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NEUROTOXICITY OF CONTINUOUS (90 DAYS) INHALATION OF TECHNICAL GRADE METHYL BUTYL KETONE IN HENS

Kamal M. Abdo, Doyle G. Graham, Phillip R. Timmons, Mohamed B. Abou-Donia

Departments of Pharmacology and Pathology,
Duke University Medical Center,
Durham, North Carolina

Neurotoxicity was produced in 1-yr-old hens (five hens per treatment) by continual 90-d exposure in inhalation chambers to atmospheres containing 50, 100, 200, or 400 ppm technical grade methyl butyl ketone (MBK) containing 70% methyl n-butyl ketone (MnBK) and 30% methyl isobutyl ketone (MiBK). A 30-d observation period followed. Severity of clinical condition and progression or improvement of neurological deficit signs were dependent on the concentration of MBK and duration of exposure. Hens exposed to the two highest levels developed ataxia and paralysis; they died or were sacrificed before the designated exposure period ended. The intermediate level of MBK (100 ppm) caused severe ataxia; most treated hens showed no change in clinical condition during the observation period. Hens exposed to 50 ppm exhibited gross ataxia, with most demonstrating partial regression of neurological deficit after the exposure ceased. Hens exposed to the lowest tested level (10 ppm) remained normal. Only hens exposed to 400 or 200 ppm showed significant weight loss. Some hens from the 50–400 ppm treatment groups showed unequivocal histopathologic changes in the spinal cord and peripheral nerves. Severity of histopathologic changes depended on the level and duration of MBK exposure. These changes were characterized by excessive swelling, phagocytosis, degeneration, and demyelination of the axons.

INTRODUCTION

Methyl *n*-butyl ketone (M*n*BK) is a hexacarbon solvent that was used extensively in the manufacture of various polymeric coatings and adhesives. It was implicated in a polyneuropathy outbreak among workers in a coated fabrics plant (Billmaier et al., 1974). Subsequent studies showed that prolonged exposure to M*n*BK vapor produced similar conditions in

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K. M. Abdo's present address is: National Toxicology Program, National Institute of Environmental Health Sciences, Post Office Box 12233, Research Triangle Park, North Carolina.

A preliminary account of this study was presented by Abdo et al. (1981).

Requests for reprints should be sent to M. B. Abou-Donia, Department of Pharmacology, Post Office Box 3813, Duke University Medical Center, Durham, North Carolina 27710.

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laboratory animals (McDonough, 1974; Mendell et al., 1974; Spencer et al., 1975). M*n*BK has been characterized as a metabolic product of *n*-hexane and is metabolized further to 2,5-hexanedione (2,5-HD) (Kramer et al., 1974; DiVincenzo et al., 1976, 1977; Abdel-Rahman et al., 1976). 2,5-HD may also be produced from the oxidation of 2,5-hexanediol (2,5-HDOH), a metabolic product of *n*-hexane (Fig. 1). It is interesting that *n*-hexane and related hexacarbons such as M*n*BK, 2,5-HD, and 2,5-HDOH produced the same neurotoxic syndrome in the rat (Spencer et al., 1978). Histopathologic lesions occurred simultaneously in both central and peripheral nervous tissues and were characterized by accumulation of multifocal giant axonal swellings accompanied by accumulation of 10-nm neurofilaments within the axon (Spencer et al., 1978).

Experimental animal studies showed differences in species senstivity to M*n*BK-induced neurotoxicity (Mendell et al., 1974). The most sensitive animal was the domestic chicken, followed in decreasing order by cats,

