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PATTERN OF NEUROTOXICITY OF *n*-HEXANE, METHYL *n*-BUTYL KETONE, 2,5-HEXANEDIOL, AND 2,5-HEXANEDIONE ALONE AND IN COMBINATION WITH *O*-ETHYL *O*-4-NITROPHENYL PHENYLPHOSPHONOTHIOATE IN HENS

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This investigation was designed to study the neurotoxicity produced in hens by the aliphatic hexacarbons n-*hexane, methyl* n-*butyl ketone (MnBK), 2,5-hexanediol (2,5-HDOH), and 2,5-hexanedione (2,5-HD) following daily dermal application of each chemical alone and in combination with* O-*ethyl* O-*4-nitrophenyl phenylphosphonothioate (EPN). Dermal application was carried out on the unprotected back of the neck. To assess whether the joint neurotoxic action of various chemicals is caused by the enhancement of absorption through the skin or by interaction at the molecular level, two additional experiments were performed. In the first experiment, EPN was dissolved in each of the aliphatic hydrocarbons prior to their topical application. In the second experiment, EPN was dissolved in acetone and applied at a different location from that of the aliphatic hexacarbons. Dermal application was carried out for 90 d followed by a 30-d observation period. The results show that (1) hens treated with EPN developed severe ataxia followed by improvement during the observation period; (2)* n-*hexane produced leg weakness with subsequent recovery, whereas the same dose of MnBK, 2,5-HDOH, or 2,5-HD produced clinical signs of neurotoxicity characterized by gross ataxia; (3) concurrent dermal application of EPN with* n-*hexane or 2,5-HDOH at the same site or at different sites produced an additive neurotoxic action; (4) simultaneous dermal application of EPN and MnBK at different sites resulted in an additive effect, whereas it caused potentiation when applied at the same site; and (5) concurrent topical application of EPN and 2,5-HD produced a potentiating neurotoxic effect. While no histopathologic lesion was produced at the end of the observation period when any test chemical was applied alone, binary treatments of EPN and aliphatic hexacarbons resulted in histopathologic changes in some hens, with morphology and distribution characteristic of EPN neurotoxicity. The joint potentiating or additive action of aliphatic hexacarbons on EPN neurotoxicity was: 2,5-HD > MnBK > 2,5-HDOH >* n-*hexane. The mechanism of this joint action seems to be related both to enhancing skin absorption of EPN and/or to its metabolic activation by* n-*hexane and its related chemicals.*

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INTRODUCTION

Toxicological studies and epidemiological evidence implicated that concurrent exposure to the organophosphorus insecticide leptophos (*O*-4-dibromo-2,5-chlorophenyl *O*-methyl phenylphosphonothioate) and the industrial solvents *n*-hexane and toluene caused an outbreak of neuropathy in workers at a Bayport, Texas, plant that produced this insecticide (Staff Report, 1976). Large quantities of *n*-hexane were used in the same factory to produce a resin called Klyrvel, and some workers reported high dermal exposure to *n*-hexane (Xinteras et al., 1978). Although clinical manifestations exhibited by these workers resembled those of organophosphorus poisoning, *n*-hexane or even toluene could not be excluded as the causative or contributing agents to the neurologic dysfunctions. Although it was suggested that the effects from leptophos and *n*-hexane could be additive, it was proposed that excessive dermal exposure with solvents may have increased the total body burden of leptophos, thus contributing to its neurotoxic effects (Xinteras, et al., 1978).

