o.	–	157
C_2H_5O	*n*-C_5H_{11}	4-NO_2-C_6H_4O	O	12 × 2[c]	i.p.	+	135
C_2H_5O	*n*-$C_{10}H_{21}$	4-NO_2-C_6H_4O	O	16	s.c.	+	9
n-$C_5H_{11}O$	*n*-C_5H_{11}	4-NO_2-C_6H_4O	O	6	s.c.	+	9
CH_3O	CH_3	2,5-di-ClC_6H_3O	O	50	s.c.	+	9
C_2H_5O	C_2H_5	2,4,5-tri-ClC_6H_2O	O	30	s.c.	+	9
Phosphonothioates							
CH_3O	C_6H_5	H	S	500	p.o.	–	157
CH_3O	C_6H_5	2-ClC_6H_4O	S	300	p.o.	+	157
CH_3O	C_6H_5	3-ClC_6H_4O	S	275	p.o.	+	157
CH_3O	C_6H_5	4-ClC_6H_4O	S	285	p.o.	+	157
CH_3O	C_6H_5	2,3-di-ClC_6H_3O	S	100	p.o.	+	157
CH_3O	C_6H_5	2,4-di-ClC_6H_3O	S	160	p.o.	+	157
CH_3O	C_6H_5	2,5-di-ClC_6H_3O	S	50	p.o.	+	69, 91, 157, 171
CH_3O	C_6H_5	2,6-di-ClC_6H_3O	S	40	p.o.	+	157

Table 4 *(Continued)*

Substituents				Dose	Route of administration[a]	Delayed neurotoxicity	References
R_1	R_2	R_3	X	(mg/kg)			
CH_3O	C_6H_5	3,4-di-ClC_6H_3O	S	100	p.o.	+	157
CH_3O	C_6H_5	3,5-di-ClC_6H_3O	S	175	p.o.	+	157
CH_3O	C_6H_5	2,5-di-Cl-4-BrC_6H_2O	S	100	p.o.	+	48, 69, 157, 172
C_2H_5O	C_6H_5	2,4-di-ClC_6H_3O	S	800	p.o.	+	68, 69, 173
C_2H_5O	C_6H_5	2,5-di-ClC_6H_3O	S	1000	p.o.	–	157
C_3H_7O	C_6H_5	2,5-di-ClC_6H_3O	S	500	p.o.	–	157
C_4H_9O	C_6H_5	2,5-di-ClC_6H_3O	S	333	p.o.	–	157
C_2H_5O	C_6H_5	2,5-di-Cl-3-BrC_6H_2O	S	1000	p.o.	–	157
C_2H_5O	C_6H_5	2,4,5-tri-ClC_6H_2O	S	300	p.o.	–, +	9, 157
2,5-di-Cl-4-BrC_6H_2O	C_6H_5	2,5-di-Cl-4-BrC_6H_2O	S	500	p.o.	–	9, 157
C_2H_5O	C_6H_5	4-NO_2-C_6H_4O	S	25	p.o.	+	64, 69, 174–177
C_2H_5O	C_6H_5	4-CN-C_6H_4O	S	10	p.o.	+	67, 69
Phosphorodiamidofluoridates							
C_6H_5NH	C_6H_5NH	F	O	10	i.m.	+	165
4-CH_3-C_6H_4NH	4-CH_3-C_6H_4NH	F	O	20	i.m.	+	165
2-CH_3-C_6H_4NH	2-CH_3-C_6H_4NH	F	O	100	i.m.	+	165
Phosphinates							
C_2H_5	C_2H_5	4-NO_2-C_6H_4O	O	10	s.c.	–	144
n-C_4H_9	*n*-C_4H_9	4-NO_2-C_6H_4O	O	10	s.c.	–	144
n-C_5H_{11}	*n*-C_5H_{11}	4-NO_2-C_6H_4O	O	10	s.c.	–	144

[a] See Table 2.
[b] Cumulative dose.
[c] Interval between successive doses is one day.
[d] Abou-Donia, M. B., unpublished data.

