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The Relative Neurotoxicities of *n*-Hexane, Methyl *n*-Butyl Ketone, 2,5-Hexanediol, and 2,5-Hexanedione following Oral or Intraperitoneal Administration in Hens[1]

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The Relative Neurotoxicities of *n*-Hexane, Methyl *n*-Butyl Ketone, 2,5-Hexanediol, and 2,5-Hexanedione following Oral or Intraperitoneal Administration in Hens. ABOU-DONIA, M. B., MAKKAWY, H. M., AND GRAHAM, D. G. (1982). *Toxicol. Appl. Pharmacol.* **62,** 369-389. The sensitivity of the hen to neurotoxicity produced by po and ip administration of *n*-hexane, methyl *n*-butyl ketone (M*n*BK), 2,5-hexanediol (2,5-HDOH), and 2,5-hexanedione (2,5-HD) was investigated. While po administration of one or two doses of these chemicals at a 21-day interval caused acute effects, it did not induce neuropathy in treated hens. Subchronic po or ip administration of *n*-hexane caused only weakness, which subsided after cessation of administration. By contrast, subchronic administration of the other three related compounds caused neurotoxicity characterized by ataxia, which progressed to paralysis in some hens. Severity of the neurotoxic effect was dependent on both the test compound and its route of administration of a similar dosage. Generally, ip injection caused more severe effects than po administration. Pathological examination of nervous system tissues of hens treated with the 2,5-HD, 2,5-HDOH, and M*n*BK showed giant paranodal axonal swelling followed by Wallerian degeneration of axons and myelin in peripheral nerve and spinal cord. Wallerian degeneration in the spinal cord was observed almost exclusively in the ventral columns of the lower spinal cord. *n*-Hexane failed to produce the characteristic pathological features produced by related compounds. The neurotoxic potency of these chemicals which considers onset and magnitude of clinical signs and severity of histopathologic changes was in descending order: 2,5-HD > 2,5-HDOH > M*n*BK > *n*-hexane when given by either method.

n-Hexane has been implicated as the cause of central and peripheral neuropathy in some industrial workers and in individuals who inhale its vapor for its euphoric effects (Yamada, 1964; McDonough, 1974; Gonzalez and Downey, 1972; Spencer *et al.*, 1980). Repetitive sc injection or continuous inhalation of *n*-hexane produces neurotoxicity in rats (Schaumburg and Spencer, 1976). Another hexacarbon, the industrial solvent methyl *n*-butyl ketone (M*n*BK), was suspected of producing an outbreak of peripheral neuropathy in workers at a coated fabrics plant in Columbus, Ohio (Billmaier *et al.*, 1974). Subsequent studies on laboratory animals showed that prolonged exposure to M*n*BK vapor produced similar effects (McDonough, 1974; Mendell *et al.*, 1974; Spencer *et al.*, 1975). *n*-Hexane (Fig. 1) is metabolized to M*n*BK and 2,5-hexanediol (2,5-HDOH), both of which are metabolized to 2,5-hexanedione (2,5-HD) (Kramer *et al.*, 1974; DiVincenzo *et al.*, 1976; Abdel-Rahman *et al.*, 1976). Recent studies have shown that *n*-hexane and its related hexa-

[1] A preliminary account of this work has been presented (Makkawy *et al.*, 1981).

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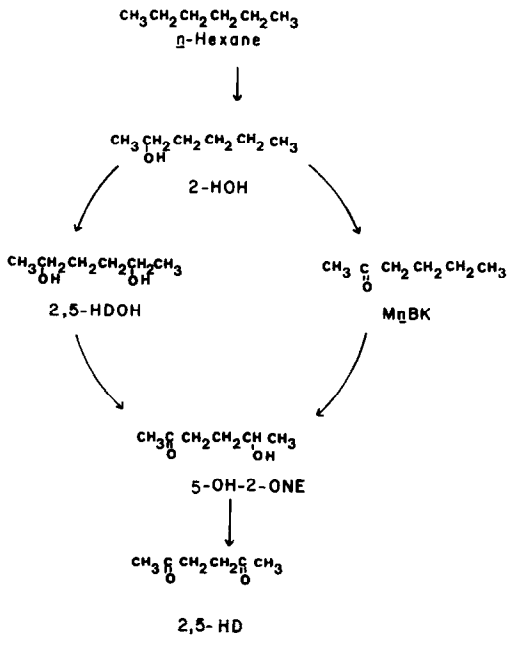

FIG. 1. Metabolic biotransformation pathways of *n*-hexane and methyl *n*-butyl ketone (Kramer *et al.*, 1974; Abdel-Rahman *et al.*, 1976; DiVincenzo *et al.*, 1976). Abbreviations are: 2-HOH, 2-hexanol; M*n*BK, methyl *n*-butyl ketone; 2,5-HDOH, 2,5-hexanediol; 5-OH-2-ONE, 5-hydroxy-2-hexanone; 2,5-HD, 2,5-hexanedione.

carbons, e.g., M*n*BK, 2,5-HDOH, and 2,5-HD, produce a typical peripheral neuropathy in the rat, characterized clinically by slowly developing weakness in the hindlimb (Spencer *et al.*, 1978). Histopathological changes produced by these chemicals were identical and occurred concurrently in both the central and peripheral nervous systems.

Between 1974 and 1975 leptophos (*O*-4-bromo-2,5-dichlorophenyl *O*-methyl phenylphosphonothioate), an insecticide causing delayed neurotoxicity, was implicated in the neurologic dysfunction that developed in some workers in the Texas factory where it was manufactured and packaged (Staff Report, 1976). These cases were further complicated, however, by the possibility that some of the workers had been exposed simultaneously to solvents such as toluene and *n*-hexane (Xintaras *et al.*, 1978).

In order to study how exposure to hexacarbon solvents contributes to delayed neurotoxicity produced by organophosphorus esters, it is essential to characterize both syndromes in one sensitive species. The adult chicken is the animal of choice to study organophosphorus ester-induced delayed neurotoxicity since rodents are not susceptible (Cavanagh, 1979; Abou-Donia, 1981). Thus, although the relative neurotoxicity of orally administered *n*-hexane-type compounds has been studied in the rat (Krasavage *et al.*, 1980), we investigated the neurotoxicity of these chemicals in the hen. Only one study has reported the neurotoxic effect of inhaled M*n*BK in the chicken (Mendell *et al.*, 1974).

The purpose of the present study was twofold: (1) to investigate the sensitivity of the chicken to neurotoxicity produced by *n*-hexane and some related compounds, e.g., M*n*BK, 2,5-HDOH, and 2,5-HD; (2) to characterize the morphology and distribution of neuropathologic lesions induced by these chemicals. These purposes were accomplished by the oral administration of either a single dose of *n*-hexane and its related chemicals or by daily subchronic doses for 90 days. A second study was carried out by subchronic (90 days) ip injections. This method avoided interfering factors arising from the absorption and metabolism of these compounds in the gastrointestinal tract. The subchronic doses were selected from results of a preliminary study with a single dose.

METHODS

Chemicals

n-Hexane (99%) was purchased from Sigma Chemical Company, St. Louis, Missouri; methyl *n*-butyl ketone (70%) (M*n*BK) containing 30% methyl *iso*butyl ketone (M*i*BK) was provided by Eastman Kodak Company, Kingsport, Tennessee; 2,5-hexanediol (90%) (2,5-HDOH) and 2,5-hexanedione (90%) (2,5-HD) were obtained from Eastman Kodak Company, Rochester, New York.

Birds

Birds used were leghorn laying hens (*Gallus gallus* domesticus), 12 months old, with mean weights ± SE

1.7 ± 0.04 kg (1.3 to 2.1 kg) (Featherdown Farm, Raleigh, N.C.). The hens were tested for and found to be free of common viral avian nervous system diseases such as Marek's disease, Newcastle disease, and avian encephalomyelitis. Hens were placed in individual cages in an air-conditioned room at 21 to 23°C with a 12-hr light cycle and allowed to adjust to the environment for 1 week before the experiment.