Leptophos belongs to a group of organophosphorus compounds capable of causing delayed neurotoxicity (Abou-Donia et al., 1974; Abou-Donia and Preissing, 1976a,b). Organophosphorus-induced delayed neurotoxicity (OPIDN) was first demonstrated in humans by tri-*o*-cresyl phosphate (TOCP) (Smith et al., 1930). Later, other species (cats, dogs, cows, mallard ducklings, and chickens) were found to be sensitive to OPIDN (About-Donia, 1981). While mice (Lapadula et al., 1985) and rats (Veronesi and Abou-Donia, 1982) are susceptible to OPIDN, they are much less sensitive. OPIDN is characterized by delayed ataxia accompanied by Wallerian-type degeneration of the central and peripheral nervous system (Abou-Donia, 1981). *n*-Hexane and its related aliphatic hexacarbons—e.g., methyl *n*-butyl ketone (M*n*BK), 2,5-hexanediol (2,5-HDOH), and 2,5-hexanedione (2,5-HD)—have been shown to produce or have been implicated in the production of neuropathy in humans or experimental animals (Spencer and Schaumburg, 1975; Abdo et al., 1982; Abou-Donia et al., 1982). *n*-Hexane is metabolized to M*n*BK, 2,5-HDOH, and 2,5-HD (Kramer et al., 1974; Abdel-Rahman et al., 1976). The descending order of neurotoxic potency of these chemicals in hens is: 2,5-HD > 2,5-HDOH > M*n*BK > *n*-hexane.

Although the production and use of leptophos has since ceased, other delayed neurotoxic organophosphorus pesticides—e.g., *O*-ethyl *O*-4-nitrophenyl phenylphosphonothioate (EPN) and *S,S,S,*-tri-*n*-butyl phosphorotrithioate (DEF) (Abou-Donia, 1981)—are being manufactured and formulated in factories where concurrent exposure to aliphatic hexacarbon solvents may be possible. The purpose of this investigation was (1) to investigate if subchronic (90 d) dermal exposure to *n*-hexane, M*n*BK, 2,5-HDOH, or 2,5-HD produces neuropathy in the hen when applied alone; (2) to determine the joint neurotoxic action resulting from binary dermal application of EPN and each of these aliphatic hexacarbons when applied at the

same site or at different sites; and (3) to characterize the morphology and distribution of the neuropathologic lesions induced by single and binary treatments.

In this study the hen was used as the test animal, since it is sensitive to neurotoxic effects produced by both classes of chemicals (Abou-Donia, 1981, Abou-Donia et al., 1982). Dermal exposure was used with both chemicals since it was implicated as a major route of body entry when workers were exposed to leptophos and *n*-hexane. Also, dermal absorption was suggested as the major entry of *n*-hexane in humans (Nomiyama and Nomiyama, 1974) and proposed as responsible for an outbreak of industrial neuropathy (Nomiyama et al., 1973). A more recent study demonstrated that significant amounts of M*n*BK can penetrate intact skin of humans and dogs (DiVincenzo et al., 1978).

METHODS

Chemicals

n-Hexane (99%), methyl *n*-butyl ketone (2-hexanone, M*n*BK, 99%), 2,5-hexanedione (2,5-HD, 97%), and 2,5-hexanediol (2,5-HDOH, 99%) were purchased from Aldrich Chemical Co., Milwaukee, Wis. Technical-grade (85%) *O*-ethyl *O*-4-nitrophenyl phenylphosphonothioate (EPN) was provided by E. I. DuPont De Nemours and Company, Inc., Wilmington, Del.

Birds

The birds used were leghorn laying hens, 14 mo old, with a mean weight (± SE) of 1.6 ± 0.04 kg and specified pathogen-free (Featherdown Farm, Raleigh, N.C.). Hens were placed in individual stainless-steel cages in a humidity-controlled (40–60%) and temperature-controlled (21–23°C) room with a 12-h artificial light, 12-h dark cycle. The hens were allowed to acclimatize to their environment for a week prior to randomization and assignment to treatment and control groups. They were supplied with feed (Layena Chicken Feed, Ralston Purina Co., St. Louis, Mo.) and water ad libitum.

Treatment of Birds

All chemicals were applied topically with a micropipette over an area of 10 cm^2 on the unprotected back of the neck for 90 d. Groups of 5 hens were treated daily with a topical dose of 1.0 mg/kg EPN in 0.1 ml acetone, 0.1 ml acetone, or 1.0 mmol/kg of *n*-hexane, M*n*BK, 2,5-HDOH, or 2,5-HD. To study the effect of concurrent dermal application of EPN and the *n*-hexane-type chemicals on hens, two additional experiments were carried out. In the first experiment, EPN at 1.0 mg/kg was dissolved in 1.0 mmol/kg of each aliphatic hexacarbon prior to their topical application.