dichlorophenyl esters were more potent in causing delayed neurotoxicity than the monochlorophenyl esters. Abou-Donia (69) has suggested that delayed neurotoxicity may be a chracteristic property of phenylphosphonothioate esters. The differential potency of halogen-containing phenylphosphonothioate esters seems to relate to lengthening the *O*-alkyl chain from methyl to ethyl: The delayed neurotoxic potency decreased as the chain lengthened. Further lengthening the chain to *n*-propyl and *n*-butyl abolished the delayed neurotoxic effect at relatively large doses. These results add more confirmative evidence to the hypothesis being proposed here—that on the active surface of the neurotoxicity protein there are hydrophobic areas of limited size which can accommodate hydrocarbon radicals of only a certain size.

A recent study has reported that the development of delayed neurotoxicity depended on the presence of the intact or only slightly changed molecular skeleton of the leptophos ester. The hydrolytic products had no delayed neurotoxic action (91). This study confirmed the theory that the initial event in delayed neurotoxicity is the phosphorylation of a nucleophilic site on the target protein. This hypothesis was further substantiated by studying the acute cholinergic and delayed neurotoxic effects of EPN stereoisomers (174) in chickens. These compounds inhibited plasma BuChE to a greater extent than brain AChE. The specificity of various EPN stereoisomers in producing delayed neurotoxicity suggests that a protein target is involved in the events leading to delayed neurotoxicity. Also, this active site must be stereochemically different from that of AChE.

TRIARYL PHOSPHATE ESTERS The following generalizations about the delayed neurotoxicity of triaryl phosphate esters (Table 5) may be made:

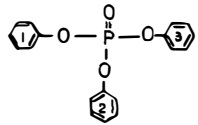
1. The unsubstituted tri-phenyl phosphate was not delayed neurotoxic at 1000 mg/kg single oral dose.

2. Delayed neurotoxicity depended on the size of the alkyl chain, the number of substituents, and the position in the ring.

3. The delayed neurotoxic potency of substituted alkyl phenyls decreased in the order $CH_3 > C_2H_5 > n\text{-}C_3H_7 > iso\text{-}C_3H_7 > sec$-butyl $\simeq$ *tert* butyl, a correlation that further confirms the hypothesis that a hydrophobic area of limited size exists near the neurotoxic site, on which the alkyl substituent is adsorbed.

4. Triaryl phosphate esters with one or more phenyl rings substituted in the 2-position (*ortho*) were generally delayed neurotoxic because of the size, configuration, and steric properties. It has been suggested that the presence of at least one hydrogen on the α-carbon atom, which allows the formation of cyclic derivative, is an essential requirement for the delayed neurotoxicity among triaryl phosphates (9). This suggestion, however, does not explain

