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ORGANOPHOSPHATE ESTER INDUCED CHRONIC NEUROTOXICITY (OPICN)

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BIOGRAPHICAL SKETCH

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ABSTRACT

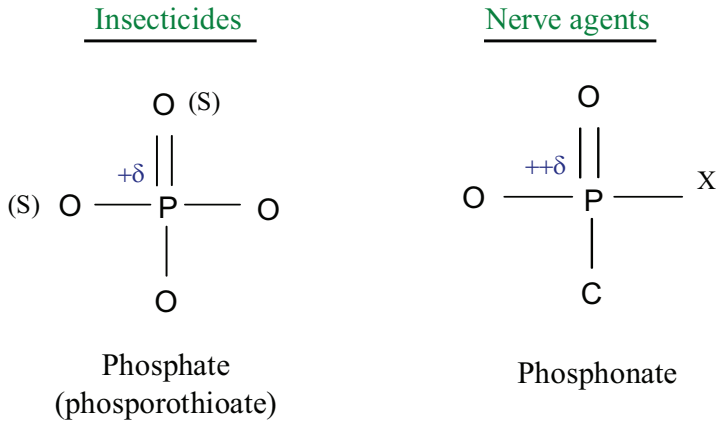
Organophosphorus compounds have been developed for use in medicine, industry, agriculture, as pesticides, and in warfare as nerve agents. Most of these chemicals are neurotoxic, and have three distinct neurotoxic actions. The primary action is the irreversible inhibition of acetylcholinesterase, resulting in the accumulation of acetylcholine and subsequent over stimulation of the nicotinic and muscarinic acetylcholine receptors leading to cholinergic effects. Following a single or repeated exposures, some of these compounds produce a delayed onset of ataxia, accompanied by a Wallerian-type degeneration of the axon and myelin in the most distal portion of the longest tracts in both the central and peripheral systems that is known as organophosphorus ester-induced delayed neurotoxicity (OPIDN). A third action that has been reported, since the introduction and extensive use of synthetic organophosphorus compounds in agriculture and industry half-a-century ago, has been recently termed organophosphorus ester-induced chronic neurotoxicity (OPICN), and is characterized by long-term, persistent, chronic neurotoxicity symptoms in individuals resulting from acute exposure to high doses that cause acute cholinergic toxicity, or from long-term, low-level, sub-clinical doses of these chemicals. Although the mechanisms of this neurodegenerative disorder are yet to be defined, available data suggest that large toxic doses of organophosphorus compounds cause acute necrotic neuronal cell death in the brain whereas sub-lethal or sub-clinical doses produce apoptotic neuronal cell death and involve oxidative stress.

INTRODUCTION

Phosphorus-containing organic compounds may be divided into two major subgroups; one has a trivalent phosphorus atom with a pyramidal

configuration and the other has a pentavalent phosphorus atom with a tetrahedral configuration. Because the trivalent phosphorus atom is electron-deficient in tri-substituted phosphorus acid (triaryl or trialkyl phosphites) such as triphenyl phosphite and tri-*iso*-propyl phosphite, are highly reactive and used as antioxidants, while *S,S,S*-tri-*n*-butyl phosphorotrithioite (merphos) is used as a cotton defoliant.[1] On the other hand, most synthetic organophosphorus compounds belong to the pentavalent group. These compounds are used in agricultural pesticides, nerve agents, pharmaceuticals, flame retardants and for industrial uses. While most organophosphorus esters are organophosphates or organophosphorothioates, nerve agents are phosphonate esters (see Figure 1).

Figure 1: Organophosphate molecules



Organophosphorus compounds have three distinctive neurotoxic actions:

1) Cholinergic neurotoxicity;

2) Organophosphorus ester-induced delayed neurotoxicity (OPIDN);

3) Organophosphorus ester-induced chronic neurotoxicity (OPICN).

CHOLINERGIC NEUROTOXICITY

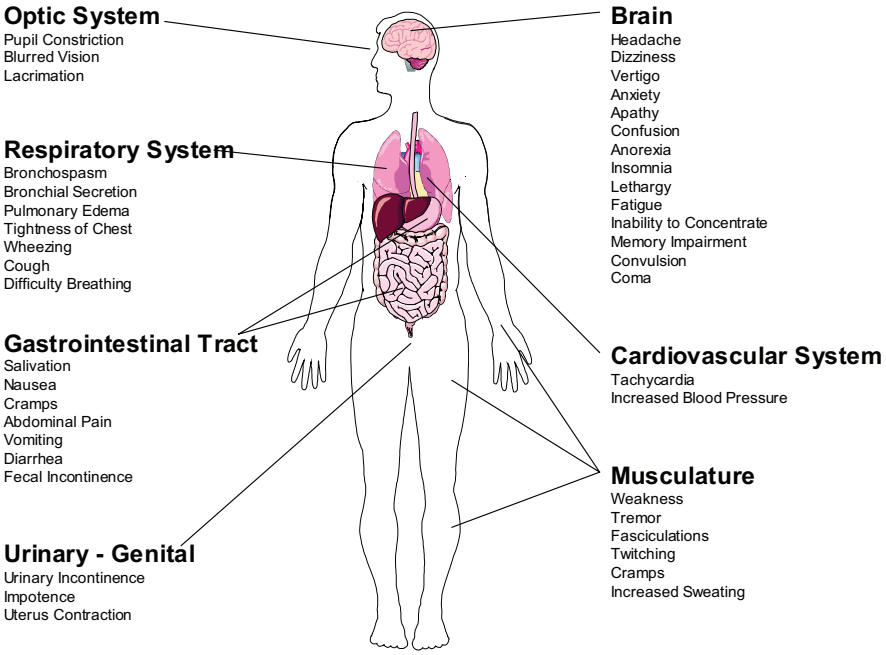
Acetylcholine (ACh) is a neurotransmitter involved in the functioning of the cholinergic nervous system. ACh is released in response to nerve stimulation and binds to post-synaptic acetylcholine receptors, resulting in a muscle contraction or a gland secretion. The action of ACh is rapidly terminated by hydrolysis with the enzyme acetylcholine esterase (AChE).[2,3] In the central nervous system (CNS), AChE is present in many areas, particularly in the cerebral cortex and striatum. In the peripheral nervous system, AChE is localized in the ganglia of the autonomic nervous system, in the parasympathetic nerve endings, and at the neuromuscular junction.[4]

Organophosphorus esters inhibit AChE by phosphorylating the serine hydroxyl group at the catalytic triad site.[3] The phosphoric or phosphonic acid ester formed with the enzyme is extremely stable and is hydrolyzed very slowly. Phosphorylated AChE also undergoes aging, a process that involves the loss of an alkyl group, resulting in a negatively charged monoalkyl

enzyme.[3] Organophosphorus compounds undergo detoxification by binding to other enzymes containing the amino acid serine. These enzymes include plasma butyrylcholinesterase,[5] and paraoxonase.[6] They are also dealkylated or dearylated by the cytochrome P450 mixed function oxidase system.

Inhibition of AChE results in the accumulation of acetylcholine (ACh) at both muscarinic and nicotinic receptors in central and peripheral nervous systems (see Figure 2).

Figure 2: Manifestations of Organophosphate Poisoning



Initially, excess ACh causes excitation, then paralysis of the cholinergic transmission, resulting in some or all of cholinergic symptoms, depending on the dose size, frequency of exposure, duration of exposure, route of exposure as well as other factors such as combined exposure to other chemicals, and individual sensitivity and susceptibility.

Symptoms of Cholinergic Toxicity Resulting from Organophosphate Poisoning

a. Central nervous system (brain and spinal cord)

Headache, dizziness, anxiety, apathy, confusion, restlessness, anorexia, insomnia, drowsiness, lethargy, fatigue, inability to concentrate, cognitive dysfunction, generalized weakness, tremors, depression of respiratory centers, depression of circulatory centers, convulsions and coma.[1]

b. Peripheral nervous system:

1 Parasympathetic autonomic postganglionic nerves (muscarinic symptoms):

a) *sweat glands:* increased sweating;

b) *salivation glands:* excessive salivation;

c) *lacrimation glands:* lacrimation (tearing);

d) *pupils:* constriction (pinpoint and miosis), spasm of accommodation;

e) *ciliary body:* blurred vision;

f) *respiratory tract:* bronchi constriction, increased bronchi secretion pulmonary edema, wheezing, tightness in chest, cough, difficult or labored breathing (dyspnea), and slow breathing (bradypnea);

g) *cardiovascular system:* bradycardia, decreased blood pressure;

h) *gastrointestinal system:* nausea, swelling and cramps, abdominal pain, vomiting, diarrhea, fecal incontinence;

i) *urinary bladder:* urinary frequency, urinary incontinence;

j) *uterus*: contraction.