In the second experiment, EPN at 1.0 mg/kg dissolved in 0.1 ml acetone was applied at a location different from that of *n*-hexane and its metabolites (1.0 mmol/kg). Following the application period, the hens were kept for a 30-d observation period. Body weights were monitored weekly, and hens were examined daily for neurologic dysfunctions.

Clinical Assessment of Neurotoxicity

Control and treated hens were examined daily to check for signs of neurotoxicity while in the cage and when forced to move outside the cage. Clinical signs were graded into four stages: T_1, mild ataxia; T_2, gross ataxia; T_3, severe ataxia; and T_4 ataxia with near paralysis (Abou-Donia, 1978). For graphical presentation, birds were assigned numerical value of 1 to 4, corresponding to T_1 to T_4 of ataxia, and normal animals were assigned the value of zero.

Histopathological Studies

At the end of the observation period, hens were anesthetized with CO_2 and perfused with 10% neutral phosphate-buffered formalin. The spinal cord and sciatic, peroneal, and tibial nerves were excised and dehydrated in graded ethanol and imbedded in paraffin. Parasaggital, longitudinal, and cross sections (8 μm) of the spinal cord and cross and longitudinal sections of the periopheral nerves were stained with hematoxylin and eosin combined with luxol fast blue (H and E–LFB). Sections from peripheral nerves were also stained with Holmes silver stain (Abou-Donia and Preissig, 1976a). Severity of histopathologic lesions was described as follows: (1) equivocal changes are characterized by rare swollen axons unaccompanied by fragmentation, phagocytosis, or loss of myelin; (2) mild to moderate degeneration is marked by degenerated axons and myelin and may contain foci of phagocytic cells; and (3) when severe lesions occurred there was almost complete destruction of axons and myelin in a given tract such as the anterior columns or within extensive areas of peripheral nerve.

Quantification of Neurotoxicity

Neurotoxicity index. Neurotoxicity was quantified by determining the neurotoxicity index (NTI), which involves the following parameters: (1) the latent period before onset of neurologic dysfunction, (2) the progression and severity of neurologic deficit, and (3) the frequency and severity of histopathologic lesions (Abou-Donia et al., 1982). Changes in the three areas were ranked beginning with minimal changes (Jonckheere, 1954). The NTI was calculated as the mean of the three ranks of hens in each of the three categories.

Coneurotoxicity coefficient. The joint neurotoxic action produced by applying dermal doses of EPN and *n*-hexane-type chemicals simul-

taneously on hens was determined by calculating the coneurotoxicity coefficient ($CNC_{EPN,\ aliphatic\ hexacarbon}$) as follows:

$$CNC_{EPN,\ hexacarbon} = \frac{NTI_{EPN,\ hexacarbon}\ (Experimental)}{NTI_{EPN} + NTI_{hexacarbon}\ (Expected)}$$

where $NTI_{EPN,\ hexacarbon}$ is the neurotoxicity index resulting from simultaneous application of EPN and a hexacarbon solvent, NTI_{EPN} is the neurotoxicity index when EPN is applied alone and $NTI_{hexacarbon}$ is the neurotoxicity index when a hexacarbon solvent is applied alone. When CNC is greater than 1, synergism or potentiation is indicated; antagonism occurs when CNC is less than 1, while additive effect takes place when CNC equals 1.

Statistics

Significance of the difference between body weight of control and treated hens was assessed by the Student's two-tailed *t*-test. A p value of 0.05 or less was considered significant.

RESULTS

General Observations

All hens treated with dermal doses of EPN or *n*-hexane-type chemicals alone or in combinations suffered a slight loss of weight at onset of ataxia, which was regained in most of the chickens during the 30-d observations period (results not shown).