Treatments

Single po dose. Oral administration was carried out with a syringe and a round-head, 7-cm needle (Popper & Sons, Inc., New Hyde Park, N.Y.). Groups of hens were given a single po dose of each of the test compounds, and the surviving birds were killed after 30 days. Other groups of hens were treated with two po doses of *n*-hexane, M*n*BK, or 2,5-HDOH, one dose at Day 0 of the experiment and the other at Day 21. The surviving birds were killed after an additional 21 days. A group of untreated hens was included as controls. After dosing, the birds were returned to their cages and supplied with feed (Layena Chicken Feed, Ralston Purina Co., St. Louis, Mo.) and water *ad libitum*. Body weights were monitored weekly and the hens were examined daily.

Daily po doses. Five groups of hens (three birds each) were given a daily dose of 100 mg/kg of either *n*-hexane, M*n*BK, 2,5-HDOH, or 2,5-HD for 90 days. Hens were handled as described above, and after the final dose, they were observed for 30 days before termination.

Daily ip injections. For 90 days, eight groups of hens (three birds each) were given a daily ip injection of 100 or 200 mg/kg dose of *n*-hexane, M*n*BK, 2,5-HDOH, or 2,5-HD. Following this period, the hens were observed for 30 days. One group was not treated and served as the control.

Because of the anatomy of the hen, the ip injections were performed with special care. Each injection was made ventrally through the body wall and abdominal air sac into the peritoneal cavity (Fig. 2). In avian species, air sacs are directly connected to the lung and consist of thin-walled fibrous, elastic tissue and muscle fibers (Fedde, 1976). The fact that no hens died from chemical pneumonitis confirmed that no injections were deposited in the air sac.

Histopathological Methods

In hens that died during the experiment, the skin was stripped off shortly after death and each sciatic nerve with its branches was exposed and covered with 10% neutral-buffered formalin. Hens that survived to the end of each experiment were anesthetized with pentobarbital and perfused with a formalin solution. Sciatic, tibial, and peroneal nerves and their branches were dissected, stretched, and pinned on cork boards. Then, these nerves, along with the spinal cord, were fixed for a minimum of 7 days in buffered formalin. Cross and parasagittally longitudinal sections near the midline were prepared from the cervical, thoracic, and lumbar regions of the spinal cord. Peripheral nerves were cut transversely, and cross and longitudinal sections were obtained. Tissues were dehydrated in graded ethanol and imbedded in paraffin. Sections 8 μ from spinal cord and peripheral nerve were stained with hematoxylin and eosin (H&E) combined with luxol fast blue (LFB). Sections from the peripheral nerves also were stained with Holmes' silver stain.

FIG. 2. The site of ip injection in the chicken.

Neurotoxicity Index

Neurotoxic potency of test compounds was measured by a neurotoxicity index that ranks hens according to three factors: (1) the clinical condition of the hen, (2) the time of onset of clinical signs, and (3) the intensity of histopathologic changes within each treatment group (Jonckheere, 1954). First, hens were sorted and assigned ranks within each of the following categories: clinical condition, onset of clinical signs, and severity of histopathological lesions. Ranks were assigned starting with the lowest clinical grade and minimal histologic alteration. In case of ties, the mean rank of the birds involved was assigned to each of these birds. The neurotoxicity index for a treatment was calculated as the mean of the three ranks of hens in each of the three factors.

Statistics

Significance of the difference between the weight of control and treated hens was assessed by Student's two-

TABLE 1

SEQUENCE OF TREATMENTS, CLINICAL SIGNS, AND CHANGE IN WEIGHT OF HENS GIVEN ONE OR TWO po DOSES OF *n*-HEXANE, METHYL *n*-BUTYL KETONE, 2,5-HEXANEDIOL, OR 2,5-HEXANEDIONE[a]

Hen No.	Treatment	Dose[b] (mg/kg)	Dosing at Day	Days after administration: Acute toxicity: Onset	Days after administration: Acute toxicity: Recovery	Days after administration: Died or killed	Percentage of initial weight at termination
1406	*n*-hexane	1000	0			30	83
1479	*n*-hexane	2000	0 and 21	0[c]	3	42	100
1483	*n*-hexane	2000	0 and 21	0	2	42	100
1484	*n*-hexane	2000	0 and 21	0	4	42	94
1460	M*n*BK	100	0			30	55
1480	M*n*BK	2000	0 and 21	0		2	[d]
1485	M*n*BK	2000	0 and 21	0	5	42	80
1497	M*n*BK	2000	0 and 21	0	4	42	100
1498	M*n*BK	2000	0 and 21	0	4	42	94
1481	2,5-HDOH	2000	0	0		0[c]	[d]
1499	2,5-HDOH	1500	0	0		0	[d]
691	2,5-HDOH	1000	0	0		0	[d]
692	2,5-HDOH	1000	0	0	6	30	94
693	2,5-HDOH	1000	0	0		0	[d]
1458	2,5-HDOH	1000	0	0	5	30	72
688	2,5-HDOH	500	0 and 21	1	5	42	95
689	2,5-HDOH	500	0 and 21	1	7	42	106
690	2,5-HDOH	500	0 and 21	1	6	42	106
1457	2,5-HD	1000	0	0		0	[d]
1482	2,5-HD	500	0	0		0	[d]
1496	2,5-HD	500	0	1	5	30	100
694	2,5-HD	500	0	1	6	30	80
695	2,5-HD	500	0	1	6	30	94

[a] Abbreviations: M*n*BK, methy *n*-butyl ketone; 2,5-HDOH, 2,5-hexanediol; 2,5-HD, 2,5-hexanedione.
[b] po administration was by gavage.
[c] Indicates shortly after administration.
[d] Hens died and necropsy was done shortly after death.

tailed *t* test. A *p* value of 0.05 or less was considered significant.

RESULTS

Body Weight

Two single po doses of 2000 mg/kg *n*-hexane had little effect on the weight of treated hens. Similar treatment with M*n*BK slightly reduced the weight of the bird, resulting at termination in a mean value ± SE of 91 ± 6% of the initial weight. A single po dose of 1000 mg/kg 2,5-HDOH caused a 17% weight loss 30 days after administration. By contrast two po doses of 500 mg/kg 2,5-HDOH at a 21-day interval caused little change in body weight. A single po dose of 500 mg/kg 2,5-HD slightly reduced the weight of three treated birds that had a mean value ± SE of 91 ± 6% at the end of the 30-day experiment (Table 1). Untreated control hens gained (mean ± SE) 123 ± 10% of initial weight during the experiment. Continuous po administration of *n*-hexane and its related compounds caused weight loss in all treated hens (Table 2). Weight loss was dependent on the chemical used. Control hens gained weight throughout the experiment.

TABLE 2

CHANGE IN WEIGHT OF HENS FOLLOWING SUBCHRONIC (90 DAYS) DAILY po[a] 100 mg/kg DOSE OF *n*-HEXANE, METHYL *n*-BUTYL KETONE, 2,5-HEXANEDIOL, OR 2,5-HEXANEDIONE[b]

Treatment	Percentage of initial weight ($\bar{x}$ ± SE) at[c,d]		
	Onset of ataxia	End of dosing	Termination
n-Hexane	93 ± 2	81 ± 5	92 ± 4
M*n*BK	91 ± 3	83 ± 3	89 ± 4
2,5-HDOH	88 ± 9	86 ± 9	93 ± 6
2,5-HD	88 ± 3	86 ± 5	91 ± 5
Control			115 ± 5

[a] A daily po dose was administered by gavage for 90 days followed by a 30-day observation period.

[b] Abbreviations: M*n*BK, methyl *n*-butyl ketone; 2,5-HDOH, 2,5-hexanediol; 2,5-HD, 2,5-hexanedione.

[c] Values are from three hens.

[d] All values of treated birds were significantly different from control values, $p < 0.05$.