2. Parasympathetic and sympathetic autonomic ganglia (nicotinic receptors):

a) *cardiovascular system:* tachycardia, increased blood pressure;

b) *skin:* pale skin (pallor).

3. Somatic motor neurons, neuromuscular junction (nicotinic receptors):

Skeletal muscles: muscle fasciculations (eyelids, fine facial muscles), twitching, muscle weakness, cramps, tightness in chest, respiratory difficulty, tremors, paralysis, cyanosis, arrest.

Severity of Symptoms

In organophosphorus ester poisoning, not all symptoms are seen in any one patient. The frequency and severity of the symptoms depend on the compound used and level, frequency, duration, and route of exposure.

Mild Poisoning: Initial symptoms are usually fatigue, dizziness, and sweating. These symptoms may also be accompanied by headache, inability to concentrate, cognitive dysfunction, weakness, anxiety, tremors of the tongue and eyelids, miosis (pupil constriction), and tightness of the chest.

Moderate Poisoning: In addition to the initial symptoms, the following symptoms may result: salivation, lacrimation, abdominal cramps, nausea, vomiting, slow pulse, bradycardia, fall in blood pressure, and muscular tremors.

Severe Poisoning: Pinpoint and non-reactive pupils, muscular twitching, wheezing, increase in bronchial secretion, respiratory difficulty, cough,

pulmonary edema, cyanosis, diarrhea, loss of sphincter and urinary bladder control, tachycardia, elevated blood pressure, convulsions, coma, heart block, and possibly death.

Acute and Chronic Exposure: Generally, the interval between a single acute toxic exposure to organophosphorus ester and onset of symptoms is very short, usually ranging from 5 to 60 minutes. Some individuals, however, may not develop the symptoms of poisoning until 24 hours after exposure.

Repeated small exposures have cumulative effects. Early symptoms of chronic organophosphorus insecticide exposure are influenza-like symptoms. As exposure continues, clinical manifestations appear until a full picture develops.[1]

Effect of Route of Exposure

Organophosphorus compounds are efficiently absorbed by inhalation, ingestion, and skin exposure. The route of entry influences the development of symptoms. In mild cases, only some of the symptoms become evident, depending upon the route of absorption. In severe poisoning, however, most of the signs appear, irrespective of the route of entry.[1]

Inhalation: Inhalation of organophosphorus esters first affects respiratory system and eyes. These effects may include: tightness of the chest, wheezing, a bluish discoloration of the skin, salivation, constriction of the pupils, aching in and behind the eyes, blurring of vision, tearing of the eyes runny nose, headache, inability to concentrate, and cognitive dysfunction.

Ingestion: Ingesting organophosphorus esters causes loss of appetite, nausea, vomiting, abdominal cramps, and diarrhea may take place within two hour of exposure.

Skin: Skin absorption results in sweating and twitching of the area affected usually within fifteen minutes to one hours of exposure.

Severe intoxication by organophosphorus esters via all routes may produce in addition to the above symptoms, body weaknesses, generalized muscle twitching, paralysis, leading to asphyxia and death. Furthermore, the following symptoms may occur: dizziness, confusion, staggering, slurs speech, generalized sweating, irregular or slow heartbeat, convulsions, and coma.

Human Exposure

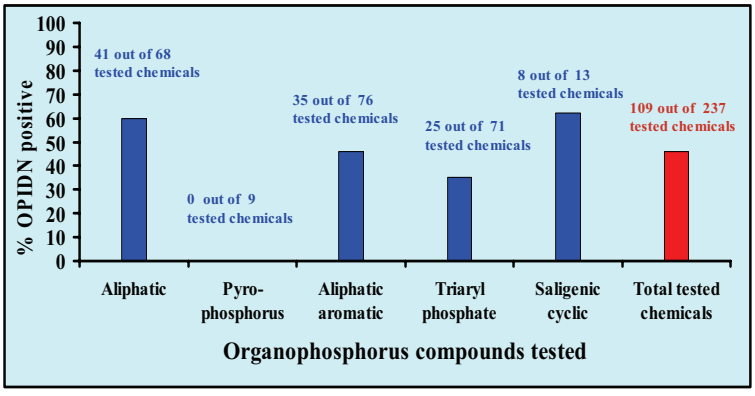
Recent human exposure, mostly via inhalation, to the organophosphorus nerve agent, Sarin has been documented in two terrorist incidents in Japan. Sarin was released at midnight in Matsumoto city on June 27, 1994.[7] Of the 600 persons who were exposed, 58 were admitted to hospitals, where seven died. While miosis was the most common symptom, severely poisoned patients developed CNS symptoms and cardiomyopathy. A few victims

complained of arrhythmia and showed cardiac contraction. Following a terrorist attack by Sarin in the Tokyo subway trains, at 8:05 am, on March 20, 1995, a total of 5,000 persons were hospitalized and 11 died.[8] Patients, with high exposure to Sarin in the Tokyo subway terrorist incident, exhibited the following symptoms: marked muscle fasciculation, tachycardia, high blood pressure (nicotinic responses), sneezing, rhenorrhea, miosis, reduced consciousness, respiratory compromise, seizures, and flaccid paralysis.[9] Patients with mild exposure complained of headaches, dizziness nausea, chest discomfort, abdominal cramps, and miosis. Interestingly, patients had pupillary constriction, even when their cholinesterase activity was normal. Furthermore, inhibition of red blood cell acetylcholinesterase activity was more sensitive than serum butrylcholinesterase activity.[10] The absence of bradycardia and excessive secretions, which are common in dermal or ingestion exposure, suggest that the major route of exposure to the Sarin gas was via inhalation. These patients were treated with atropine eye drops for marked miosis, and pralidoxime iodide (2-PAM).

ORGANOPHOSPHORUS PESTICIDE-INDUCED DELAYED NEUROTOXICITY (OPIDN)

Although many organophosphorus esters cause cholinergic neurotoxicity, only some of these compounds are capable of producing OPIDN. The results of studies that tested 237 organophosphorus compounds for the potential to produce OPIDN, showed that only 109 compounds were positive. Figure 3 shows these chemicals according to their chemical structure.

Figure 3: Percentage of Positive Organophosphorus Compounds Tested for OPIDN



Characteristics of OPIDN

OPIDN is a neurodegenerative disorder characterized by a delayed onset of prolonged ataxia and upper motor neuron spasticity from a single or repeated exposure to organophosphorus esters.[11,12,13,14] The neuropathological lesion is a central-peripheral distal axonopathy, caused by a chemical transection of the axon known as a Wallerian-type degeneration of the axon, followed by myelin degeneration of distal parts of long and of large-diameter tracts of the central and peripheral nervous systems.

Incidents of OPIDN have been documented for over a century (Table 1).