Hens treated with a 1.0-mg/kg daily dermal dose of EPN developed severe ataxia (T_3), which improved to gross ataxia (T_2) during the observation period (Fig. 1). Also, hens exposed to a daily dermal 1-mmol/kg dose of M*n*BK, 2,5-HDOH, or 2,5-HD developed gross ataxia. On the other hand, daily dermal applications of *n*-hexane caused only leg weakness after 67 d with subsequent recovery 110 d after beginning of administration. In every case simultaneous application of EPN and *n*-hexane or its related chemicals, on the same site or at different sites, caused an increase in the severity of clinical signs to severe ataxia (T_3), which regressed to T_2 during the 30-d observation period (Fig. 1).

Necropsy Examination

At sacrifice, major tissues were examined. No changes were observed when treated and control birds were compared for size, shape, weight, or color.

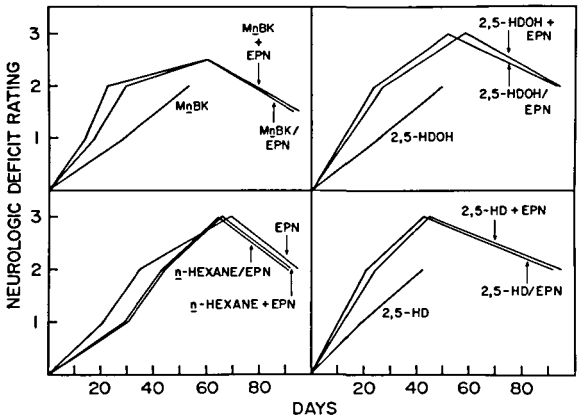

FIGURE 1. Neurologic deficits in hens exposed to daily dermal doses of EPN at 1 mg/kg, 1 mmol/kg *n*-Hexane, M*n*BK, 2,5-HDOH, or 2,5-HD, or combinations. Neurologic deficit rating is described in the methods. The results show days before onset of each stage of neurologic deficit and represent the mean from five hens.

Histopathological Changes

Histopathological examination was carried out on spinal cord and peripheral nerves from all treated and control hens.

Spinal cord. Histopathologic alterations observed in the spinal cord were dependent on the treatment. Neither subchronic treatment with daily

FIGURE 2. Longitudinal section of spinal cord from a hen treated with a daily dermal dose of 1.0 mmol/kg M*n*BK for 90 d. Swollen axons are evident in spinal cord long tract. H and E-LFB, X250.

dermal doses of EPN nor *n*-hexane produced any histopathologic lesions in the spinal cord. Equivocal histologic changes were present in spinal cords of two hens from the groups treated with dermal doses of M*n*BK and 2,5-HDOH, and in one hen treated with 2,5-HD. These histopathologic changes are characterized by swollen axons without obvious fragmentation of axon or myelin, similar to the longitudinal section of spinal cord from a hen treated with M*n*BK (Fig. 2).

A daily concurrent topical application of EPN at 1 mg/kg and 1 mmol/kg of an aliphatic hexacarbon produced equivocal and unequivocal histopathologic changes in some treated hens. When EPN was applied at the same site or at different sites from *n*-hexane, one hen from each of these groups showed unequivocal changes in the spinal cord. These changes consisted of internodal axonal swelling and degenerated axons and myelin characteristic of EPN-induced lesions seen in the longitudinal (Fig. 3) and cross (Fig. 4) sections from hens treated with EPN and *n*-hexane.

Hens treated with daily dermal doses of EPN dissolved in M*n*BK exhibited changes in the spinal cord of one hen and unequivocal changes in another hen. On the other hand, EPN and M*n*BK applied at different sites produced one equivocal and three unequivocal histopathologic changes in the spinal cord. A focus of axonal degeneration with phagocytes containing myelin debris is seen in the long tract of spinal cord from one of these hens (Fig. 5).