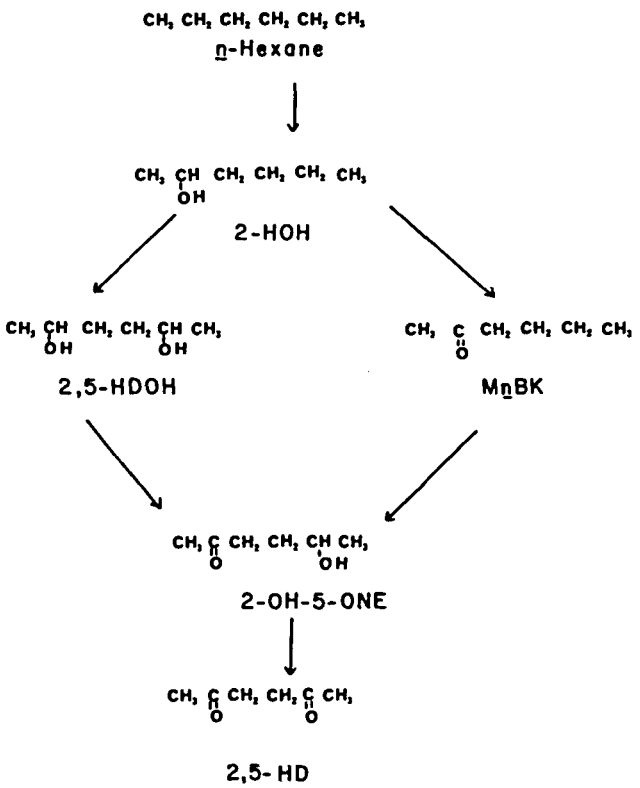

FIGURE 1. Metabolic pathways of *n*-hexane and related hexacarbons. Abbreviations: 2-hexanol, 2HOH; methyl *n*-butyl ketone, M*n*BK; 2,5-hexanediol, 2,5-HDOH; 2-hydroxy-5-hexanone, 2-OH-5-one; 2,5-hexanedione, 2,5-HD. From Kramer et al. (1974), DiVincenzo et al. (1976), and Abdel-Rahman et al. (1976).

rats, and mice. Similarly, species selectivity is a characteristic feature of organophosphorus ester-induced delayed neurotoxicity. As early as 1930 it was recognized that certain species (chickens, cats, dogs, water buffalo, cows, and sheep) are sensitive, while others (rodents, rats, mice, guinea pigs, hamster, and gerbils) are not (Smith et al., 1930; Abou-Donia, 1981). The consistency and reproducibility of neuropathologic disorders in adult chickens make them appropriate animal models in screening organophosphorus esters for delayed neurotoxicity.

Since there has been only one report of the neurotoxic effect of a high concentration of inhaled M*n*BK (Mendell et al., 1974), an investigation was conducted to determine the effect of subchronic inhalation (90 d) of various levels of technical grade methyl butyl ketone (MBK) on the development of different stages of neurotoxicity and on the morphology and distribution of histopathologic lesions.

METHODS

Chemicals

Technical grade MBK containing 70% M*n*BK and 30% methyl isobutyl ketone (MiBK) was provided by Eastman Kodak Co., Kingsport, Tenn. This mixture will be referred to as MBK mixture or MBK.

Exposure of Hens to Methyl Butyl Ketone

Care and Treatment of Birds Adult leghorn laying hens (*Gallus gallus domesticus*), 12 mo old, weighing 1.60 ± 0.24 kg (mean ± SE) were used (Featherdown Farm, Raleigh, N.C.). When tested against common viral avian nervous system diseases such as Marek's disease, Newcastle disease, and avian encephalomyelitis, these hens showed negative responses. Groups of five hens were housed in single-tier, stainless steel inhalation chambers in an air-conditioned room and allowed to adjust to their environment for 1 wk. Five groups of hens were continually exposed for 90 d to vapors containing various concentrations (10, 50, 100, 200, and 400 ppm) of MBK mixture. A control group of hens was placed in an inhalation chamber in the absence of MBK vapor. The hens were supplied with food (Layena Chicken Feed, Ralston Purina Co., St. Louis, Mo.) and water *ad libitum*. Body weights were monitored weekly, and hens were examined every other day for neurological signs of MBK neurotoxicity. At the end of the exposure period, the surviving and control birds were removed from the inhalation chambers and kept in individual cages in an air-conditioned room for a 30-d observation period before termination of the experiment.

Animal Inhalation Exposure Chamber A portable-type stainless steel animal inhalation exposure chamber (Young and Bertke Co., Cincinnati, Ohio) on an iron stand was used. The chamber had a cubic configuration with top and bottom cones. The length, width, and height between cones

were 27 in. Cross-sectional area and total volume were 5 ft^2 (0.46 m^2) and 11.4 ft^3 (0.32 m^3), respectively. The floor was 2 X 2 in stainless steel woven wire mesh. The chamber was equipped with observation windows in the door and the back, a high-pressure autoclave-type door, an air exhaust, and a spray ring flushing system in the bottom cone. Temperature and relative humidity inside the chamber were 26°C and 60%, as measured by a 3-in dial stainless steel thermometer with a 4-in stem and a 6-in brass hygrometer, respectively (Fig. 2).