Table 1: Chronology of TOCP Induced OPIDN

Year	Country	Incidence	Cases
1899	France	Creosote	59
1930-31	USA	Contaminated Ginger Extract	Approx 50,000
1925-34	France, Germany, Switzerland	Apiol Abortifacient	200-500
1937	South Africa	Contaminated Cooking Oil	600
1940	Switzerland	Contaminated Cooking Oil	80
1942	United Kingdom	Manufacturing	3
1945	United Kingdom	Contaminated Cottonseed Oil	17
1943-47	Germany	Used as Cooking Oil	10-20
1947	Switzerland	Contaminated Food	73
1952	Switzerland	Contaminated Olive Oil	80
1955	South Africa	Contaminated Water	11
1955	Morocco	Used as Cooking Oil	10,000
1960	India	Contaminated Cooking Oil	58
1966	Rumania	Contaminated Alcohol	12
1967	Fiji	Contaminated Flour	56
1973	Morocco	Shoe Glue Exposure	40
1977-78	Sri Lanka	Contaminated Sesame Oil	23
1988	India	Contaminated Cooking Oil	2

The earliest recorded cases were attributed to the use of tri-*ortho*-cresyl phosphate (TOCP)-containing creosote oil for treatment of pulmonary tuberculosis in France in 1899.[13,14] In 1930, TOCP was identified as the chemical responsible for an estimated 50,000 cases of OPIDN in the Southern and Midwestern regions of the United States.[11-14] More recently, Himuro *et al*[15] reported that a 51-year old man who was exposed to Sarin during the Tokyo subway incident and survived its acute toxicity, died 15 months later. Neuropathological alterations and neurological deficits were consistent with dying back degeneration of the nervous system characteristic of OPIDN. This incident indicates that humans are more sensitive than experimental animals to Sarin-induced OPIDN, since it required 26-28 daily doses of LD_{50} (25 µg/kg, i.m.) Sarin to produce OPIDN in the hen.[16]

OPIDN has been classified into three classes: Type I caused by phosphates and phosphonates as well as their sulfur analogs, and Type II, produced by phosphites. Recently, previously unknown neurotoxicity produced by phosphines has been classified as Type III OPIDN.[13,14]

Factors Involved in the Development of OPIDN

To evaluate the potential for an organophosphorus compound to produce OPIDN, several factors should be considered (see Table 2).

Table 2: Threshold single and daily doses of organophosphorus compounds for the production of OPIDN in hens

Compound	Single Dose		Repeated Doses (mg/kg)			Single/ Daily	Single/ Total Repeated
	mg/kg	Route	Daily	Total	Route		
TOCP	250	Oral[17]	0.5	36	Oral[17]	500	6.9
Leptophos	200	Oral[18]	1.0	64	Oral[19]	200	3.1
Leptophos			0.5	25	Dermal[20]	400	4[8]
EPN	25	Oral[21]	0.1	1.9	Oral[22]	250	13
EPN			0.01	0.2	Dermal[23]	2,500	125
DEF	100	Dermal[24]	0.5	36	Dermal[25]	200	2.8

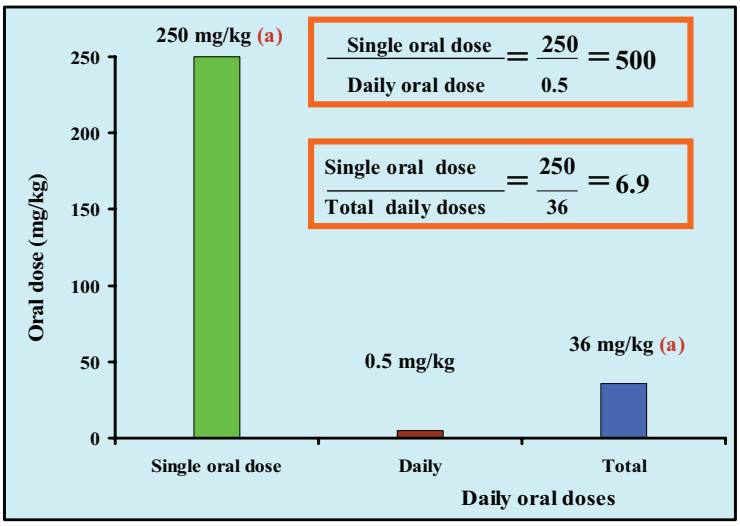
Species

Although humans are very susceptible to OPIDN, not all animal species are sensitive.[11,13] The susceptible species include cows, sheep, water buffaloes, dogs, cats, and chickens, while rodents are much less sensitive.[13] Also, since the young of susceptible species are not sensitive, the adult hen has become the animal model to study this disorder. Thus, a positive result that an organophosphorus insecticide can produce OPIDN in the hen is indicative that this compound is capable of causing this effect in humans. On the other hand, a negative result in the hen screening does not indicate that the test compound will not induce OPIDN in humans.[14] This conclusion is supported by several clinical reports indicating that some organophosphorus pesticides are capable of causing OPIDN in humans, despite the result that they did not produce it in the hen. These pesticides include: omethoate, trichloronate, trichlorfon, parathion, methamidophos, fenthion, and malathion. Subsequent studies have shown that malathion can produce OPIDN in hens and cats.[13,14]

Dose Size

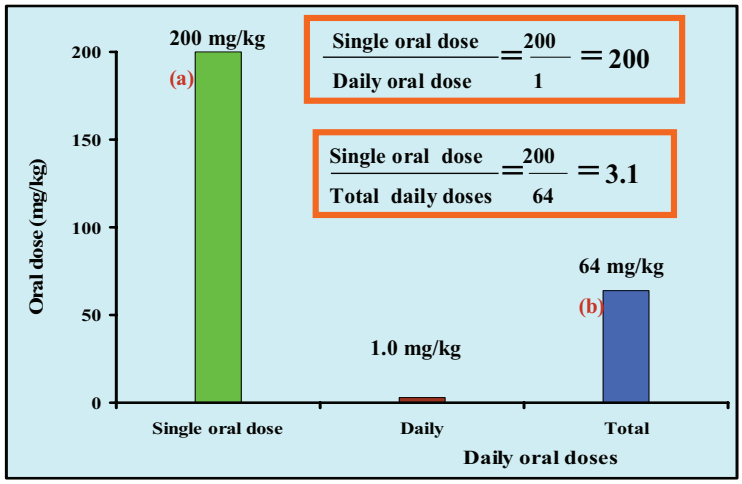
Chronic or subchronic exposures to small daily doses of organophosphorus compounds are more toxic and efficient in producing OPIDN than large single doses (Table 2, Figure 4). While the threshold for a single oral dose of the organophosphorus ester, TOCP that produced OPIDN in hens was 250 mg/kg,[19] a total of 36 daily 0.5 mg/kg doses induced OPIDN,[19] indicating that daily small doses were 7 times as effective as a single oral dose in producing OPIDN.

Figure 4: Threshold Single and Daily Oral Doses of TOCP for Producing OPIDN, (from [19])



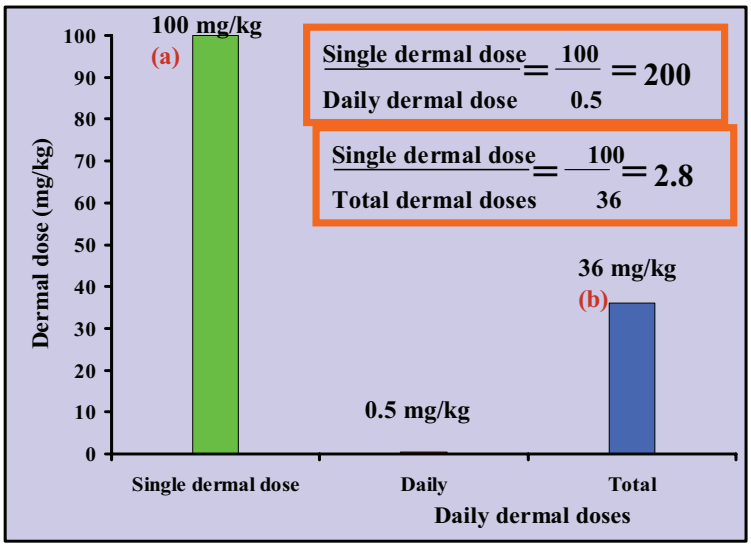
Also, while 200 mg/kg was the maximum oral dose of leptophos was the minimum single oral dose required to produce OPIDN,[24] it took 64 daily 1.0 mg/kg doses of leptophos, totaling 64 mg/kg to produce OPIDN (Table 2, Figure 5), demonstrating that daily small oral doses of leptophos were 3 times as effective as a single oral dose to produce OPIDN.[19]

Figure 5: Threshold Single and Daily Doses of Leptophos for Producing OPIDN (from[19,24])