Treatment with EPN on the same or at different sites from 2,5-HDOH produced an equivocal and an unequivocal histopathologic alteration in

FIGURE 3. Longitudinal section of spinal cord from a hen treated with a daily dermal dose of EPN at 1.0 mg/kg in 1.0 mmol/kg *n*-hexane. Axonal swelling is evident. H and E-LFB, X163.

FIGURE 4. Cross section of spinal cord from a hen treated with a daily dermal dose of EPN at 0.1 mg/kg in 0.1 ml acetone and of 1.0 mmol/kg *n*-hexane. Multiple swollen axons are present in anterior column of spinal cord adjacent to anterior horn. H and E-LFB, X163.

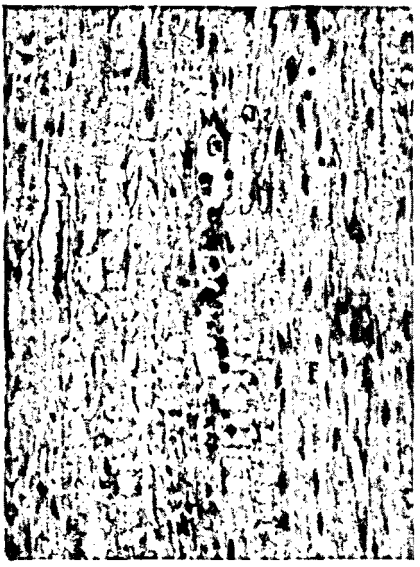

FIGURE 5. Longitudinal section of spinal cord from a hen treated with a daily dermal dose of EPN at 1 mg/kg in 0.1 ml acetone and of 1.0 mmol/kg M*n*BK. A focus of axonal degeneration with phagocytes containing myelin debris in long tract in spinal cord. H and E-LFB, X260.

each group of hens. A longitudinal section of spinal cord of a hen from the group dermally treated with EPN dissolved in 2,5-HDOH exhibits multiple foci of axonal degeneration with phagocytes containing myelin debris (Fig. 6).

Topical application of EPN at 1 mg/kg dissolved in 2,5-HD produced unequivocal changes in 2 hens and equivocal changes in 3 hens. Axonal swelling in multiple internodal segments of the same axon is seen in the longitudinal section of spinal cord of a hen from this group (Fig. 7). When dermal doses of EPN and 2,5-HD were applied at different sites, they produced unequivocal changes in one hen and equivocal alterations in three hens. Large axonal swelling with phagocytosis is seen in a longitudinal section of spinal cord from one of these hens (Fig. 8).

Peripheral nerves. In this study only two hens that were treated with daily dermal doses of EPN dissolved in M*n*BK showed unequivocal degeneration of axons and myelin (Table 1, Fig. 9).

Relative Neurotoxicity

The relative neurotoxic potency of daily dermal doses of 1 mg/kg EPN, or 1 mmol/kg *n*-hexane, M*n*BK, 2,5-HDOH, or 2,5-HD, or combinations was quantified by determining the neurotoxicity index (NTI) for each treatment (Table 1). The NTI includes the onset and progression of neurologic deficits as well as the severity and frequency of neuropathologic changes. Control birds had an NTI value of zero. The NTI values indicated

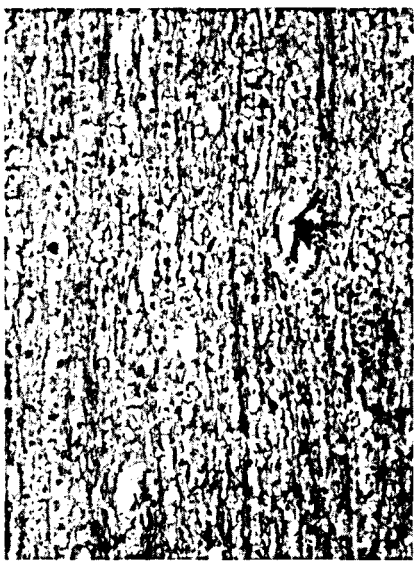

FIGURE 6. Longitudinal section of spinal cord of a hen treated with a daily dermal dose of EPN at 1 mg/kg in 1.0 mmol/kg 2,5-HDOH. Multiple foci of axonal degeneration with phagocytes in spinal cord, some containing myelin debris. H and E-LFB, X163.