The chamber had an inlet orifice and a damper that allowed one total chamber volume change every 5 min. Airflow through the chamber was calibrated with a flow computer (model 100E Autotronic Controls, El Paso, Tex.). A negative static pressure of 0.3 in H_2O was maintained in the chamber and measured with a negative gauge connected to one of the front port openings and regulated with a butterfly damper located in the exhaust pipe.

Room air supplied to the chamber was filtered through a 1-in-thick, 12 X 12 in fiberglass prefilter and then a glove box filter. The self-contained absolute filter was 8 in^2 and 10 in deep with a 3-in connection of polyvinyl chloride (PVC) pipe on each side of the plywood frame. The prefilter was held in a stainless steel square-to-round funnel and was easily slipped out of a slot on the face of the filter box for changing.

Air was exhausted through a 2-in tube on the side of the lower cone connected to an Aerovent type PPB hood exhaust fan (Aerovent, Piqua, Ohio) with a $\frac{1}{2}$-horsepower d-c motor (Indiana General, El Paso, Tex.). A 2-in quick-opening valve at the bottom of the lower cone was permanently

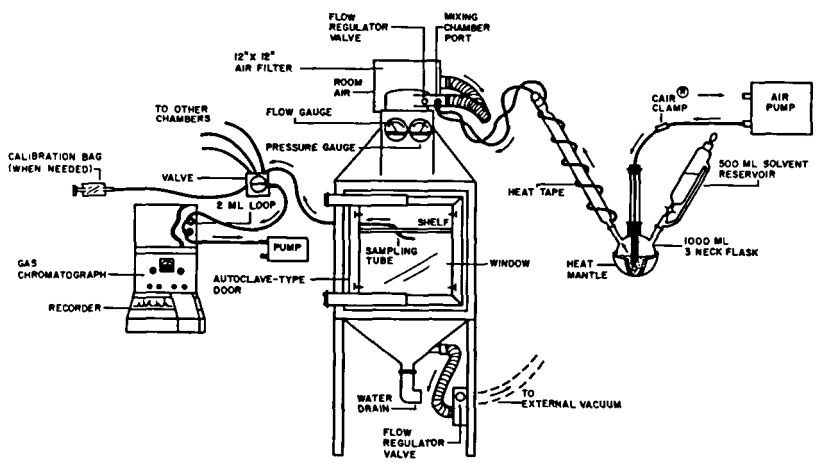

FIGURE 2. Exposure system for MBK vapor: (1) generation of MBK vapor, (2) inhalation exposure chamber, and (3) monitoring of MBK vapor concentration. For a detailed description see the Methods section.

connected to the floor drain, allowing the chamber to be drained when it was necessary to wash down the interior.

Generation of MBK Mixture Vapors MBK vapor was generated in a 1-l three-neck round-bottom flask placed in a heating mantle. The flask was half-filled with technical grade MBK. One neck was connected to a tank of compressed pure breathing air, another to a 500-ml separatory funnel that served as a reservoir of MBK, and the third to a 400-mm condenser. The condenser was connected to the chamber with Teflon tubing. Both the condenser and the Teflon tubing were wrapped with heating tape that was heated to 50°C to prevent condensation of MBK mixture vapor. The vapor was swept into the chamber by airflow from the air tank. The desired concentration of vapor was maintained by adjusting the heating temperature and airflow through liquid MBK. No heat was needed to generate 10, 50, or 100 ppm MBK vapors. However, for 200 or 400 ppm MBK, the heating mantle was at 34°C (Fig. 2).

Monitoring of MBK Mixture Vapor Concentration MBK mixture vapor concentration was monitored five times a day with a Gow-Mac gas chromatograph (series 750, Gow-Mac Instruments, Bound Brook, N.J.). Teflon tubing was used when drawing air samples from the center of the cage. The Teflon tubing was connected to a 2-mm injection loop attached to a valve for injecting the air into the gas chromatograph. The gas chromatograph was equipped with a 6 ft X 2 mm OD coiled glass column packed with Tenax GC 60/80 mesh, a flame ionization detector, and a chart recorder. The column was conditioned at 250°C with purified He at 70 ml/min for 24 h. Column temperature was 150°C and that of the detector and injector block was 160°C. Gas flow rates (cm^3/min) were: carrier gas (He), 30; H_2, 20; and air, 300. A standard curve was constructed for both M*n*BK and MiBK with 500-ml plastic bags (Anspec Co., Ann Arbor, Mich.). A series of M*n*BK or MiBK concentrations was prepared by injecting these solvents with a 10λ Hamilton syringe into a plastic bag through a rubber septum. Air was then pumped into the bag with a needle. Airflow was determined with a Fisher Mark IV flowmeter, using a 1/16 in stainless steel ball (Fisher Scientific Co., Raleigh, N.C.). After the air and solvent vapor were mixed, the bag was connected to the injection loop and the vapor was injected into the gas chromatograph. Standard curves were obtained by plotting concentration against peak areas (Fig. 2).