Table 5 *Triaryl phosphate esters tested for delayed neurotoxicity in chickens*



Substituents			Dose	Route of administration[a]	Delayed neurotoxicity	References
1	2	3	(mg/kg)			
H	H	H	1000 (60)[b]	p.o.	–	16, 167, 168, 176
2-CH_3	H	H	50	p.o.	+	76
2-CH_3	2-CH_3	2-CH_3	25 (1.5)[b]	p.o.	+	16, 167, 168, 178
2-CH_3	2-CH_3	3-CH_3	250	p.o.	+	177
2-CH_3	2-CH_3	4-CH_3	25	p.o.	+	26
2-CH_3	3-CH_3	3-CH_3	50	p.o.	+	26
2-CH_3	3-CH_3	4-CH_3	50	p.o.	+	26
2-CH_3	4-CH_3	4-CH_3	50	p.o.	+	26, 167, 178, 179
2-CH_3	3,5-di-CH_3	3,5-di-CH_3	1000	p.o.	+	9, 176, 177
2,2-di-CH_3	2,2-di-CH_3	2,2-di-CH_3	(12)[b]	p.o.	–	168
2,3-di-CH_3	2,4-di-CH_3	2,3-di-CH_3	40 x 1000[c]	p.o.	+	180
2,4-di-CH_3	2,4-di-CH_3	2,5-di-CH_3	8 x 2500[c]	p.o.	+	180
2,5-di-CH_3	2,5-di-CH_3	2,5-di-CH_3	18 x 2500[c]	p.o.	–	180
2,6-di-CH_3	2,5-di-CH_3	2,6-di-CH_3	18 x 2500[c]	p.o.	–	180
2,3-di-CH_3	3,5-di-CH_3	3,5-di-CH_3	50 x 900[c]	p.o.	–	76
2,4-di-CH_3	3,5-di-CH_3	3,5-di-CH_3	28 x 900[c]	p.o.	–	76
2,5-di-CH_3	3,5-di-CH_3	3,5-di-CH_3	50 x 900[c]	p.o.	–	76
2,6-di-CH_3	3,5-di-CH_3	3,5-di-CH_3	30 x 900[c]	p.o.	+	76
2,4-di-CH_3	2,4-di-CH_3	3,5-di-CH_3	13 x 900[c]	p.o.	+	86
2,6-di-CH_3	2,6-di-CH_3	3,5-di-CH_3	50 x 900[c]	p.o.	+	76
2-CH_3-4-C_2H_5	2-CH_3-4-C_2H_5	2-CH_3-4-C_2H_5	2 x 700[c]	p.o.	–	9

2-C_2H_5	2-C_2H_5	2-C_2H_5	4 × 1200[c]	p.o.	+	140
2-C_2H_5	2-C_2H_5	4-CH_3	1000	p.o.	+	178, 179
2-C_2H_5	4-CH_3	4-CH_3	50	p.o.	+	178, 179
2-C_2H_5	3-C_2H_5	3-C_2H_5	50	p.o.	+	9
2-C_2H_5	3,5-di-CH_3	3,5-di-CH_3	500	p.o.	+	178, 179
2-*n*-C_3H_7	4-C_2H_5	4-C_2H_5	100	p.o.	+	178, 179
2-*n*-C_3H_7	2-*n*-C_3H_7	4-CH_3	4 × 500[c]	p.o.	+	179
2-*n*-C_3H_7	4-C_2H_5	4-C_2H_5	100	p.o.	+	179
2-*n*-C_3H_7	2-*n*-C_3H_7	2-*n*-C_3H_7	1000	p.o.	–	9, 178
2-*n*-C_3H_7	3,5-di-CH_3	3,5-di-CH_3	1000	p.o.	–	179
2-*iso*-C_3H_7	H	H	10 (12)[b]	p.o.	+	9, 168
2-*iso*-C_3H_7	2-*iso*-C_3H_7	H	600	p.o.	–	9
2-*iso*-C_3H_7	2-*iso*-C_3H_7	2-*iso*-C_3H_7	1000 (12)[b]	p.o.	–	9, 168
2-*sec*-C_4H_9	H	H	1200	p.o.	–	9
2-*tert*-C_4H_9	H	H	1200	p.o.	–	9
2-OCH_3	2-OCH_3	2-OCH_3	3000	p.o.	–	16
2-C_6H_5	H	H	(120)[b]	p.o.	–	168
2-C_6H_5	2-C_6H_5	2-C_6H_5	1000	p.o.	–	167
2-Cl	H	H	1000	p.o.	–	167
2-Cl	2-Cl	H	1000	p.o.	–	167
2-Cl	2-Cl	2-Cl	1000	p.o.	–	178
3-CH_3	3-CH_3	3-CH_3	1200	p.o.	–	9, 16
			25 × 250[c]	p.o.	+	181
3-CH_3	3-CH_3	4-CH_3	2500	p.o.	–	26
3-CH_3	4-CH_3	4-CH_3	2500	p.o.	–	26
3,5-di-CH_3	3,5-di-CH_3	4-C_2H_5	1000	p.o.	–	9, 178
3,4-di-CH_3	3,4-di-CH_3	3,4-di-CH_3	18 × 2500[c]	p.o.	–	180
3,5-di-CH_3	3,5-di-CH_3	3,5-di-CH_3	18 × 2500[c]	p.o.	–	180
3,4-di-CH_3	3,5-di-CH_3	3,5-di-CH_3	50 × 900[c]	p.o.	–	76
3-C_2H_5	H	H	1200	p.o.	–	9