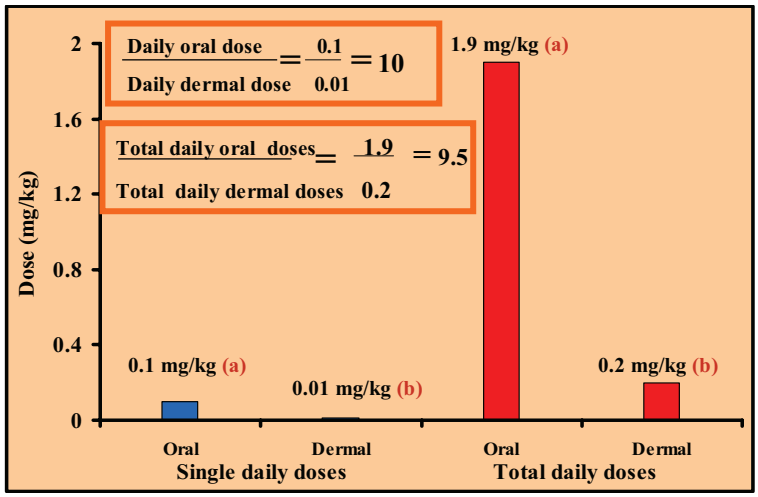
Similarly, daily dermal doses of small doses of the delayed neurotoxic organophosphorus compound DEF (Table 2, Figure 6) were three times as effective as a single dermal dose.[21,25]

Figure 6: Threshold Single and Daily Doses of DEF for Producing OPIDN (from [21,25])



While the threshold for a single oral dose of OP insecticide EPN to produce OPIDN in hens was 25 mg/kg,[21] it took 20 daily oral doses of 0.1 mg/kg to reach the same condition (Table 2, Figure 7).[22] Thus, the minimum daily oral dose and the cumulative total dose of EPN required to cause OPIDN are 250 and 13 times less than that of the single oral dose, respectively.

Figure 7: Threshold Daily Oral and Daily Dermal Doses of EPN for Producing OPIDN (from [22,23])

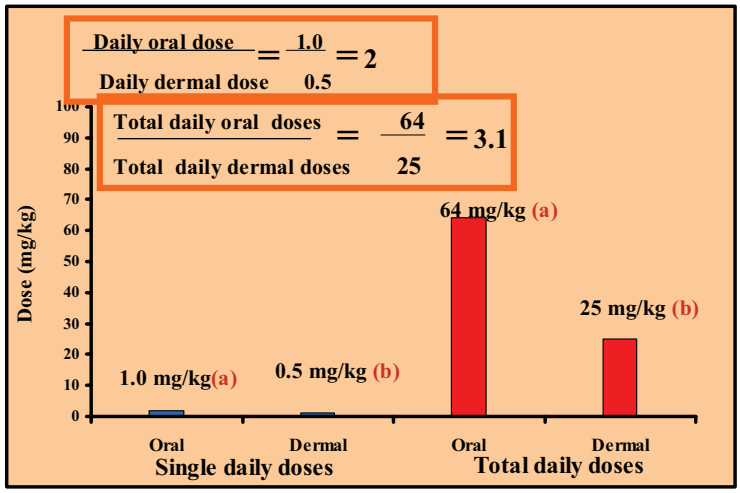


Route of Exposure

Organophosphorus compounds have more access to the neurotoxicity target through inhalation, and skin penetration than the gastrointestinal tract with inhalation being the most effective route of entry, preceded only by intravenous injection. The results of our studies show that dermal exposure to be a more effective route for the development of OPIDN (Table 2, Figure 8). Thus, while daily oral administration of 1.0 mg/kg leptophos for 64 days produced OPIDN in hens,[19] only 25 daily dermal applications of 0.5 mg/kg

caused OPIDN. The results indicate that dermal application was eight times as effective as oral administration in causing OPIDN.

Figure 8: Threshold Daily Oral and Daily Dermal Doses of Leptophos for Producing OPIDN (from [19,22])

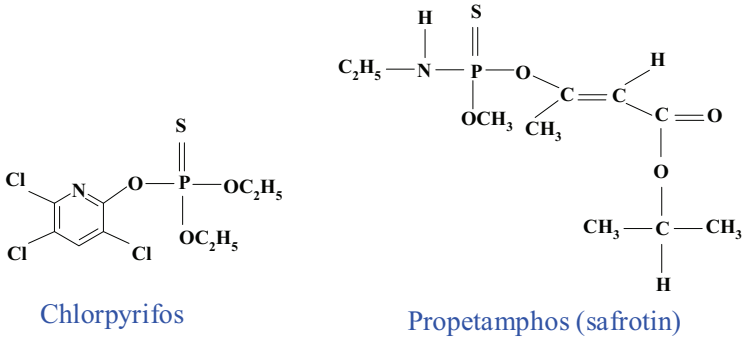


Also, the threshold daily oral administration of EPN that produced OPIDN was 0.1 mg/kg/day topical dose for 20 days.[23] These studies indicate that the minimum total daily dermal dose was 10 times as effective as daily oral administration in producing OPIDN.

Exposure to Other Chemicals

Concurrent exposure to organophosphorus compounds and other chemicals may increase their potency to induce OPIDN. The non-neurotoxicant solvent methyl isobutyl ketone given either via inhalation or dermal application, increased the severity of OPIDN induced by EPN.[26,27] Also, propetamphos, an OP that is capable of producing OPIDN (Figure 9), decreased the threshold oral dose of chlorpyrifos to induce the disorder.[28]

Figure 9: Factors involved in the Development of OPIDN: Combined Exposure with other Chemicals



Chlorpyrifos

Propetamphos (safrotin)

Oral LD_{50} in rats 150 mg/kg
Causes OPIDN at lethal doses

Oral LD_{50} in rats 119 mg/kg
Does not cause OPIDN

Other chemicals, such as the insect repellent DEET, may enhance the transdermal delivery of other pesticides[29] that compete with OPs for blood

and liver esterases such as arylesterases and aliesterases decrease the body's ability to detoxify these OPs, allowing larger concentrations of them reach in the neurotoxicity target. Recent studies have demonstrated that combined exposure to pyridostigmine bromide, DEET, and permethrin[30] DEET and permethrin,[31] and malathion, DEET, and permethrin[30] increased the neurotoxic action of individual compounds.

Neurological Dysfunction of OPIDN in Humans

OPIDN is characterized by a motor-sensory deficit resulting from Wallerian-type degeneration of the axon followed by demyelination of the central and peripheral nervous systems. Early changes in OPIDN result from degeneration of the peripheral nerves leading to flaccid paralysis. Long lasting effects are followed by degeneration of the central nervous system producing spasticity. The course of neurological deficits of OPIDN in humans may be divided into the following distinct phases.[13,14] Usually, not all the signs and symptoms are exhibited in one patient all the time.

Latent Period

Following exposure to organophosphorus compounds, there is a delay before onset of neurological deficits. The length of this latent period varies from a few days to weeks depending on the following factors:

1. The nature of the chemical
2. Route of exposure
3. Dose size and duration and frequency of exposure
4. Exposure to other chemicals
5. Individual differences

Progressive Phase

Early stage of OPIDN is a peripheral neuropathy and is characterized by:

1. Symmetric cramping, burning and/or stinging pain in the calves of the legs and less often in the ankles and feet.
2. Numbness and tingling in the feet and legs.
3. Bilateral dragging of the toes on the floor (foot-drop).
4. Next, the weakness spreads symmetrically to the hand.
5. "Glove-and-stocking" type decreased insensitivity.
6. Steppage gait
7. Positive Rhomberg
8. Absence of Achilles and ankle joint reflexes.
9. Neurological dysfunction may progress to flaccid paralysis.
10. Some patients exhibit urinary and bowel irregularities.

Stationary Phase

After early progression, symptoms, neurological deficits become stationary. During this phase, bilateral paraplegia or quadriplegia persists.

Improvement Phase

During this phase sensory symptoms disappear first followed by improvement of motor function, with hands and arms recovering before feet and legs. As improvement resulting from regeneration of the peripheral nervous system occurs, central nervous system damage becomes unmasked and are characterized by spasticity and exaggerated knee jerk.