Clinical Assessment of Neurotoxicity

Control and treated hens were examined every other day to check for signs of neurotoxicity. Ataxic hens were graded by classifying the degree of their ataxia before paralysis 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). In T_1 there is diminished leg movement and reluctance to walk,

with hens tending to slide on the floor or fly; T_2 is characterized by a change in gait and disturbance of leg movement; in T_3 severe leg weakness is manifested by unsteadiness and occasional falling on the floor; and T_4 is marked by inability to walk. In the paralysis stage, hens were unable to stand.

Histopathology of Nerve Tissues

The spinal cord and the sciatic, peroneal, and tibial nerves were excised from hens that died during the experiment or were killed by heart puncture and exsanguination. Hens whose clinical condition deteriorated to paralysis were killed before the end of the experimental period. Nerve tissues were fixed in phosphate-buffered 10% Formalin solution (pH 7.0) for at least 10 d (Abou-Donia and Preissig, 1976). Parasaggital longitudinal and cross sections of the spinal cord and cross and longitudinal sections of the peripheral nerves were stained with hematoxylin & eosin combined with luxol fast blue (H&E-LFB). Sections from peripheral nerves were also stained with Holmes silver stain. Severity of lesions was defined by the following criteria. (1) Rare swollen axons without fragmentation, phagocytosis, or loss of myelin staining were designated as equivocal histological changes. (2) Occasional degenerative changes of axons and myelin in peripheral nerve or within the spinal cord, which may contain nests of phagocytic cells, were termed mild to moderate degeneration. (3) Lesions were considered severe when 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. Only gross examination was carried out for all other tissues.

Statistics

The significance of differences between weight of control and treated hens was assessed by a Student's two-tailed *t*-test. A p value of 0.05 or less was considered significant.

RESULTS

Analysis of Methyl Butyl Ketone Vapor Mixture in Chambers

The retention times for M*n*BK and MiBK were 2.5 and 1.8 min, respectively. The M*n*BK concentration in the chamber was 70 ± 5.14% (mean ± SE). The concentration of MBK vapor mixture in the chamber varied only 5%.

Body Weight Changes

Only hens exposed to one of the highest two concentrations of MBK, 400 or 200 ppm, lost significant weight at the onset of ataxia (Table 1);

TABLE 1. Change in Weight of Hens following Subchronic (90 d) Continuous Inhalation of MBK[a]

MBK concentration (ppm)	Initial weight (kg)	Percent of initial weight[b]			
		Onset of ataxia	Onset of paralysis	End of treatment	Termination
400	1.75 ± 0.08	73.2 ± 6.3	48.0 ± 7.4	48.0 ± 7.4[c]	48.8 ± 7.4
200	1.53 ± 0.09	72.5 ± 5.2	63.1 ± 5.5	57.7 ± 4.2[d]	57.7 ± 4.2
100	1.51 ± 0.09	114.9 ± 6.0	*e*	91.7 ± 2.1	108.3 ± 7.0
50	1.46 ± 0.09	100.6 ± 11.0	*e*	104.1 ± 11.0	108.2 ± 12.0
10	1.64 ± 0.11	96.6 ± 6.0	*e*	96.1 ± 6.7	100.0 ± 4.0
0	1.70 ± 0.13	*f*	*f*	110.3 ± 7.5[g]	116.0 ± 5.0

[a]Hens were continuously exposed to technical grade MBK (70% M*n*BK and 30% MiBK) in inhalation chambers, and surviving birds were kept out of the exposure chambers for a 30-d observation period.

[b]Values are means ± SE of three hens.

[c]Two of these hens died after 27 d and the remaining three were killed on d 30 after their condition deteriorated.