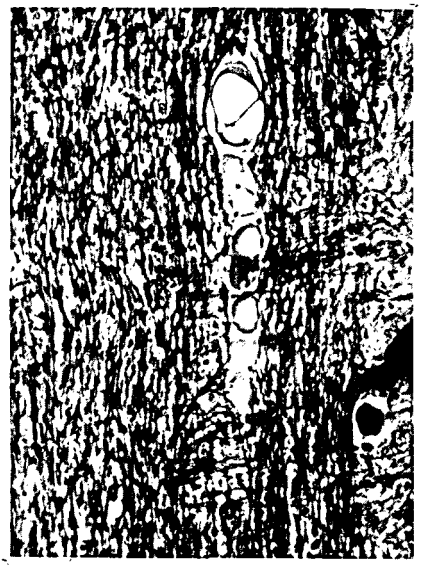

FIGURE 7. Longitudinal section of spinal cord from a hen treated with a daily dermal dose of EPN at 1.0 mg/kg in 1.0 mmol/kg 2,5-HD shows axonal swelling in multiple internodal segments of the same axon. H and E-LFB, X163.

FIGURE 8. Longitudinal section of spinal cord from a hen treated with a daily dermal dose of EPN at 1.0 mg/kg in 0.1 ml acetone and 1.0 mmol/kg 2,5-HD shows large axonal swelling with phagocytosis. H and E-LFB, X260.

TABLE 1. Histopathological changes, Neurotoxicity Index (NTI),[a] and Coneurotoxicity Coefficient (CNC)[a] in Hens Treated Dermally with EPN, Aliphatic Hexacarbons, or Combinations[b]

Aliphatic hexacarbon mmol/kg		EPN (mg/kg)	Acetone (ml)	Histopathological changes[c] Spinal cord +	Spinal cord ±	Peripheral nerves +	Peripheral nerves ±	NTI	CNC
None		1.0	0.1	0	0	0	0	4.2	–
n-Hexane	2.5	0	0	0	0	0	0	1.2	–
n-Hexane	2.5	1.0	0	1	0	0	0	6.3	1.1
n-Hexane	2.5	1.0	0.1	1	0	0	0	5.2	1
M*n*BK	1.0	0	0	0	2	0	0	3.5	–
M*n*BK	1.0	1.0	0	1	1	2	0	10.2	1.4
M*n*BK	1.0	1.0	0.1	1	3	0	0	8.8	1.1
2,5-HDOH	1.0	0	0	0	2	0	0	4.2	–
2,5-HDOH	1.0	1.0	0	1	1	0	0	9.8	1.1
2,5-HDOH	1.0	1.0	0.1	1	1	0	0	9.5	1.1
2,5-HD	1.0	0	0	0	1	0	0	4.7	–
2,5-HD	1.0	1.0	0	2	3	0	0	12.7	1.4
2,5-HD	1.0	1.0	0.1	1	3	0	0	11.5	1.3

[a]NTI and CNC are defined in the methods.

[b]Daily dermal doses were applied for 90 d, followed by a 30-d observation period.

[c]Number of hens showing histopathological changes. The severity of lesions is described in the methods: + = unequivocal, ± = equivocal.

that *n*-hexane was the least effective chemical in producing neurotoxicity when applied dermally, followed by, in ascending order from least effective to most effective, *n*-hexane < M*n*BK < 2,5-HDOH = EPN ≪ 2,5-HD.