Prognosis

The prognosis of patients with OPIDN depends on the severity of neurological deficits resulting from nervous system damage. Patients with mild cases of OPIDN will show clinical improvement and recovery in some cases, as peripheral nerves regenerate. In contrast, severe cases of OPIDN that involve damage to the central nervous system would persist as central nervous system does not regenerate. On the other hand, reversible changes in the central nervous system, such as edema, may subside with time. Also, other neurons may take over the function of damaged neurons, resulting in functional improvement. Patients with severe neurological dysfunction may suffer permanent neurological deficits despite the regeneration of their peripheral nerves.

Mechanisms of OPIDN

Previous studies eliminated the involvement of acetylcholinesterase[32] and butyrylcholinesterase[33] in the mechanisms of OPIDN.[34] The hypothesis that inhibition and ageing of neurotoxicity target esterase (NTE), an enzymatic activity preferentially inhibited by organophosphorus compounds, results in OPIDN has not been proven.[35] The most convincing evidence against this hypothesis is the recent finding that NTE-knockout mice are sensitive to the development OPIDN,[36,37] indicating that this enzyme is not involved in the mechanisms of OPIDN.

We hypothesized that the increased aberrant protein kinase mediated phosphorylation of cytoskeletal proteins could result in the destabilization of microtubules and neurofilaments (NF) leading to their aggregation and deregulation in the axon. Protein kinases are able to amplify and distribute signals, since a single protein kinase is able to phosphorylate many different target proteins. Several protein kinases are turned on by second messengers. For example, calcium/calmodulin-dependent protein kinase II (CaM kinase II) is inactive until it is bound by the calcium- calmodulin complex that induces conformational changes and causes the enzyme to unfold an inhibitory domain from its active site.[38]

We have demonstrated that aberrant hyperphosphorylation of cytoskeletal proteins is central to the pathogenesis of OPIDN. Our results showed that aggregated cytoskeletal proteins are not only a feature of OPIDN, but also a mediator of axonal dysfunction.[39,40,41,42,43,44,45,46,47,48,49,50,51] Sustained hyperphosphorylation of cytoskeletal proteins perturbs the dynamics of cytoskeleton and disrupts axonal functions and stability.

ORGANOPHOSPHATE-INDUCED CHRONIC NEUROTOXICITY (OPICN)

Numerous epidemiological studies have demonstrated that individuals who were exposed to a single large toxic dose or small sub-clinical doses of organophosphorus compounds have developed a long-term, neurological deficits that last for years after exposure, that was distinct from both cholinergic and OPIDN effects. These studies described a nervous system disorder induced by organophosphorus compounds, that involves neuronal degeneration and subsequent neurological, neurobehavioral, and neuropsychological consequences that has been referred to as "organophosphate ester-induced chronic neurotoxicity" or OPICN.[52]

Characteristics of OPICN

The concept of OPICN encompasses structural, functional, physiological, neurological, and neurobehavioral abnormalities, including neuropsychiatric alterations. OPICN has the following characteristics:

1. It is produced by exposure to large acutely toxic or small sub-clinical doses of organophosphorus compounds.
2. Clinical signs consist of neurological and neurobehavioral abnormalities.
3. Persistent, long-term clinical signs continue for a prolonged time, ranging from weeks to years after exposure.
4. Nervous system damage is present in the peripheral (PNS) and central nervous systems (CNS), with more involvement of the latter.
5. In the brain, neuropathological lesions are seen in various regions including the cortex, hippocampal formation, and cerebellum.
6. The lesion is characterized by neuronal cell death resulting from early necrosis or delayed apoptosis.
7. Neurological and neurobehavioral alterations are exacerbated by combining exposure with stress or other chemicals that cause neuronal cell death or oxidative stress.
8. Because CNS injury predominates, improvement is slow and complete recovery is unlikely.

Neurological and neurobehavioral alterations

Although the symptoms of OPICN are caused by damage to the peripheral (PNS) and central nervous systems (CNS) they are primarily related to injury

of the CNS system leading to neurological and neurobehavioral abnormalities. Studies on the effects of exposure to organophosphorus compounds over the past half-a-century have shown that chronic neurological and neurobehavioral symptoms include headache, drowsiness, dizziness, anxiety, apathy, mental confusion, restlessness, labile emotion, anorexia, insomnia, lethargy, fatigue, inability to concentrate, memory deficits, depression, irritability, confusion, generalized weakness and tremors.[53,54,55,56,57] Respiratory, circulatory and/or skin problems may be present as well in cases in chronic toxicity.[1] It should be noted that not every patient exhibits all of these symptoms. In 1997, Jamal carried out an extensive review of the health effects of organophosphorus compounds, and concluded that either acute or long-term, low-level exposure to these chemicals produce a number of chronic neurological and psychiatric abnormalities that he called organophosphate induced neuropsychiatric disorder, or COPIND.[58]

OPICN following large toxic exposures to organophosphorus compounds

Numerous studies have reported that some individuals who were exposed to large toxic doses of organophosphorus compounds and experienced severe acute poisoning but subsequent recovery, have eventually developed the long-term and persistent symptoms of OPICN that were not related to AChE inhibition.[59] Individuals with a history of acute organophosphate exposure reported an increased incidence of depression, irritability, confusion and social isolation.[60] Such exposure resulted in a decrease in verbal attention, visual memory, motoricity and affectivity.[61]

Exposure to Organophosphorus Pesticides

In 1991, Rosenstock et al reported that even a single exposure to organophosphates requiring medical treatment was associated with a persistent deficit in neuropsychological functions.[62] A study of the long-term effects in individuals who had acute toxicity with organophosphorus insecticides indicated a decrease in sustained visual attention and vibrotactile sensitivity that were dose-dependent.[63] In another study, one-fourth of the patients who were hospitalized following exposure to methamidophos exhibited an abnormal vibrotactile threshold between 10 and 34 months after hospitalization.[64] Callender et al,[65] have described a woman with chronic neurological sequelae after acute exposure to a combination of an organophosphorothioate insecticide, pyrethrin, piperonyl butoxide, and petroleum distillates and initial development of symptoms of acute, cholinergic toxicity. Twenty-eight months after exposure, she developed "delayed sequelae of gross neurological symptoms" consisting of coarse tremors, intermittent hemiballistic movements of the right arm and leg, flaccid fasciculations of muscle groups, muscle cramps, and sensory disturbances. Colosio et al,[66] reviewed the literature on the neurobehavioral toxicity of pesticides, and reported that some individuals who were acutely poisoned with organophosphorus compounds developed long-term impairment of their

neurobehavioral performance that was "an aspecific expression of damage and not of direct neurotoxicity". These results are consistent with neuronal necrosis induced by the organophosphorus insecticide, fenthion.[67]

Exposure to Organophosphorus Nerve agents

Organophosphorus nerve agents such as Sarin and Soman cause damage to the blood brain barrier leading to neuronal cell death with subsequent neurological deficits.[68,69] In the 1995 Tokyo subway Sarin incident, some victims of who developed acute cholinergic acute neurotoxicity, also developed long-term, chronic neurotoxicity characterized by CNS neurological deficits and neurobehavioral impairments.[70] Six to eight months after the Tokyo poisoning, some victims showed delayed effects on psychomotor performance, the visual nervous system, and the vestibule-cerebellar system.[71] Furthermore, females were more sensitive than males in exhibiting delayed effects on the vestibular-cerebellar system. Three years after the Matsumoto attack in Japan, some patients complained of fatigue, shoulder stiffness, weakness and blurred vision.[72] Others complained of insomnia, had bad dreams, husky voice, slight fever, and palpations.

Petras (1981) investigated the neuropathological alterations in rat brains, 15–28 days after intramuscular injections of large, acutely toxic doses (79.4–114.8 μg/kg) of the nerve agent Soman.[73] He reported that the brain damage in all four animals that developed seizures, was comparable to that present in three of the four animals that only exhibited limb tremor. Neuropathological lesions were characterized by axonal degeneration, seen in the cerebral cortex, basal ganglia, thalamus, subthalamic region, hypothalamus, hippocampus, fornix, septum, preoptic area, superior colliculus, pretectal area, basilar pontine nuclei, medullary tegmentum and corticospinal tracts. Although the mechanism of Soman-induced brain injury was not known, he noted that the lesions did not resemble those present in fetal hypoxia[74] or OPIDN.[75] These results are consistent with latter findings obtained after acute exposure to Soman[76,77] or Sarin.[78] Although Petras indicated that Soman-treated-animals did not need to have a seizure to develop lesions in rat brain lesions,[73] other investigators have recently reported that only mice exhibiting long-lasting convulsions developed neuropathological alterations in the brain.