While a daily 100 mg/kg ip dose of *n*-hexane resulted in neither decrease nor increase in body weight of hens, the daily administration of 200 mg/kg ip *n*-hexane dose produced significant loss of weight and death by the end of the dosing period (Table 3). Similar results were obtained with M*n*BK and 2,5-HDOH, with the latter compound causing the greatest decrease of any chemical tested. Both dose levels of 2,5-HD resulted in significant weight loss. This loss was greater with the 200 mg/kg dose than with the 100 mg/kg dose. Control hens gained weight throughout the experiment.

Acute Effects

Single po dose. While a single po dose of 1000 mg/kg *n*-hexane caused no signs of acute toxicity, a po dose of 2000 mg/kg produced mild weakness on the day of administration, followed by full recovery in 2 to 4 days (Table 1).

Similar results were obtained with M*n*BK. However, in this treatment, one hen given a dose of 2000 mg/kg M*n*BK abruptly died without showing any signs of acute toxicity.

Two hens given a single po dose of 2000 or 1500 mg/kg 2,5-HDOH died shortly after administration. Four hens given a single po

TABLE 3

CHANGE IN WEIGHT OF HENS FOLLOWING SUBCHRONIC (90 DAYS) DAILY ip INJECTION OF *n*-HEXANE, METHYL *n*-BUTYL KETONE, 2,5-HEXANEDIOL, OR 2,5-HEXANEDIONE[a]

Treatment	Dose[b] (mg/kg/day)	Percentage of initial weight[c] at		
		Onset of ataxia	End of dosing	Termination
n-hexane	100		98 ± 5	102 ± 7
n-hexane	200		79 ± 8[d]	79 ± 8[d,e]
M*n*BK	100	98 ± 5	89 ± 10[d]	96 ± 13[f]
M*n*BK	200	95 ± 3	88 ± 4[d]	84 ± 5[d,f]
2,5-HDOH	100	104 ± 2	92 ± 6	100 ± 3
2,5-HDOH	200	73 ± 5[d]	52 ± 5[d]	52 ± 5[d]
2,5-HD	100	73 ± 3[d]	79 ± 6[d]	87 ± 9
2,5-HD	200	93 ± 4	66 ± 3[d]	66 ± 3[d,f]
Control	0			117 ± 3

[a] Abbreviations: M*n*BK, methy *n*-butyl ketone; 2,5-HDOH, 2,5-hexanediol; 2,5-HD, 2,5-hexanedione.

[b] A single daily ip injection was administered for 90 days followed by a 30-day observation period to groups of three hens.

[c] Values are $\bar{x}$ ± SE of three hens.

[d] Significantly different from control values, $p < 0.05$.

[e] These hens died between 51 and 91 days (see Table 5).

[f] One hen from each of these groups died before the end of the 90-day dosing period (see Table 5).

TABLE 4

SEQUENCE OF TREATMENTS AND ONSET OF CLINICAL SIGNS IN HENS FOLLOWING SUBCHRONIC (90 DAYS) po[a] 100 mg/kg DOSE OF *n*-HEXANE, METHYL *n*-BUTYL KETONE 2,5-HEXANEDIOL, OR 2,5-HEXANEDIONE[b]

Hen No.	Treatment	Ataxia[c]:	Days after beginning of administration			
			T_1	T_2	T_3	T_4
1	*n*-Hexane		Leg weakness at 35, recovery at 95			
2	*n*-Hexane		Leg weakness at 31, recovery at 97			
3	*n*-Hexane		Leg weakness at 30, recovery at 96			
4	M*n*BK		13	26 (107)[d]	50	
5	M*n*BK		12	25 (109)	49	
6	M*n*BK		11	24 (108)	51 (91)	83
7	2,5-HDOH		11	24	80 (102)	87
8	2,5-HDOH		10	23	78	
9	2,5-HDOH		9	22	79	
10	2,5-HD		10	22		
11	2,5-HD		8	20		
12	2,5-HD		11	21		

[a] A daily po dose was administered by gavage for 90 days followed by a 30-day observation period to groups of 3 hens.

[b] Abbreviations: M*n*BK, methyl *n*-butyl ketone; 2,5-HDOH, 2,5-hexanediol; 2,5-HD, 2,5-hexanedione.

[c] Clinical grades are: T_1, mild ataxia; T_2, gross ataxia; T_3, severe ataxia; and T_4, ataxia with near paralysis (Abou-Donia, 1978).

[d] Hens improved and had a lesser grade of ataxia at days in parentheses.

dose of 1000 mg/kg 2,5-HDOH developed severe signs of acute toxicity which consisted of general weakness, tremors, and gasping for breath. Two of these hens died shortly after dosing while the other two recovered and survived the 30-day post-treatment period. Three hens given two po doses of 500 mg/kg 2,5-HDOH at a 21-day interval showed signs of severe acute poisoning 1 day after administration of the first dose. These hens were weak, could not stand, and had difficulty breathing. The clinical condition of these hens improved with time, and they completely recovered within 5 to 7 days. It is interesting to note that none of these hens showed any signs of acute poisoning following the second po dose of 500 mg/kg 2,5-HDOH at 21 days.

One hen given a single po dose of 1000 mg/kg 2,5-HD died shortly after administration. One of four hens given a single po dose of 500 mg/kg 2,5-HD died shortly after the administration. The three remaining hens in this group initially showed general weakness but completely recovered 5 to 6 days after the administration. The clinical condition of these hens was less severe than those treated with a similar dose of 2,5-HDOH.

Subchronic po and ip administration. Hens given po or ip doses of 100 mg/kg *n*-hexane, M*n*BK, 2,5-HDOH, and 2,5-HD for 90 consecutive days showed no sings of acute effect.

Clinical Assessment of Neurotoxicity

The sequence of treatment and onset of clinical signs in treated hens are shown in Tables 4 and 5. The degree of ataxia before paralysis developed was given one of four grades (Abou-Donia, 1978): T_1, mild ataxia; T_2 gross ataxia; T_3, severe ataxia; T_4, ataxia with near paralysis.

po administration. Hens did not develop

TABLE 5

SEQUENCE OF TREATMENTS AND ONSET OF CLINICAL SIGNS IN HENS FOLLOWING SUBCHRONIC (90 DAYS) DAILY ip INJECTION OF *n*-HEXANE, METHYL *n*-BUTYL KETONE, 2,5-HEXANEDIOL, OR 2,5-HEXANEDIONE[a]

Hen No.	Compound	Dose[b] (mg/kg/day)	Ataxia[c]: Days after beginning of administration T_1	T_2	T_3	T_4	Paralysis	Died or killed
43	*n*-Hexane	100	Leg weakness at day 35, recovery at day 95					120
44	*n*-Hexane	100	Leg weakness at day 36, recovery at day 93					120
45	*n*-Hexane	100	Leg weakness at day 33, recovery at day 94					120
76	*n*-Hexane	200	Leg weakness at day 26					91[d]
77	*n*-Hexane	200	Leg weakness at day 28					51[d]
78	*n*-Hexane	200	Leg weakness at day 25					69[d]
46	M*n*BK	100	12	18 (115)[e]	29 (98)	77		120
47	M*n*BK	100	11	19	20	22	28	30
48	M*n*BK	100	13	17	29 (95)	35		120
79	M*n*BK	200	10	17	22	28		65[d]
80	M*n*BK	200	11	18	20	29 (115)		120
81	M*n*BK	200	10	16	30	35		120
50	2,5-HDOH	100	13	20 (114)	29 (97)	66		120
51	2,5-HDOH	100	11	21	30			120
55	2,5-HDOH	100	12	22 (112)	32 (95)	73		120
82	2,5-HDOH	200	11	19	29	53	67	81
83	2,5-HDOH	200	10	18	28	46	68	81
84	2,5-HDOH	200	10	17	25	27	34	35
52	2,5-HD	100	7	9	24	59		120
53	2,5-HD	100	8	10	55	77	115	120
54	2,5-HD	100	6	8	9	10	13	35
85	2,5-HD	200	7	9	18	24	26	28
86	2,5-HD	200	5	8	19	22	26	28
87	2,5-HD	200	8	7	15	17	22	25[d]

[a] Abbreviations: M*n*BK, methy *n*-butyl ketone; 2,5-HDOH, 2,5-hexanediol; 2,5-HD, 2,5-hexanedione.