The joint neurotoxic action of a dermal dose of EPN at 1 mg/kg and 1 mmol/kg of the aliphatic hexacarbons was determined by calculating the coneurotoxicity coefficient (CNC) described in the methods section and listed in Table 1. Concurrent dermal application of EPN and *n*-hexane at the same site or at different sites produced an additive effect, since CNC values were 0.96 and 1.1. Similar results were obtained when EPN was applied with 2,5-HDOH. However, while dermal application of EPN and M*n*BK at different sites produced a slight increase in neurotoxicity, dermal application of EPN dissolved in M*n*BK resulted in 40% poentiation. Also, 2,5-HD potentiated the neurotoxic effect of EPN to a greater extent when applied with EPN (40% increase) than when applied at separate sites (30% increase).

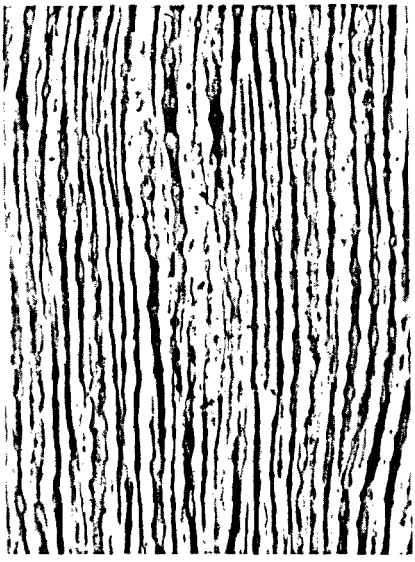

FIGURE 9. Longitudinal section of peripheral nerve from a hen treated with a daily dermal dose of EPN at 1.0 mg/kg in 1.0 mmol/kg M*n*BK. This section shows axonal degeneration in the center light area of the field. Holmes, X211.

DISCUSSION

This report describes the pattern of neurotoxicity produced in hens, following subchronic daily topical application of EPN, *n*-hexane, M*n*BK, 2,5-HDOH, or 2,5-HD alone and when EPN was applied in combination with each of these neurotoxic aliphatic hexacarbons. In an attempt to approximate conditions of human exposure, in this study dermal application was carried out on unprotected skin. Furthermore, occlusive dressing (Draize et al., 1944) causes reversible hydration of the skin, which results in an increase of the absorption rate of dermally applied compounds (Wurster and Kramer, 1961), exaggerating the toxic effects of dermally applied chemicals (McElligott, 1972).

Daily dermal application of 1.0 mg/kg EPN produced clinical signs of delayed neurotoxicity as described previously (Abou-Donia et al., 1983). Also, while subcutaneous dermal administration of *n*-hexane caused only leg weakness with subsequent recovery, after cessation of administration, topical dosing of its related chemicals—e.g., M*n*BK, 2,5-HDOH, and 2,5-HD—produced moderate neurologic deficits. Neurotoxic potency of dermally applied aliphatic hydrocarbons was similar to that when given via oral administration, but less than when applied intraperitoneally, and was, in ascending order: *n*-hexane < M*n*BK < 2,5-HDOH < 2,5-HD (Abou-Donia et al., 1982).

None of the hens treated with single compounds showed unequivocal

histopathologic lesions in the central or peripheral nervous systems. The absence of histopathologic changes in the peripheral nerves may be attributed to regeneration of these nervous tissues during the 30-d observation period. On the other hand, since the central nervous system does not regenerate, it is possible these chemicals may have induced changes of an acute and reversible nature, such as edema, which later subsided. Another explanation is that neurologic deficits might have resulted from an extensive effect at the molecular level that was not observed by light microscopy in the present investigation.

An important finding in this study is the joint neurotoxic action between EPN, a delayed neurotoxic organophosphorus compound, and neurotoxic aliphatic hexacarbons. Concurrent application of daily dermal doses of EPN and *n*-hexane, at the same site or at different sites, produced an additive neurotoxic effect, since it caused neurotoxicity equalling the action expected from the summation of the effect of each chemical alone. Similar results were obtained following the topical application of EPN and 2,5-HDOH and when EPN and M*n*BK were applied at different sites.