[d]One died after 72 d and the remaining four were killed on d 73 after their condition deteriorated.

[e]These hens did not develop paralysis.

[f]Control hens remained normal.

[g]Weight after 90 d from the beginning of the experiment.

weight loss for these two groups continued, and the hens exposed to 400 and 200 ppm MBK weighed 48.0 ± 7.4 and 63.1 ± 5.5% (mean ± SE) of the initial weights, respectively, at the onset of paralysis. Although the group exposed to 100 ppm MBK gained some weight at the onset of ataxia, they lost 24.4% of their initial weight after 69 d of exposure. This weight loss coincided with the development of severe ataxia. This treatment group, however, regained all lost weight by the end of the 30-d observation period. No appreciable change in weight was observed in groups exposed to 50 and 10 ppm MBK.

Clinical Assessment of Neurotoxicity

None of the hens continually exposed to MBK vapor showed any signs of acute toxicity that are attributed to the narcotizing effects of MBK on the central nervous system.

All hens continually exposed to 50–400 ppm MBK vapor through inhalation developed ataxia after a latent period of 6–30 d, depending on the MBK concentration (Table 2). Those exposed to 400 ppm progressed to paralysis, and two died 27 d after the beginning of exposure. The remaining three chickens were sacrificed at d 31 because their condition had deteriorated; they became too weak, had difficulty breathing, and could not reach food or water. All hens exposed to 200 ppm MBK developed paralysis 64–72 d after the beginning of the exposure; one of

TABLE 2. Sequence of Treatments and Onset of Clinical Signs in Hens following Subchronic (90 d) Continuous Inhalation of Technical Grade MBK[a]

MBK concentration[b] (ppm)	Days after beginning of exposure (mean ± SE)					
	Ataxia[c]					
	Mild	Gross	Severe	Near paralysis	Paralysis	Termination
400	11 ± 1.3	17 ± 1.4	23 ± 2.0	26 ± 1.0	28 ± 1.0	29.4 ± 1.0[d]
200	15 ± 0.8	34 ± 0.5	49 ± 3.0	58 ± 2	68 ± 2	73 ± 0.2[e]
100	21 ± 0.5	57 ± 2.0	69 ± 2.0	101		120 ± 0.0
50	27 ± 2.0	60 ± 4.0	89 ± 1.0 (98 ± 1)[f]	89 ± 1[f]		120 ± 0.0

[a]Hens were continuously exposed to MBK mixture (70% M*n*BK and 30% MiBK) vapors in inhalation chambers, and surviving birds were kept out of the chambers for a 30-d observation period.

[b]Hens exposed to 10 ppm were not different from controls exposed to ambient air in an exposure chamber for 90 d, then kept outside the chamber for a 30-d observation period.

[c]Clinical grades are defined in the Methods section.

[d]Two hens died after 27 d and the other three were sacrificed after their condition deteriorated and they had difficulty breathing.

[e]One hen died after 72 d and the other four were sacrificed on d 73 after their condition deteriorated and they had difficulty breathing.

[f]Three hens developed ataxia with near paralysis (T_4) and their condition regressed to severe paralysis (T_3).

these hens died at d 72 and the other four were sacrificed the following day. Four of the hens inhaling 100 ppm MBK developed severe ataxia (T_3), while the fifth bird progressed to ataxia with near paralysis (T_4). Three hens of the group exposed to 50 ppm MBK showed severe ataxia (T_3), while the other two developed only gross ataxia (T_2). The clinical condition of all hens in this group was gross ataxia (T_2) at termination. All hens exposed to 10 ppm MBK remained normal. Figure 3 shows that the number of days of exposure to MBK mixture vapors before the onset of ataxia was dependent on and inversely proportional to the concentration of MBK. Thus when hens were exposed to 50 ppm MBK, 27 ± 2 d (mean ± SE) were required to produce ataxia, compared with 11 ± 1 d when 400 ppm MBK was inhaled.

Necropsy Observations

All tissues were grossly examined at sacrifice. No changes in tissues were observed when treated and control hens were compared for size, shape, and color.