[b] A single daily intraperitoneal injection was administered for 90 days and surviving hens were kept for a 30-day observation period.

[c] Clinical grades are: T_1, mild ataxia; T_2, gross ataxia; T_3, severe ataxia; and T_4, ataxia with near paralysis.

[d] Hens died and were dissected shortly after death.

[e] Hens improved and had a lesser grade of ataxia on day in parentheses.

clinical signs of hexacarbon neurotoxicity when given *n*-hexane, M*n*BK, 2,5-HDOH, or 2,5-HD in a single po dose or in two po doses administered 21 days apart. Subchronic po administration of these compounds, however, cased neurologic dysfunction (Table 4). Continuous po administration of *n*-hexane caused leg weakness after 30 to 35 days. These hens recovered after the daily administration was discontinued.

One of three hens given subchronic 100 mg/kg po doses of M*n*BK developed all successive stages of ataxia, while the other two showed severe ataxia. All three improved to a stage of gross ataxia after cessation of M*n*BK administration. Three hens treated with a daily po dose of 100 mg/kg 2,5-HDOH showed severe ataxia, and one of these hens progressed to ataxia with near paralysis. This hen, however, improved after

the administration had stopped, and its condition, along with the other two hens, was classified as severe ataxia at the end of the 30-day observation period. Continuous po administration of 100 mg/kg 2,5-HD produced gross ataxia 20 to 22 days after the first dose, and the hens remained in this condition throughout the experiment.

ip administration. A daily ip dose of 100 mg/kg of *n*-hexane caused leg weakness in hens after 33 to 36 days of administration. Their condition was manifested by their reluctance to walk and their tendency to fly (Table 5). These hens returned to normal activity shortly after cessation of administration. Hens given a daily ip dose of 200 mg/kg *n*-hexane developed leg weakness earlier, after 25 to 28 days. These hens died between 51 and 91 days after the beginning of administration without further deterioration in their clinical condition.

Hens given a daily 100 mg/kg ip injection of M*n*BK progressed through all successive stages of ataxia; the clinical conditions of two of them improved after treatment was stopped, while the third hen progressed to paralysis and died after 30 days of administration. Daily ip injection of 200 mg/kg M*n*BK produced ataxia with near paralysis (T_4) which progressed to paralysis in one hen. The clinical condition of this hen, however, reverted to grade T_4 after cessation of administration.

Daily ip injection of 100 mg/kg 2,5-HDOH caused severe ataxia in one hen and ataxia with near paralysis in two. Administration of a 200 mg/kg single ip dose of 2,5-HDOH produced paralysis in all three tested hens. All these hens were killed within 35 to 81 days after their clinical conditions deteriorated.

Two hens given a daily 100 mg/kg ip injection of 2,5-HD progressed to paralysis while the third developed ataxia with near paralysis. All hens given daily ip 200 mg/kg injections of 2,5-HD showed paralysis quite early after 22 to 26 doses. One of these hens died at Day 25 while the other two were killed at Day 28, when they became moribund. None of the hens treated with 2,5-HD showed any improvement throughout the experiment.

Necropsy Observations

When tissues of treated and control birds were compared for size, shape, and color, no differences were observed. All tissues including inoculation site, air sacs, lungs, and the peritoneum were examined.

Histopathological Changes

po administration. Histological examination was done on tissues from spinal cord and peripheral nerves of control and treated hens. Administration of one or two po doses at a 21-day interval of all test compounds did not induce any histopathological changes in the spinal cord or peripheral nerves. Similar results were obtained in tissues from control hens and from hens given 90 consecutive daily po doses of *n*-hexane. Equivocal histological changes were seen in the spinal cord from two of the three hens given daily po doses of M*n*BK (Table 6). These lesions consisted of occasional swollen axons without obvious fragmentation, phagocytosis, or myelin loss. Although these lesions may represent the earliest histopathological changes in M*n*BK neurotoxicity, they have been found on rare occasions in spinal cords of normal birds, and thus may be artifactual in origin. Sections from the spinal cord of the third hen of this group exhibited unequivocal histopathologic degeneration of axons and myelin. Sections from the peripheral nerves of all M*n*BK-treated hens were normal.

Spinal cord sections from all three hens treated with 2,5-HDOH showed unequivocal histopathologic degeneration of axons and myelin. The cross section of the thoracic

TABLE 6

DURATION OF INTOXICATION AND HISTOPATHOLOGICAL CHANGES IN NERVE TISSUES FROM HENS FOLLOWING SUBCHRONIC (90 DAYS) DAILY po[a] 100 mg/kg DOSE OF *n*-HEXANE, METHYL *n*-BUTYL KETONE, 2,5-HEXANEDIOL, OR 2,5-HEXANEDIONE[b]

			Histopathological changes[d]									
			Spinal cord									
			Cervical			Thoracic			Lumbar			
Hen No.	Treatment	Days of intoxication[c]	VC	LC	DC	VC	LC	DC	VC	LC	DC	Peripheral nerve
1	*n*-Hexane	60	−	−	−	−	−	−	−	−	−	−
2	*n*-Hexane	66	−	−	−	−	−	−	−	−	−	−
3	*n*-Hexane	66	−	−	−	−	−	−	−	−	−	−
4	M*n*BK	107	−	−	−	±	−	−	±	−	−	−
5	M*n*BK	108	−	−	−	±	−	−	−	−	−	−
6	M*n*BK	109	−	−	−	+	−	−	−	−	−	−
7	2,5-HDOH	109	+	−	−	+	−	−	+	−	−	−
8	2,5-HDOH	110	++	+	±	++	−	−	++	−	−	−
9	2,5-HDOH	111	+	−	−	+	+	−	±	−	−	−
10	2,5-HD	113	+	+	−	++	+	−	+	−	−	+
11	2,5-HD	112	++	−	−	++	+	−	+	−	−	−
12	2,5-HD	114	+	−	−	+	+	−	+	−	−	−
575	Control	0	−	−	−	−	−	−	−	−	−	−
576	Control	0	−	−	−	−	−	−	−	−	−	−
578	Control	0	−	−	−	−	−	−	−	−	−	−

[a] Abbreviations: M*n*BK, methyl *n*-butyl ketone; 2,5-HDOH, 2,5-hexanediol; 2,5-HD, 2,5-hexanedione.

[b] A daily po dose was administered by gavage for 90 days followed by a 30-day observation period.

[c] Days of intoxication between onset of clinical signs and recovery or termination.

[d] The following symbols are used: NE, tissue not examined; −, changes absent; ±, equivocal histological changes (rare swollen axons without fragmentation, phagocytosis, or loss of myelin staining); +, mild to moderate degeneration of axons and myelin; ++, lesions are termed severe when there is almost complete destruction of axons and myelin in a given tract, such as the anterior columns, or within extensive areas of peripheral nerve; VC, ventral column; LC, lateral column; DC, dorsal column.

spinal cord from Hen 8 (Fig. 3) shows severe degeneration of axons and myelin within the ventral columns on either side of the midline. It also shows numerous enlarged axons. Figure 4 represents a longitudinal section from the ventral column of the same bird showing two of the large swollen axons. The constrictions near the areas of enlargement may represent nodes of Ranvier. In this figure many axons appear degenerated, and axonal and myelin debris can be seen within the phagocytes. No histopathological changes were detected in peripheral nerves in hens given subchronic po doses of 2,5-HDOH.