Table 5 *(Continued)*

Substituents			Dose	Route of administration[a]	Delayed neurotoxicity	References
1	2	3	(mg/kg)			
3-C_2H_5	3-C_2H_5	3-C_2H_5	1200	p.o.	–	9, 177, 178, 168
3-C_2H_5	3-C_2H_5	4-C_2H_5	1200	p.o.	–	9, 179
3-*iso*-C_3H_7	3-*iso*-C_3H_7	3-*iso*-C_3H_7	1000	p.o.	–	9
4-CH_3	3-CH_3	4-CH_3	1200	p.o.	–	9, 16, 178
4-CH_3	4-CH_3	4-C_2H_5	2500	p.o.	–	179
4-CH_3	4-C_2H_5	4-C_2H_5	1200	p.o.	–	9
4-C_2H_5	4-C_2H_5	4-CH_3-CH(OH)	50	i.m.	+	182, 183
			70	s.c.	–	9
			50	i.v.	+	9
4-C_2H_5	4-C_2H_5	4-CH_3-CO_2	100	p.o.	+	10, 11, 182, 183
4-C_2H_5	4-CH_3CO_2	4-CH_3CO_2	25	i.m.	+	184
4-CH_3-CO_2	4-CH_3CO_2	4-CH_3-CO_2	100	p.o.	+	183, 185
4-CH_3-CO_2	H	H	1000	p.o.	–	185
4-*iso*-C_3H_7	H	H	1000 (120)[b]	p.o.	–	9, 168
4-*iso*-C_3H_7	4-*iso*-C_3H_7	H	1000	p.o.	–	9
4-*iso*-C_3H_7	4-*iso*-C_3H_7	4-*iso*-C_3H_7	1000	p.o.	–	9
4-*sec*-C_4H_9	H	H	1200	p.o.	–	9
4-*tert*-C_4H_9	H	H	1000 (120)[b]	p.o.	+ (–)	9, 168
4-*tert*-C_4H_9	4-*tert*-C_4H_9	H	1000	p.o.	–	9
4-*tert*-C_4H_9	4-*tert*-C_4H_9	4-*tert*-C_4H_9	450	p.o.	–	9
4-*n*-C_6H_{13}	H	H	(120)[b]	p.o.	–	168
4-nonyl acid	H	H	(120)[b]	p.o.	–	168

[a] See Table 2.
[b] Cumulative dose.
[c] Interval between successive doses is one day.

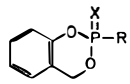
the finding that tri-2,5-di-CH_3-phenyl phosphate and several other esters containing hydrogen on the α-carbon atom (180) failed to cause delayed neurotoxicity at high doses. Also, the delayed neurotoxic activity of 4-*tert* -butyl diphenyl phosphate does not fit into this proposal. Even though the initial hydroxylation should not occur, this compound was reported to show a definite small delayed neurotoxic effect (9, 168). It is possible, however, that the delayed neurotoxic effect of this ester can be attributed to the 1.5% unidentified impurities in the test chemical (9). Increasing substitution in the phenyl ring markedly reduced the neurotoxic potency. Thus, introduction of 3,5-demethyl and similar substituents abolished or decreased delayed neurotoxicity. This may be explained by the steric hindrance effect of these groups which interfere with the adsorption of the organophosphorus ester with the neurotoxic site.

5. Triaryl phosphate esters with one or more phenyl rings substituted in the 3-position (*meta*) and lacking 2-substituent did not produce delayed neurotoxicity, even at large oral doses. Only the ester tri-3-tolyl phosphate was reported to cause delayed neurotoxicity following the oral administration of large doses (181).