Abdel-Rahamn et al,[69] demonstrated neuropathological alterations in rat brain 24 h after administration of an intramuscular LD_{50} dose (100 μg/kg) of Sarin. Neuronal degeneration was present in the cerebral cortex, dentate gyrus, CA1 and CA3 subfields of the hippocampal formation, and the Purkinje cells of the cerebellum. In these animals, both superficial (layers I-III) and deeper (IV-V) layers of the motor cortex and somatosensory cortex showed degenerating neurons. In the deeper layers of the cortex, degenerating neurons were seen in layer V. The layers III and IV neurons in the cortex are the source of axons of the corticospinal tract which is the largest descending fiber tract (or motor pathway) from the brain controlling movement of contralateral muscle group. Thus, Sarin-induced death of layer V neurons of the motor cortex could lead to considerable motor and sensory abnormalities, ataxia, weakness and loss

of strength. Furthermore, disruption of hippocampal circuitry because of the degeneration of neurons in different subfields can lead to learning and memory deficits.[79,80,81,82] Lesions in the cerebellum could result in gait and coordination abnormalities. Because the severely affected areas, such as the limbic system, corticofugal system and central motor system, are associated with mood, judgment, emotion, posture, locomotion, and skilled movements, humans exhibiting acute toxicity symptoms following exposure to large doses of organophosphates may develop psychiatric and motor deficits. Since the damaged areas of the brain do not regenerate, these symptoms are expected to be long-term effects.[68,83,84] The 0.50 × LD_{50} Sarin dose did not cause motor convulsions and only caused some Purkinje neuron loss. The dose 0.1 and 0.01 × LD_{50} Sarin did not cause any alterations at 24 hours after dosing. These results indicate that Sarin-induced acute brain injury is dose-dependent.

Shih et al,[85] have demonstrated that lethal doses (2 × LD_{50}) of all tested nerve agents (that is, Tabun, Sarin, Soman, Cyclosarin, VR and VX) induced seizures accompanied by neuropathological lesions in the brains of guinea pigs similar to those reported for Soman in other species.[86,87,88,89,90,91] Recent reports indicated that anticonvulsants protected guinea pigs against Sarin- and Soman-induced seizures and the development of neuropathological lesions.[92,93] Time-course studies also have reported that Sarin-induced brain lesions exacerbated over time and extended into brain areas that were not initially affected.[†,78] Similar results have been reported in a variety of species.[85,94,95] A subcutaneous dose of 104 µg/kg Soman, induced *status epileptus* in rats followed by degeneration of neuronal cells in piriform cortex and CA3 of the hippocampus.[91] Only mice treated with a subcutaneous dose of 90 µg/kg of Soman and developed long-lasting convulsive seizures exhibited neuropathological alterations.[96] Twenty-four hours after dosing, there were numerous eosinophilic cells and DNA fragmentation (TUNEL-positive) cells in the lateral septum, the endopiriform and entorhinal cortices, the dorsal thalamus, the hippocampus and amygdala. Animals that had only slight tremors and no convulsions did not show any lesions. Guinea pigs given a subcutaneous dose of 200 µg/kg Soman (2 × LD_{50}) developed seizures and exhibited neuropathological lesions between 24-48 h in surviving animals in the amygdale, the substantia nigra, the thalamus, the piriform, entorhinal and perirhinal cortices and hippocampus.[92] Male guinea pigs developed epileptiform seizure after receiving 2 × LD_{50} subcutaneous doses of the following nerve agents (µg/kg): Tabun 240, Sarin 84, Soman, 56, Cyclosarin 114, VX 16, or VR 22 accompanied by necrotic death of neuronal cells, with the amygdale having the most severe injury, followed by the cortex and caudate nucleus.[85]

† Unpublished results.

Exposure to Other Organophosphates

Kim et al,[97] reported that an intraperitoneal injection of 9 mg/kg (1.8 × LD_{50}) DFP in rats protected with pyridostigmine bromide and atropine nitrate, caused tonic-clonic seizures followed by prolonged mild clonic epilepsy, accompanied by early necrotic and delayed apoptotic neuronal degeneration. Early necrotic brain injury was seen between 1 and 12 hours after dosing, in the hippocampus and piriform/entorhinal cortices. On the other hand, typical apoptotic (TUNEL-positive) cell death began to appear at 12 hours in the thalamus. An intraperitoneal injection of 9 mg/kg (1.8 × LD_{50}) diisopropyl phosphorofluridate (DFP) caused severe early (15-90 min) tonic-clonic limbic seizures followed by prolonged mild clonic epilepsy.[97] Necrotic cell death was seen one hour after DFP administration mostly in the CA1 and CA3 subfields of the hippocampus and piriform/entorhinal cortices, which was exhibited as degeneration of neuronal cells and spongiform of neuropils. While the severity of hippocampal injury remained the same up to 12 hours, damage to piriform/entrohinal cortices, thalamus, and amygdala continued to increase up to 12 hours. Furthermore, apoptotic death (TUNEL-positive) of neuronal cells was seen in the thalamus at 12 h and peaked at 24 h. Rats that survived 1 × LD_{50} Sarin (95 μg/kg) exhibited persistent lesions mainly in the hippocampus, piriform cortex, and thalamus.[78] Furthermore, brain injury was exacerbated by time and three months after exposure, other areas that were not initially affected became damaged.

OPICN following Sub-clinical Exposures to Organophosphorus Compounds

Reports on OPICN in individuals following long-term, sub-clinical exposures, without previous acute poisoning have been documented in humans and animals.

Exposure to Low-level Organophosphorus Insecticides

Professional pesticide applicators and farmers who had been exposed to organophosphorus pesticides showed elevated levels of anxiety, impaired vigilance and reduced concentration.[98] A significant increase in hand vibration threshold was reported in a group of pesticide applicators.[99] Male fruit farmers who were chronically exposed to organophosphorus insecticides showed significant slowing of their reaction time.[100] Female pesticide applicators exhibited longer reaction times, reduced motor steadiness, and increased tension, depression, and fatigue compared to controls.[101] Workers exposed to the organophosphorus insecticide quinalphos during its manufacture exhibited alterations in the function of the central nervous system that were manifested as memory, learning, vigilance and motor deficits, despite having normal AChE activity.[102]

Kaplan et al,[103] reported persistent long-term cognitive dysfunction and defects in concentration, word finding, and short-term memory in individuals exposed to low sub-clinical levels of the organophosphorus insecticide

chlorpyrifos. These neurological deficits are in agreement with a recent study that evaluated the effects of chronic low-level exposure to the organophosphorus insecticide, chlorpyrifos in 22 patients for neurobehavioral impairments.[104] The study demonstrated, for the first time, the presence of an association between chlorpyrifos sprayed inside homes and offices and neurophysiological impairments of the body balance, visual fields, color discrimination, hearing, reaction time, and grip strength. Furthermore, these patients also had psychological impairments of verbal recall and cognitive function and two-thirds of them had been prescribed antidepressant drugs. The patients exhibited excessive respiratory symptoms that were accompanied by airway obstruction. Other chlorpyrifos-induced neurotoxicity incidents in humans have been reported.[105]

Published results of chlorpyrifos-induced OPICN in humans are consistent with a recent report that daily dermal application of 1.0 mg/kg chlorpyrifos to adult rats resulted in sensorimotor deficits.[106] Also, maternal exposure to 0.1 mg/kg chlorpyrifos during gestational days 4-20, caused an increased expression of glial fibrillary acidic protein (GFAP) in the cerebellum and hippocampus of offspring on postnatal day 30.[107] A major component of astrocytic intermediate neurofilament, GFAP is upregulated in response to reactive gliosis resulting from insults, such as trauma, neurodegenerative diseases, and exposure to neurotoxicants.[108] Also, daily dermal administration of $0.01 \times LD_{50}$ of malathion for 28 days caused neuronal degeneration in the rat brain that was exacerbated by combined exposure to the insect repellent DEET and/or the insecticide, permethrin.[109]

Exposure to "Sheep dip" Pesticides

A significant cognitive and neuropsychological deficits have been found in sheep dippers who had been exposed to organophosphorus insecticides.[110] Pilkington et al,[111] reported a strong association between chronic low-level exposure to organophosphate concentrates in sheep dips and neurological symptoms in sheep dippers, suggesting that long-term health effects may occur in at least some sheep dippers exposed to these insecticides over their working lives.