All hens treated with 2,5-HD showed unequivocal histopathologic changes in the spinal cord. Two of these hens exhibited severe alterations. A longitudinal section of a

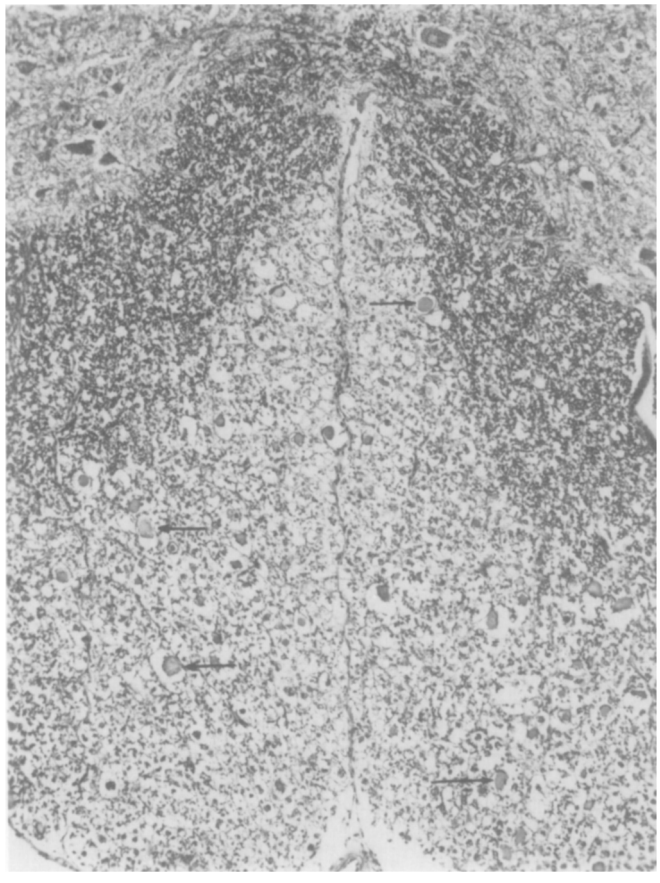

FIG. 3. Thoracic spinal cord from Hen 8 (given 90 daily po doses of 100 mg/kg 2,5-HDOH). This cross section shows severe degeneration of axons and myelin within the anterior columns on either side of the midline. The loss of myelin can be appreciated by comparing the intensity of staining with that of the white matter between the sites of degeneration and the anterior horns (both upper corners). Among the degenerated axons, scattered axons remain, many of which are markedly enlarged (arrows). H&E-LFB, ×100.

ventral column of thoracic spinal cord from Hen 11 shows degenerated and markedly enlarged axons with deep eosin staining of the axonal contents (Fig. 5). Sections from peripheral nerves of Hen 10 show degenerated axons and myelin (Fig. 6). They also show macrophages containing axonal and myelin debris. Marked enlargement of the axonal diameter is seen among myelinated nerves in the longitudinal section of peripheral nerve from Hen 10; the swelling was paranodal (Figure 6). A section from peripheral nerve of Hen 10 showing swollen axons and phagocytic cells which indicate sites of Wallerian degeneration is represented in Fig. 7.

ip injections. Neither the control hens nor five of the *n*-hexane treated hens showed unequivocal histopathologic alterations in the central or peripheral nervous system (Table 7). One hen (No. 77) treated with 200 mg/kg ip dose of *n*-hexane showed equivocal histopathologic changes in the peripheral nerves.

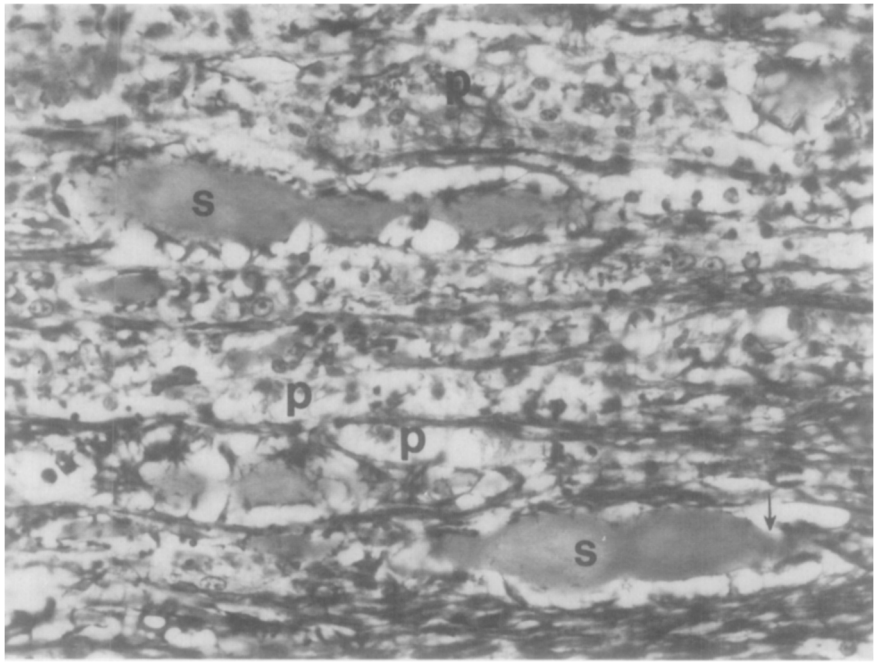

FIG. 4. Thoracic spinal cord from Hen 8 (given 90 daily po doses of 100 mg/kg 2,5-HDOH). Two of the large swollen axons from the anterior column are seen here in longitudinal secton. The constrictions near the areas of enlargement may represent nodes of Ranvier (arrow). Many of the intervening axons have degenerated, and axonal and myelin debris can be identified with phagocytes (P). H&E-LFB, ×400.

Spinal cords from hens given daily ip 100 mg/kg injections of M*n*BK did not exhibit any histopathological changes. One of these hens (No. 47), however, showed unequivocal histopathologic changes in the peripheral nerves. Figure 8 shows a longitudinal section of the degenerated axons. The sites of axonal degeneration were accompanied by myelin degeneration, and macrophages were observed containing debris with the staining properties of myelin. Although none of the hens given 200 mg/kg ip injections of M*n*BK showed histopathologic alterations in peripheral nerves, two (Nos. 80 and 81) of these hens developed unequivocal histopathologic lesions in the spinal cord. One was not examined. A longitudinal section from the ventral column of the thoracic spinal cord from Hen no. 81 shows axons with prominent swellings (Fig. 9). These swellings have the morphologic configuration of the paranodal swelling that suddenly ends at the nodes of Ranvier. A longitudinal section of the thoracic spinal cord from Hen No. 80 demonstrated extensive degeneration in the ventral column and a markedly swollen axon and nests of macrophages.

Hens treated with a daily 100 mg/kg ip injection of 2,5-HDOH demonstrated no peripheral nerve lesions; however, all these hens showed unequivocal histopathologic alterations in the spinal cord. A cross section from the thoracic spinal cord from Hen No. 55 exhibited numerous swollen axons and loss of myelin in the ventral columns. Daily ip injection of 200 mg/kg 2,5-HDOH caused unequivocal histopathologic changes both in the spinal cord of two hens and in the peripheral nerves of all the three treated hens. A longitudinal section of the peripheral

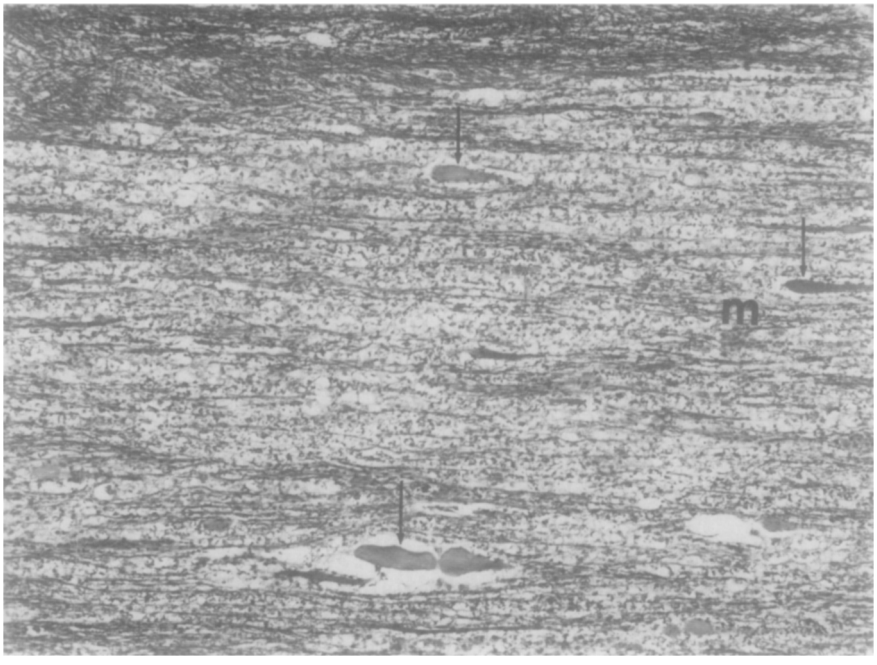

FIG. 5. Thoracic spinal cord from Hen 11 (given 90 daily oral doses of 100 mg/kg 2,5-HD). This longitudinal section of an anterior column shows preservation of white matter just below the anterior horns (top) which can be compared with that in the lower 90% of this figure. Here few myelinated axons remain between those which have undergone Wallerian degeneration. The latter sides are densely populated by macrophages containing axonal and myelin debris. One collection of macrophages is seen below the letter "m". Many of the remaining axons demonstrate marked enlargement of axonal diameter, with deep eosin staining of the axonal contents (arrows). H&E-LFP, ×100.