6. Delayed neurotoxicity of triarylphosphate esters with one or more phenyl rings substituted in the 4-position (*para*) and lacking 2-substituent depends on the size of the 4-substituent. While esters containing tri-4-methyl or combinations of 4-methyl and 4-ethyl groups did not cause OPIDN, esters with 4-ethyl, 4-α-hydroxy-ethyl, or 4-acetyl groups were potent delayed neurotoxicants (9, 168). The results that triaryl organophosphorus esters with 2- and 4-substituents, but not 3-substituents, are delayed neurotoxic agents, suggests that two hydrophobic areas exist, separated by some hydrophilic group on the neurotoxicity active site. The first area can accommodate 2-methyl phenyl substituent while the distant area is compatible with 4-ethyl phenylphosphate residue.

SALIGENIN CYCLIC PHOSPHORUS ESTERS The number of aliphatic derivatives tested was too small to allow any generalization (Table 6). In the aryl series, however, the delayed neurotoxic potency increased as the position of the methyl group changed from 2- to 3-. The most active compound was found to be the 4-methyl phenyl phosphate derivative (186, 187). This increase in delayed neurotoxic potency can be attributed to the improved adsorption of the organophosphorus compound onto the neurotoxic site. Further substitution with 3,5-dimethyl groups decreased the potency. These results are in accord with the hypothesis that there are, on the surface of neurotoxicity site, some hydrophobic folds or pockets on which the saligenin cyclic phosphate can be adsorbed in a complementary manner.

Table 6 Saligenin cyclic phosphorus esters tested for delayed neurotoxicity in chickens



R	X	Dose (mg/kg)	Route of administration[a]	Delayed neurotoxicity	References
CH_3O	O	12	i.p.	–	186
CH_3O	S	80	i.p.	–	187
C_6H_5O	O	2	i.p.	+	186
2-CH_3-C_6H_4O	O	5	i.p.	+	186
3-CH_3-C_6H_4O	O	2	i.p.	+	186
4-$CH_3C_6H_4O$	O	0.5	i.p.	+	186
3,5-di-CH_3-C_6H_3O	O	8	i.p.	+	186
2-Cl-C_6H_4O	O	25	i.p.	+	186
C_2H_5	O	2	i.p.	–	186
$ClCH_2$	O	25	i.p.	–	186
C_6H_5	O	200	i.p.	+	186
C_6H_5	S	100	i.p.	+	186
$(CH_3)_2N$	O	10	i.p.	–	186

[a] See Table 2.

MISCELLANEOUS ARYL PHOSPHORUS ESTERS Table 7 lists miscellaneous arylphosphorus esters that were tested and found not to be delayed neurotoxic at large single oral doses (167). Again, the esters belonging to each series were too few to draw general correlations.

CONCLUSIONS The data described above indicate that it is very difficult to be certain that organophosphorus esters that did not cause delayed neurotoxicity under test conditions were truly not delayed neurotoxic. It seems that many of the organophosphorus esters that are potent inhibitors of esterases can cause delayed neurotoxicity; however, only those that are relatively stable and moderately toxic will produce the delayed neurotoxic syndrome. This is because the dosage required usually must be built up to a threshold level at the neurotoxic site. Rapid metabolism or overwhelming acute reactions do not permit this buildup.

These results allow us to formulate a general idea about the active center of the neurotoxicity target. In this model, there is a nucleophilic group (e.g. serine hydroxyl) on the active center of the neurotoxicity target protein which is stereochemically different from that of acetylcholinesterase. The site on this protein seems to have two hydrophobic areas. The first, nearer to the nucleophilic group (at approximately 5.7 Å to 7.7 Å from the nucleophilic group) is strictly limited and is complementary to three to four

Table 7 Miscellaneous aryl phosphorus esters tested for delayed neurotoxicity in chickens