Exposure to Low-level Sarin

Rescue workers and some victims who did not develop any acute neurotoxicity symptoms nevertheless complained of a chronic decline of memory, three years and nine months after the Tokyo attack.[112] Upon their return from the Persian Gulf War, thousands of American and British veterans complained of a range of unexplained illnesses including chronic fatigue, muscle and joint pain, headaches, loss of concentration, forgetfulness, and irritability.[113] Many of the military personnel were exposed to low-level of the nerve agent Sarin that was released into the atmosphere in the region at Khamisiya, following the destruction of enemy's arsenal during the war.[114] Follow up studies in rats have established that large toxic doses of Sarin caused acute necrotic death of brain neurons,[69] whereas small doses resulted

in delayed apoptotic neuronal cell death.† Thus, OPICN can explain the report that Persian Gulf War Veterans are at an almost two-fold greater risk of developing ayotrophic lateral sclerosis (ALS) than other veterans.[115] This also is in agreement with the suggestion that the increase in ALS is "a war related environmental trigger".[116]

Exposure to Hydraulic Fluids and Jet Engine Lubricating Oils

Hydraulic fluids and Jet engine lubricating oils have been identified as possible contaminants in the recent incidents of smoke in the cabins of aircrafts.[117] For example, a total of 760 incidents involving 900 flight attendants subsequent to 1989, have been reported.[118] The components of these fluids, that include several organophosphates, have been identified and are listed in Table 3).

Table 3: Components of Some Jet Engine Oils and Hydraulic Fluids

Product	Components (wt%)
Engine lubricating oils	
Mobil Jet Oil 254	Tricresyl phosphate (TCP, 3%)
Mobil Jet Oil II	Tricresyl phosphate (TCP, 3%), N-Phenyl-1-naphthtylamine (PAN, 1%)
Hydraulic fluids	
Skydrol 5 (Solutia Inc.)	Triisobutyl phosphate, Triphenyl phosphate, Epoxy-modified alkyl ester
Skydrol 500B (Solutia Inc.)	Tributyl phosphate, Dibutyl phenyl phosphate, Butyl diphenyl phosphate, Epoxy-modified alkyl ester, 2,6-Di-tert-butyl-p-cresol
Skydrol LD-4 (Solutia Inc.)	Tributyl phosphate, Dibutyl phenyl phosphate, Epoxy-modified alkyl ester
Hyjet IV-A (Chevron)	Tributyl phosphate (79%), Cyclic aliphatic epoxide (<2.9%), Additives (<21%)

Although the main components of tri-cresyl phosphate (TCP) are approximately 15-25% tri-*meta*-cresyl phosphate, 5-10% tri-*para*-cresyl, 60-75% mixed *meta*- and *para*-cresyl phosphates, and small amounts of *ortho*-cresyl isomers (mainly in the mono-*ortho*-cresyl form with low amounts of di-*ortho*-cresyl isomers and minute amounts of the tri-*ortho*-cresyl isomer, resulting in more than ten cresyl isomers. Because jet oils contain up to 3% tri-cresyl phosphate as anti-wear agent, inhalation exposure to the chemical constituents in this product is likely. Although the cholinergic neurotoxicity of TCP isomers is low, six members of this group of chemicals contain one or more *ortho*-cresyl moiety and are capable of causing OPIDN (Table 4).

† Unpublished results.

Table 4: Isomers of Tri-cresyl phosphate (TCP)

There are ten possible TCP isomers:

***Ortho* content**	**Isomers**	**OPIDN**
Tri-*ortho*-TCP	o,o,o	✓
Di-*ortho*-TCP	o,o,m, o,o,p	✓
Mono-*ortho*-TCP	o,m,m, o,m,p, o,p,p	✓
Non-*ortho*-TCP	m,m,m, m,m,p, m,p,p, p,p,p	×

Consistent with this is the finding that inhalation exposure to it, in a manufacturing plant produced toxic polyneuritis.[119] Furthermore, jet engine lubrication oils contain up to 3% TCP, including 0.1% of TOCP,[120] the potent OPIDN producing isomer. Also, long-term inhalation exposure of chickens to concentrations between 23 and 110 mg/m^3 produced neurotoxic effects.[121] It has been suggested, also that humans are 10 to 100 times as susceptible to developing OPIDN as chickens.[122]

Available information suggest that inhalation of jet cabin contaminated air may be related to induction of organophosphate-induced chronic neurotoxicity. Air crew members including, pilots and flight attendants have consistently complained of neurological illnesses, such as headache, dizziness, cognitive dysfunction, difficulty concentrating, tremors and generalized weakness, lack of motor control, typical of OPICN. Although the neurotoxic effects of TCPs have been associated with the ortho isomer, results of experimental studies cannot be explained by the presence of the ortho isomer alone. A recent study reported an unexpected high neurotoxic potency of aviation engine lubricants containing 3% TCP levels and less than 0.02% of the ortho isomer.[123] In addition to the ortho isomer, the presence of TPCP has been confirmed in the two jet engine lubricating oils, Castrol 5000 and Exxon 2380.[124] Furthermore, our preliminary results showed that dermal exposure to each of the three isomers: that is, TOCP, TMCP, and TPCP caused sensorimotor deficits in rats and neuropathological lesions in the brain.[†] Although most of the investigations of cabin air-induced illnesses have focused on OPIDN, TCP and its constituent isomers, other components of the hydraulic fluids and engine lubricating oils should also be studied for their action in producing OPICN. These chemicals include: tributyl phosphate, tri-isobutyl phosphate, butyl diphenyl phosphate, dibutyl phenyl phosphate, and triphenyl phosphate. These chemicals may cause OPICN or contribute to its occurrence.

Neuronal and Glial Autoantibodies as Biomarkers for Neuronal Injury Induced by OPICN

Alterations of the cytoskeletal structure are prominent features in some neurological diseases and chemically induced neurological disorders. Neurofilament (NFP) and Tau proteins are major constituents of the axon and

[†] Unpublished results.

microtubule associated protein-2 (MAP-2) are mostly present in the dendrites. Increased autoantibodies of these proteins are indicative of axonal degeneration. Also, increased autoantibodies against myelin basic protein (MBP) are consistent with axonal demyelination. The increase of glial fibrillary acidic protein (GFAP) autoantibodies is suggestive of neuronal injury.

Sera obtained from eight flight crew members and from healthy adults (controls) were assayed for the presence of autoantibodies against proteins associated with neurogenesis, that is, high molecular weight neurofilament protein (NFP-200), MAP-2, and Tau proteins; myelinogensis, that is, MBP; and gliogenesis, that is, GFAP, that have been used as markers for injury to the central nervous system. Autoantibodies against tubulin, a protein present in all tissues, including the nervous system, have been determined as markers for global tissue damage. Finally, autoantibodies against the glial calcium-binding protein S-100 were determined as markers for acute traumatic brain injury. Autoantibodies against neuronal proteins, NFP-200, MAP-2, Tau proteins, and MBP and those against the protein associated with gliogenesis, GFAP, that were increased in some sera correlated with the neurological condition of the patients.[†] Autoantibodies against the global protein, tubulin, were not significantly higher than controls. Autoantibodies against S-100 protein are used as an internal standard to determine the precision of the assay. The results show that the level of these autoantibodies was low in the patient and controls. The results indicate the high precision of the results. They also suggest the absence of acute traumatic brain injury in the cases and controls.