nerve from Hen No. 84 contained foci of Wallerian degeneration.

In hens given a daily 100 mg/kg ip 2,5-HD, No. 53 was the only one in which unequivocal degeneration of axons and myelin in the peripheral nerves was observed. The spinal cords from all hens of this group showed unequivocal histopathologic alteration. A longitudinal section of the ventral column of thoracic spinal cord from Hen 52 showed extensive degeneration of axons and myelin. Macrophages and markedly enlarged axons were evident. Severe degeneration of axons and myelin was shown in the ventral columns of the thoracic spinal cord from Hen No. 52. One hen given a daily 200 mg/kg ip injection of 2,5-HD exhibited unequivocal histopathologic lesions in the peripheral nerve while another hen showed equivocal changes. The third hen was not examined.

As can be seen from Tables 6 and 7, spinal cord lesions were found consistently within the ventral columns. These foci of axonal swelling and degeneration were most severe in the lower levels of the spinal cord. Lateral column lesions were seen less often than those in the ventral column and were also less striking. The lateral column lesions were somewhat more common in the cervical and thoracic regions than in the lumbar. Unequivocal dorsal column lesions were notably absent in this study.

The results of histopathological examination of nervous system tissues from hens treated with M*n*BK, 2,5-HDOH, and 2,5-

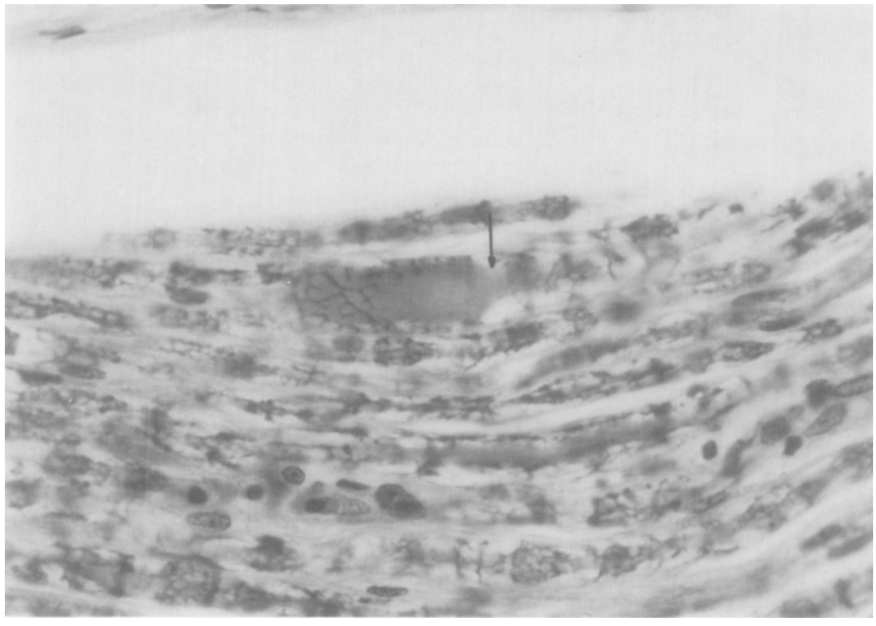

FIG. 6. Peripheral nerve from Hen 10 (given 90 daily po doses of 100 mg/kg 2,5-HD). Among normal myelinated nerves in this longitudinal section one nerve is seen which contains marked enlargement of the axonal diameter. Since myelin can be seen around the swollen (left) and normal (right) portions of the axon, the point marked with an arrow would be a node of Ranvier, identifying the swelling as paranodal. H&D-LFB, ×600.

HD showed that the presence of histopathologic changes was generally dependent on the duration of intoxication. Histopathologic changes were present in the peripheral nerves of hens that died early, while the changes in the spinal cord appeared in hens that survived longer.

Relative Neurotoxicity

The relative neurotoxic potency of *n*-hexane and related chemicals was quantitated according to the neurotoxicity index (NTI) for each compound (Table 8). The NTI encompasses the degree and onset of the clinical conditions as well as the severity of neuropathologic lesions. In this study control hens had an NTI value of zero. The NTI values indicated that the chemical's in descending order (from most effective to least effective) were 2,5-HD > 2,5-HDOH > M*n*BK > *n*-hexane when administered po or ip in hens.

It is realized that the difference in the neurotoxic potency between 2,5-HDOH and 2,5-HD may not be significant since their NTI values were close and no statistical analysis could be conducted with these values.

DISCUSSION

This report describes the sensitivity of the chicken to neurotoxicity induced by *n*-hexane and related compounds. It also characterizes the distribution and morphology of the neuropathologic lesions caused by these chemicals in the central and peripheral nervous tissues of treated hens.

Administration of one or two po doses of

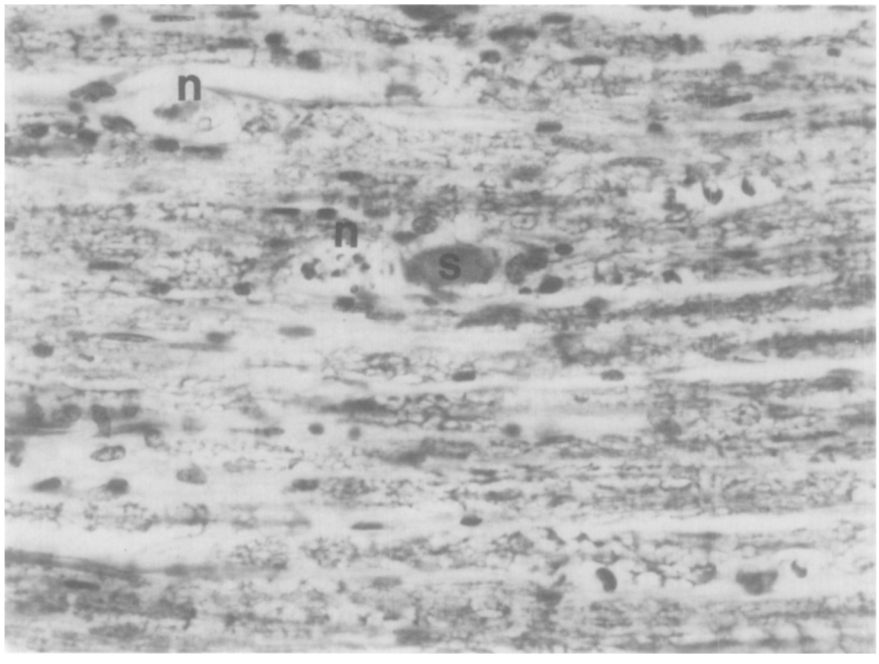

FIG. 7. Peripheral nerve from Hen 10 (given 90 daily po doses of 100 mg/kg 2,5-HD). In addition to a swollen axon (s), nests (n) of phagocytic cells mark sites of Wallerian degeneration. H&E-LFB, ×520.

n-hexane, M*n*BK, 2,5-HDOH, or 2,5-HD at a 21-day interval did not cause neurotoxicity in hens. Instead, these treatments caused signs of acute toxicity resulting from the narcotizing effect of high doses of these chemicals on the central nervous system. The intensity of the clinical signs of acute effect was dependent on both the dose and the compound tested. The potencies for the acute effect of these chemicals were in the descending order of 2,5-HDOH > 2,5-HD > M*n*BK > *n*-hexane. Treatment with a *n*-hexane-related compound killed some hens immediately, presumably due to chemical pneumonitis. However, all surviving hens recovered from the acute effect. No histopathological lesions were detected in the central or peripheral nervous tissues in any of these treated hens.