Substituents				Dose	Route of	Delayed	
R_1	R_2	R_3	X	(mg/kg)	administration[a]	neurotoxicity	References
Phosphite							
2-CH_3-C_6H_4O	2-CH_3-C_6H_4O	2-CH_3-C_6H_4O	none	1000	p.o.	–	167
Phosphine oxide							
2-CH_3-C_6H_4O	2-CH_3-C_6H_4O	2-CH_3-C_6H_4O	O	1000	p.o.	–	167
Phosphoric acid							
2-$CH_3C_6H_4O$	2-CH_3-C_6H_4O	OH	O	2500	p.o.	–	167
Phosphorochloridate							
2-CH_3-C_6H_4O	2-CH_3-C_6H_4O	Cl	O	2500	p.o.	–	167
3,5-di-$CH_3C_6H_3O$	3,5-di-CH_3-C_6H_3O	Cl	O	2500	p.o.	–	167
Phosphorodichloridate							
2-CH_3-C_6H_4O	Cl	Cl	O	1000	p.o.	–	167
Phosphitechloridate							
3,5-di-CH_3-C_6H_3O	3,5-di-CH_3-C_6H_3O	Cl	none	5000	p.o.	–	167

[a] See Table 2.

methylene groups from the phosphorus atom in the aliphatic esters and to 2-alkyl phenyl or *o*-saligenin cyclic phosphate in triaryl phosphates. The second, more distant hydrophobic (at approximately 10.2 Å from the nucleophilic group) area is suitable for the adsorption of 4-ethyl phenyl radicals.

Working Hypothesis of the Mode of Action of OPIDN

Available evidence suggests that OPIDN is caused by a direct attack by neurotoxic organophosphorus esters on the axons rather than by a damage to neuronal perikaryon. Damage to nerve cell body is inconsistent with the observed axonal regeneration during intoxication and with the presence of distal and nonterminal disruption of multifocal degeneration. It is proposed that delayed neurotoxic organophosphorus esters phosphorylate the active center of neurotoxicity target proteins (enzymatic or structural) in the axons. These proteins have functions related to energy production and utilization required for normal axoplasmic transport. Such phosphorylation would result in a localized disruption of axoplasmic transport and the accumulation of mitochondria at the distal parts of the axons. Broken down mitochondria will release calcium ion into the axoplasm. This will disrupt the membrane control mechanisms regulating intracellular/extracellular ionic gradients, which leads to focal swelling in internodes, followed by focal degeneration, that spreads somatofugally to involve the entire distal axon. If exposure to organophosphorus esters continues, this process will progress into the proximal portions of the axon as the result of phosphorylation of more proteins. However, if exposure to these compounds ceases, the axons will begin to be supplied with unphosphorylated proteins, allowing some regeneration and restoration of axonal function. Consistent with this mechanism are some results of morphological, electrophysiological, and biochemical studies.

SUMMARY

In certain animals, including humans, exposure to some organophosphorus esters causes delayed neurotoxicity (OPIDN). The clinical condition becomes manifest after a delay period, first as ataxia, followed by paralysis. Lesions are characterized by degeneration of axons with subsequent secondary degeneration of myelin in the peripheral and central nervous systems. Recovery is only likely in mild cases, whereas more severe cases show symptoms of an upper motor neuron lesion in the lower limbs.

The risk of use of these chemicals is related not only to human sensitivity to this syndrome, but also to the fact that in most disasters involving OPIDN, humans were the prime victims. Therefore, the neurotoxic action

of a chemical is of great significance, since pesticides with this property are not recommended for use.

Although OPIDN has been recognized for over half a century, its mechanism of action is still unknown. It is believed, however, that the initial target in OPIDN is the phosphorylation of a neurotoxicity target protein in the nervous system. Study of the relationship between the chemical structure of organophosphorus esters and their neurotoxic potencies suggests that two hydrophobic areas may be present in the vicinity of the active site of the neurotoxicity protein.

This article attempts to present an up-to-date overview of OPIDN. Despite the difficulties attributed to experimental variations of the reported studies, I feel that several significant points have come forth from the data.

ACKNOWLEDGMENTS

The secretarial work of Ms. Marcine Basden is greatly appreciated. This review was supported in part by the NIEHS Grants No. ESO1186 and ESO2211 and NIOSH Grant No. OHOO823.

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