Many neurotoxicants, such as organophosphorus esters, as well as other insecticides, solvents and heavy chemicals cause neuronal cell death and axonal degeneration and over-expression of GFAP, with subsequent release of neuronal, myelin, and glial proteins into circulation, followed by the formation of autoantibodies against these proteins. While not diagnostic for specific disease, the presence of circulating autoantibodies against neuronal and glial proteins, at higher levels in patients who had been exposed to neurotoxic chemicals and developed neurological deficits, over that of controls, can be used as further confirmation for chemical-induced nervous system injury. The low level of autoantibodies of S100 protein in the serum indicates that the neuronal condition is not related to an acute injury, but is rather a chronic condition.

The serum profile of increased autoantibodies against nervous system proteins in flight crew members, is consistent with neurological deficits and in the absence of other neurological diseases, it is concluded that it is consistent with chemical such as TCP-induced nervous system injury.

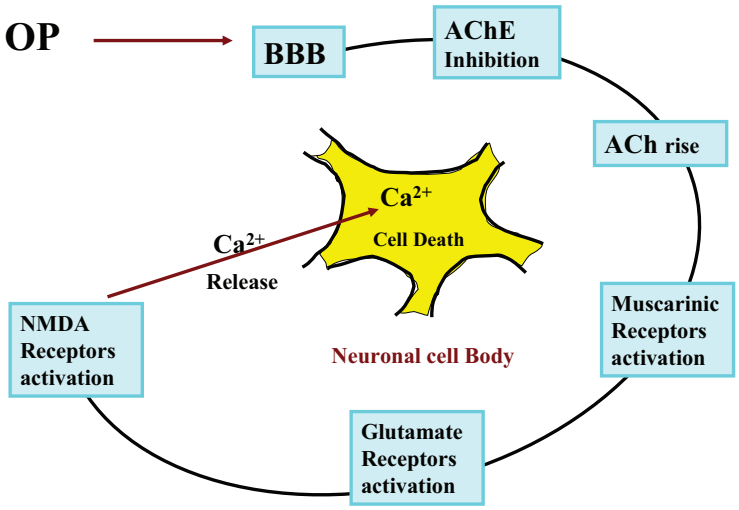
Mechanisms of OPICN

Recent studies have shown that large toxic doses of organophosphorus compounds cause early convulsive seizures and subsequent encephalopathy,

leading to the necrotic death of brain neuronal cells, whereas small doses produce delayed apoptotic death. Pazdernik et al,[91] have proposed the following five phases that result in organophosphorus compound-induced cholinergic seizures: initiation, limbic *status epilepticus*, motor convulsions, early excitotoxic damage, and delayed oxidative stress.

Necrosis: In addition to breaking down the blood brain barrier and producing early seizures, large toxic doses of organophosphorus compounds result in the activation of the glutamatergic system and the involvement of Ca^{2+}-related excitotoxic process,[119,120] possibly mediated by the *N*-methyl-*D*-aspartate (NMDA) sub-type of glutamate receptors.[125,126] Accumulated ACh, resulting from acute inhibition of AChE by organophosphorus compounds, leads to activation of glutamatergic neurons and the release of the excitatory L-glutamate amino acid neurotransmitter,[92] that is a major agonist of NMDA receptors and a major excitatory neurotransmitter in the CNS as well as being a potent excitotoxin.[127] This leads to increased depolarization and subsequent activation of the NMDA subtype of glutamate receptors, and the opening of NMDA ion channels, resulting in massive Ca^{2+} fluxes into the postsynaptic cell and the disruption of postsynaptic calcium homeostasis.[128] This results in the production of free radicals and degradation of intracellular components and mitochondrial damage, and causing neuronal degeneration (see Figure 10).[129]

Figure 10: Cholinergic Cascade Following a Large Toxic Dose of Organophosphate (OP)



Activation of nitric oxide synthase, following stimulation of NMDA receptor increases the level of nitric oxide, which functions as a signaling or cytotoxic molecule responsible for neuronal cell death.[130] As a retrograde messenger, nitric oxide induces the release of several neurotransmitters including excitatory amino acid L-glutamate (131) which alters neurotransmitter balance and affects neuronal excitability.[131] The production of nitric oxide is enhanced in AChE inhibitor-induced seizure.[132,133] Kim et al,[97] have demonstrated the involvement of nitric oxide in organophosphate-induced

seizures and the effectiveness of nitric oxide synthesis inhibitors in preventing such seizures.

Apoptosis: Small doses of organophosphorus compounds cause delayed neuronal cell death that involves free radical generation, that is, reactive oxygen species (ROS). Organophosphates that cause mitochondrial damage/dysfunction, cause depletion of ATP and increased generation of ROS, which results in oxidative stress.[134,135] ROS cause fatal depletion of mitochondrial energy (ATP), induction of proteolytic enzymes and DNA fragmentation, leading to apoptotic death.[131,136,137] These results are consistent with the DNA damage detected in the lymphocytes in peripheral blood in eight individuals, following residential exposure to the organophosphorus insecticides chlorpyrifos and diazinon.[138] The brain is highly susceptible to oxidative stress-induced injury for several reasons: its oxygen requirements are high; it has a high rate of glucose consumption; it contains large amounts of peroxidisable fatty acids; and it has relatively low antioxidant capacity.[136,137] A single sub-lethal dose of $0.5 \times LD_{50}$ Sarin, that did not induce seizures, nevertheless caused delayed apoptotic death of rat brain neurons in the cerebral cortex, hippocampus, and Purkinje cells of the cerebellum 24-h after dosing.[90,†] Furthermore, rats treated with a single $0.1 \times LD_{50}$ dose of Sarin which did not exhibit brain histopathological alterations 1, 7 or 30 days after dosing, nevertheless showed apoptotic death of brain neurons in the same areas mentioned above, one year after dosing.[69,†] These results are consistent with the sensorimotor deficits exhibited by Sarin-treated animals three months after exposure; the animals continued to deteriorate when tested six months after dosing.

Increased AChE gene expression: Recent studies suggested that AChE may play a role in the pathogenesis of OPICN, similar to that reported for Alzheimer disease.[139,140] We have demonstrated that Sarin induced AChE gene in the same regions of the brain that underwent neuronal degeneration.[141] AChE has been shown to be neurotoxic *in vivo* and *in vitro*, and accelerate assembly of amyloid peptide in Alzheimer's fibrils, leading to death through apoptosis.[142] Further studies demonstrated increased AChE expression in apoptotic neuroblastoma SK-N-SH cells after long-term culture.[142] These results support the association between AChE and neuronal apoptosis in Alzheimer's disease. Brain AChE was shown to be toxic to neuronal (Neuro 2a) and glial-like (B12) cells.[140] Also, transgenic mice over-expressing human AChE in brain neurons underwent progressive cognition deterioration.[143] The results suggest that Sarin provokes an endogenous cell suicide pathway in susceptible neurons such as caspase-3 pathway, resulting in the release of AChE into adjacent brain tissues. AChE aggregates and initiates more apoptotic neuronal death. Thus this cascade amplification results in the progressive neuronal loss that is the hallmark of Sarin-induced chronic neurotoxicity. It is noteworthy, that a common symptom of both OPICN and

† Unpublished results.

Alzheimer's disease is memory deficit, suggesting that OPICN accelerate aging process following exposure to organophosphorus compounds.

CONCLUSIONS

Previous reports have indicated that after exposure to organophosphorus compounds, an individual could develop acute cholinergic neurotoxicity, followed by OPICN. In a few cases, OPIDN may occur with or without the development of cholinergic neurotoxicity and then latter, OPICN ensues. Furthermore, OPICN may take place after long-term, low-level exposure to organophosphorus compounds and without the development of acute neurotoxicity. Because the long-term, persistent effects of OPICN result from neuronal degeneration of the peripheral and central nervous systems induced by organophosphates, it is unlikely that improvement is the consequence of the regeneration of brain neurons, since such repair phenomenon is not typical of the CNS. Clinical improvement may take place, however, through the repair of the PNS. Also, reversible changes in the CNS that might be initially present (for example, edema), could later subside, and result in the appearance of repair. Furthermore, if the damage is not too extensive, other neurons having the same function could meet the added demands and maintain normal activity. When the central nervous system is severely damaged, neither of these repair mechanisms is possible and some loss of function could occur.

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