In this study, subchronic po or ip administration of *n*-hexane for 90 days caused only leg weakness followed by recovery when the treatment stopped. By contrast the three *n*-hexane related compounds tested produced neurotoxicity in the following order of decreasing potency: 2,5-HD > 2,5-HDOH > M*n*BK. While the subchronic po administration of these chemicals produced only ataxia, ip injections caused paralysis in most treated hens. This difference might be attributed to better absorption and/or less metabolic inactivation of the injected chemicals compared to those given orally. The clinical signs were accompanied by histological changes in the central and peripheral nervous systems, including degeneration of the axons with secondary degeneration of the myelin. It is noteworthy that the clinical condition of neurotoxicity produced by *n*-hexane-type compounds appeared identical to that caused by delayed neurotoxic organophosphorus esters in the hen (Abou-Donia, 1978).

In the present study, pure chemical grades

TABLE 7

HISTOPATHOLOIGCAL CHANGES IN NERVOUS TISSUES FROM HENS GIVEN SUBCHRONIC (90 DAYS)[a] DAILY ip INJECTIONS OF *n*-HEXANE, METHYL *n*-BUTYL KETONE, 2,5-HEXANEDIOL, OR 2,5-HEXANEDIONE[b]

		Histological changes[c]									
		Spinal cord									
		Cervical			Thoracic			Lumbar			
Hen No.	Compound	VC	LC	DC	VC	LC	DC	VC	LC	DC	Peripheral nerve
43	*n*-Hexane	−	−	−	−	−	−	−	−	−	−
44	*n*-Hexane	−	−	−	−	−	−	−	−	−	−
45	*n*-Hexane	−	−	−	−	−	−	−	−	−	−
76	*n*-Hexane	−	−	−	−	−	−	−	−	−	−
77	*n*-Hexane	−	−	−	−	−	−	−	−	−	±
78	*n*-Hexane	−	−	−	−	−	−	−	−	−	−
46	M*n*BK	−	−	−	−	−	−	−	−	−	−
47	M*n*BK	−	−	−	−	−	−	−	−	−	±
48	M*n*BK	−	−	−	−	−	−	−	−	−	−
79	M*n*BK	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
80	M*n*BK	+	±	−	+	+	−	+	±	−	−
81	M*n*BK	±	±	−	+	±	−	+	−	−	−
50	2,5-HDOH	±	−	−	−	+	−	+	±	−	−
51	2,5-HDOH	+	±	−	−	±	−	+	−	−	−
55	2,5-HDOH	+	±	−	+	±	−	+	−	−	−
82	2,5-HDOH	+	−	−	+	±	−	+	−	−	+
83	2,5-HDOH	−	−	−	−	−	−	+	−	−	+
84	2,5-HDOH	−	−	−	−	−	−	−	−	−	+
52	2,5-HD	±	+	−	+	+	−	++	+	−	−
53	2,5-HD	+	±	−	+	+	−	+	+	−	+
54	2,5-HD	+	−	−	±	−	−	+	−	−	−
85	2,5-HD	−	−	−	−	−	−	−	−	−	±
86	2,5-HD	±	−	−	−	−	−	−	−	−	+
87	2,5-HD	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE

[a] Abbreviations: M*n*BK, methyl *n*-butyl ketone; 2,5-HDOH, 2,5-hexanediol; 2,5-HD, 2,5-hexanedione.

[b] A single daily ip injection was administered for 90 days followed by a 30-day observation peiod.

[c] The following symbols are used: NE, tissue not examined; −, changes absent, ±, equivocal histological changes (rare swollen axons without fragmentation, phagocytosis, or loss of myelin staining); +, mild to moderate degeneration of axons and myelin; ++, lesions are termed severe where there is almost complete destruction of axons and myelin in a given tract, such as the anterior columns, or wihtin extensive areas of peripheral nerve; VC, ventral column; LC, lateral column; DC, dorsal column.

of *n*-hexane, 2,5-HDOH, and 2,5-HD were used, while a mixture of M*n*BK and M*i*BK (7:3) was employed. It is unlikely that neurotoxicity produced by this mixture was the result of M*i*BK since chronic exposure of cats to commercial grades of methyl ethyl ketone (MEK) and M*i*BK (9:1) did not cause neuropathy or histological lesions in the nervous system (Spencer and Schaumburg, 1976). However, the possibility that

FIG. 8. Peripheral nerve from Hen 47 (given 30 ip injections of 100 mg/kg M*n*BK). This longitudinal section contains two axons undergoing Wallerian degeneration. Macrophages with debris having the staining properties of myelin are indicated with arrows. H&E–LTB, ×400.

M*i*BK might have potentiated the neurotoxicity of M*n*BK cannot be excluded. Such potentiation has been reported to occur in animals concurrently exposed to the non-neurotoxic MEK and the neurotoxic M*n*BK (Abdel-Rahman, *et al.*, 1976; Saida *et al.*, 1976) or *n*-hexane (Alterkirch *et al.*, 1977).

Hens that were treated subchronically with po administrations or ip injections of *n*-hexane and related compounds lost some weight. The basis of the weight loss is not clear. In this study, food consumption was not measured, but a loss of appetite was noted as the clinical condition worsened.

It is interesting to postulate explanations for the differential neurotoxic potencies of hexacarbon solvents in hens. Similar results were obtained in rats (Spencer *et al.*, 1978; Egan *et al.*, 1980; Krasavage *et al.*, 1980). The major difference in chemical structure between *n*-hexane and its related chemicals is the substitution of hydrogen atoms on the 2 and/or 5 position in the *n*-hexane aliphatic chain by hydroxyl or carbonyl group (Fig. 1). It has been demonstrated that *n*-hexane is metabolized to M*n*BK, 2,5-HDOH, and 2,5-HD (Abdel-Rahman *et al.*, 1976; DiVincenzo *et al.*, 1976). In rats, *n*-hexane is among the least active neurotoxicants, and its effects have been attributed to its active metabolic products (Spencer *et al.*, 1980). Of these metabolites, 2,5-HD is the most persistent biotransformation product found as a metabolite in serum *in vivo*; this compound is also the most potent neurotoxic hexacarbon (DiVincenzo *et al.*, 1976). A good correlation has been shown between the neurotoxic potency of *n*-hexane and related hexacarbons in the rat and their ability to be metabolized to 2,5-HD.

Our results agree with the report of Krasavage *et al.* (1980) that following po ad-

FIG. 9. Thoracic spinal cord from Hen 81 (given 90 ip injections of 200 mg/kg M*n*BK). This longitudinal section from a ventral column contains axons with prominent swellings. The enlargement of axonal diameter at the bottom of the figure has the morphologic configuration of a paranodal swelling. The arrow marks the ostensible node of Ranvier where the enlargement suddenly ends. H&E–LFB, ×250.

ministration of *n*-hexane and related chemicals in rats the relative neurotoxicity of the test compounds in decreasing order of potency was: 2,5-HD > 2,5-HDOH > M*n*BK > *n*-hexane. It has been suggested that M*n*BK and 2,5-HD interfere with energy metabolism in the axons by inhibiting the sulfhydryl enzymes glyceraldehyde-3-phosphate dehydrogenase (GADPH) and phosphofructokinase (Spencer *et al.*, 1979; Sabri *et al.*, 1979a, b). This inhibition presumably results in a decrease in axoplasmic transport, which then leads to axonal degeneration. We (Graham and Abou-Donia, 1980), have shown, however, that 2,5-HD is a very weak sulfhydryl reagent and have suggested that it is unlikely to inhibit GADPH through sulfhydryl group binding. We postulated that 2,5-HD neurotoxicity is attributed to its capacity to form stable conjugated Schiff bases with amino groups of proteins. Graham (1980) has recently provided evidence for the latter.