In contrast, simultaneous dermal application of EPN and M*n*BK at the same site or concurrent topical dosing of EPN and 2,5-HD resulted in potentiation, since the joint neurotoxic action produced by applying the two compounds was greater than that of each chemical (Rentz, 1932; Hodgson, 1980). The increased neurotoxic effect of EPN and M*n*BK or 2,5-HD when applied at the same site compared to when applied at different sites may partially be explained by these solvents enhancing EPN skin penetration. This explanation is in agreement with the results that M*n*BK and 2,5-HD greatly enhanced skin absorption of [^{14}C]EPN in hens (M. B. Abou-Donia, unpublished data). The potentiating effect of EPN and M*n*BK or 2,5-HD when applied at different sites suggests that the mechanism of this potentiation is at the molecular level. Previous studies, however, have established that the molecular pathogenesis of OPIDN is distinctly different from aliphatic hexacarbon-induced neurotoxicity, despite the fact that both classes of neurotoxic chemicals produce Wallerian-type degeneration. Delayed neurotoxicity of EPN in presumed to result from phosphorylation of neurotoxic esterase (NTE), the putative delayed directoxicity target protein (Johnson, 1969, 1982). Following the initial phosphorylation step, Ca^{2+} modulation in the axon has been implicated in the enhanced phosphorylation of neurotubules and neurofilaments, leading first to their condensation into aggregates and then changing into more solid disordered masses (Abou-Donia et al., 1985c; Patton et al., 1983). These initial changes accompanied by proliferation of rough endoplasmic reticulum (Bischoff, 1967) can be seen by electron microscopy. These subcellular changes result in internodal swelling of the axon followed by degeneration of axons and myelin. On the other hand, Wallerian-type degeneration of *n*-hexane and related chemicals results from the accumulation of 10-mm neurofilaments within small axons in the spinal cord and in the peripheral

nerves. The accumulation of neurofilaments above the nodes of Ranvier in the axon results in the formation of giant axonal swelling (Spencer and Schaumberg, 1975).

A significant finding in this investigation is that histopathological alterations seen in the central and peripheral systems in hens concurrently treated with EPN and *n*-hexane and related chemicals were characteristic of EPN-induced histopathologic lesions (Abou-Donia, 1983; Abou-Donia et al., 1983). Thus, while degenerated axons were generally present in the ventral columns of the spinal cord, significant lesions were often found in the dorsal and lateral columns.

Since the molecular pathogenesis of EPN and *n*-hexane-type chemical-induced neurotoxicity are different, the potentiating effect between EPN and M*n*BK and 2,5-HD when applied at different sites could not have resulted from interaction at the neurotoxicity target per se. Previous studies have demonstrated that chicken hepatic microsomes contain a small amount of cytochrome P-450 (Lasker et al., 1982; Lapadula et al., 1984), which was highly induced by EPN, M*n*BK, and 2,5-HD. Thus, M*n*BK or 2,5-HD may potentiate the effect of EPN by inducing the hen liver microsomal cytochrome P-450, which results in its metabolism to the more potent neurotoxicant EPN oxon (*O*-ethyl *O*-4-nitrophenyl phenylphosphonate) (Abou-Donia et al., 1985a,b).

Another explanation for the joint neurotoxic action of EPN and aliphatic hexacarbons is the possibility that the entry of EPN or its active metabolite from circulation to the neurotoxicity target is enhanced by increasing vascular permeability as the result of local trauma produced by M*n*BK or 2,5-HD, as has been suggested for 2,5-HD (Simonati et al., 1983).

The present report demonstrates that persistent dermal exposure to small daily doses, without skin protection, of EPN, M*n*BK, 2,5-HDOH, or 2,5-HD induces neurotoxicity in hens. Since humans are also susceptible to neurotoxicity produced by these chemicals, these results emphasize the need to prevent skin contact in industrail and agricultural workers required to handle these types of neurotoxic chemicals. Furthermore, since concurrent dermal application of EPN and *n*-hexane and its metabolites produced either additive or potentiating neurotoxic action, it is important that both management and workers handling these compounds be aware of the hazards resulting from washing skin contaminated with organophosphorus compounds.

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