Species sensitivity of hexacarbon-induced neurotoxicity is noteworthy. Mendell *et al.* (1974) studied neurotoxicity produced by inhaled M*n*BK in several species. They reported that chickens developed clinical signs of neurotoxicity earliest, closely followed by cats, while rats were the least vulnerable. The present study, however, shows that while chickens were very sensitive to neurotoxicity from po or ip treatment of 2,5-HD, 2,5-HDOH, and M*n*BK, they were less sensitive to the neurotoxic effect of *n*-hexane. Nevertheless, it is possible that a higher concentration of *n*-hexane and/or a longer period of exposure to this chemical may cause neurotoxicity in hens. If this proves to be the case, the leg weaknesses produced by

TABLE 8

NEUROTOXICITY INDEX (NTI) OF *n*-HEXANE, METHYL *n*-BUTYL KETONE, 2,5-HEXANEDIOL, AND 2,5-HEXANEDIONE[a] FOLLOWING SUBCHRONIC (90 DAYS) DAILY po DOSING OR ip INJECTION IN HENS

Treatment	Dose (mg/kg/day)	Group ranking within[b]			NTI[c]
		Clinical condition	Onset of clinical signs	Severity of histopathological changes	
		po administration			
n-Hexane	100	2 ± 0.6	2 ± 0.6	2 ± 0.6	2 ± 0
M*n*BK	100	11 ± 0.6	5 ± 0.6	4 ± 0.6	6.6 ± 2.2
2,5-HDOH	100	8 ± 0.6	8 ± 0.6	8 ± 0.6	8 ± 0
2,5-HD	100	5 ± 0.6	11 ± 0.6	11 ± 0.6	9 ± 2.0
		ip injection			
n-Hexane	100	2 ± 0.6	2 ± 0.6	4 ± 1.0	2.7 ± 1.5
n-Hexane	200	4 ± 0.6	4 ± 0.6	5.5 ± 1.5	4.8 ± 0.4
M*n*BK	100	11.3 ± 6.6	9.8 ± 1.5	5.5 ± 1.5	8.9 ± 1.7
M*n*BK	200	10.0 ± 0.6	15.2 ± 1.3	16.5 ± 1.5	13.9 ± 2.0
2,5-HDOH	100	10.3 ± 2.2	9.7 ± 1.5	15.7 ± 0.7	11.9 ± 1.9
2,5-HDOH	200	17.0 ± 0.6	15.2 ± 1.3	17.7 ± 2.9	16.6 ± 0.7
2,5-HD	100	17.3 ± 2.9	21.3 ± 1.0	18.0 ± 2.6	18.9 ± 1.2
2,5-HD	200	21.0 ± 0.6	21.7 ± 1.3	10.5 ± 0.4	17.7 ± 3.6

[a] Abbreviations: M*n*BK, methyl *n*-butyl ketone; 2,5-HDOH; 2,5-hexanediol; 2,5-HD, 2,5-hexanedione.

[b] Hens were assigned ranks within the following categories: clinical condition, onset of clinical signs, and severity of histopathological changes.

[c] Neurotoxicity index (NTI) was calculated as the $\bar{x}$ ± standard error of the three ranks of hens of each of the three factors. Standard error type calculations were made to provide an indication of the variability among the values contributing to each mean.

n-hexane in this study might be an early stage of *n*-hexane neurotoxicity in hens.

The present histopathologic findings agree with other results following the inhalation of M*n*BK vapor in chickens, cats, and rats (Mendell *et al.*, 1974; Spencer *et al.*, 1975). The morphology of histopathologic lesions was identical after exposure to M*n*BK, 2,5-HDOH, or 2,5-HD. The severity and frequency of lesions varied, however, and were generally dependent upon: (a) the chemical used, (b) the size of the administered dose, (c) the severity of the clinical condition, (d) the length of the period between beginning of administration and termination, and (e) the site of tissue sampling.

Damage to the peripheral nervous system tissues was seen in hens that were killed or died early. It is postulated that peripheral lesions had occurred in the tissues of the hens that survived longer, but that those lesions had been repaired or regeneration had occurred. Thus, peripheral nerve damage was not seen at termination. In contrast, lesions in the spinal cord were more evident in hens that survived longer. The most likely explanation of these results is that peripheral axons, unlike central nervous system axons, can regenerate.

It is noteworthy that, in hens, as in other species, e.g., rats and cats (Mendell *et al.*, 1974; Spencer *et al.*, 1975), axonal swelling was the earliest lesion observed in hexacarbon neurotoxicity. There were numerous

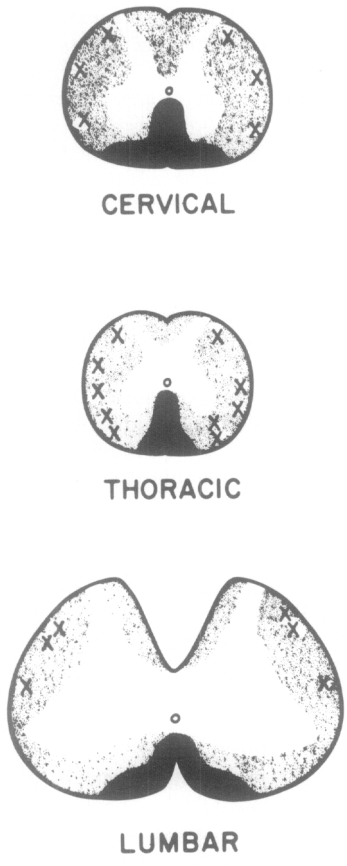

FIG. 10. Diagram showing the distribution of axonal enlargement (x) and degeneration (black areas) of axons and myelin in the spinal cord of hens treated orally or intraperitoneally with methyl *n*-butyl ketone, 2,5-hexanediol, and 2,5-hexanedione. Greatly swollen axons, with axonal degeneration and phagocytosis of axons, and myelin debris appeared almost exclusively in the ventral region of the lower spinal cord. In the lateral columns at all levels, scattered swollen axons were observed, while the dorsal columns remained free of axonal enlargement or degeneration.

swollen axons in the spinal cord, and these were found even more frequently within the ventral columns. Furthermore, in the chicken, hexacarbon solvents induced giant swelling that appeared distinctly paranodal, similar to that reported in other species. Wallerian degeneration followed axonal swelling and was observed almost exclusively in the ventral column of the lower spinal cord (Fig. 10). The axonal swellings in the hexacarbon-exposed hens were observed less frequently in the peripheral nerves than in their spinal cords.

In the light of the questions raised about industrial exposures to the neurotoxic organophosphorus insecticide, leptophos, and *n*-hexane simultaneously (Xintaras *et al.*, 1978), it is noteworthy that in the hen: (a) the clinical conditions produced by leptophos (Abou-Donia and Pressig, 1976a) and *n*-hexane-type compounds appeared to be identical, (b) while a single dose of leptophos is capable of causing delayed neurotoxicity (Abou-Donia *et al.*, 1974), one or two po doses of *n*-hexane or related chemicals do not cause neurotoxicity, (c) subchronic po administration of leptophos (Abou-Donia and Pressig, 1976b) or *n*-hexane-type compounds results in neurotoxicity, (d) although histopathologic lesions are characterized by swollen axons followed by Wallerian degeneration in both types of neurotoxicity, there are distinct differences in the morphology and distribution of the neuropathologic lesions.

This report has demonstrated that, although the hen is generally sensitive to hexacarbon-induced neurotoxicity, it exhibits selectivity toward specific compounds within this chemical group. The neurotoxic effectiveness of these chemicals in decreasing order was 2,5-HD > 2,5-HDOH > M*n*BK > *n*-hexane.

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