Histological Changes

Spinal Cord Histological examination was performed on spinal cord and peripheral nerve tissues from MBK-exposed and control hens (Table 3). Lesions in the spinal cord were dependent on MBK concentration,

duration of exposure, and duration of intoxication. Thus when hens were exposed to 400 ppm MBK, they died or were killed after 18 d of intoxication; only one of these hens exhibited unequivocal lesions in the spinal cord. By contrast, hens exposed to 100 ppm MBK survived longer; all exhibited clinical signs of neurotoxicity for 99 ± 2 d, and all five hens showed unequivocal changes in the spinal cord. Although hens exposed to 50 ppm MBK were poisoned for a mean of 97 d, only four of these hens had unequivocal changes in the spinal cord.

Two of the hens exposed to 400 ppm MBK did not exhibit any histopathologic alterations, while another two showed equivocal changes. Also, one hen from each group exposed to 200 and 50 ppm had equivocal histopathologic lesions in the spinal cord. Occasional swollen axons in the absence of fragmentation or phagocytosis of the axons without myelin loss was designated as equivocal because it has been observed on rare occasions in the spinal cord of untreated hens. However, these alterations may represent the earliest histopathologic changes in MBK neurotoxicity. Large axonal swelling is seen in the longitudinal section of the ventral columns of thoracic spinal cord from hen 204 (Fig. 4). A cross section of the thoracic spinal cord from hen 208 illustrates numerous greatly swollen axons in the ventral columns. Fewer swollen axons were observed in the lateral and dorsal columns (Fig. 5). Extensive axonal degeneration is seen in the longitudinal section of the ventral columns (Fig. 6).

Peripheral Nerves The presence of histopathologic lesions in peripheral nerves was a function of both the level of MBK inhaled and, particularly, the total dose inhaled. Although all five hens exposed to 100 ppm for 90 d survived until termination on d 120, they showed gross to severe ataxia and each had unequivocal lesions in peripheral nerves. Hens given high

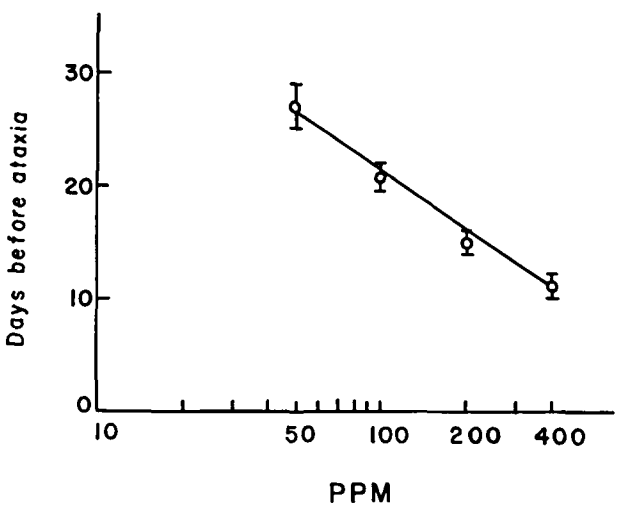

FIGURE 3. Effect of MBK concentration on time before onset of ataxia in hens.

TABLE 3. Duration of Exposure, Intoxication, and Histopathologic Changes in Nerve Tissues from Hens following Subchronic (90 d) Continuous Inhalation of MBK[a]

Hen	MBK concentration (ppm)	Duration (d)[b]		Histopathologic change[c]	
		Exposure	Intoxication	Spinal cord	Peripheral nerve
224	400	27[d]	19	—	—
225	400	31	17	±	+
226	400	27[d]	18	—	—
227	400	31	17	+	+
228	400	31	21	±	—
209	200	73	59	±	—
210	200	72[d]	55	NE	NE
211	200	73	59	+	±
212	200	73	59	+	±
213	200	73	58	+	+
204	100	90	99	+	+
205	100	90	100	+	+
206	100	90	98	++	+
207	100	90	97	+	+
208	100	90	99	+	+
234	50	90	90	+	—
235	50	90	94	+	—
236	50	90	96	±	—
237	50	90	98	+	—
238	50	90	87	+	±

[a]Hens were continuously exposed to technical grade MBK (70% M*n*BK and 30% MiBK) in inhalation chambers, and surviving birds were kept out of the exposure chambers for a 30-d observation period. Nervous tissues from hens exposed to 10 ppm MBK mixture vapors or control hens showed no neuropathologic changes.

[b]Days between onset of ataxia and death or killing.

[c]Abbreviations: NE, tissue not examined; —, changes absent; ±, equivocal histologic changes (rare swollen axons without fragmentation, phagocytosis, or loss of myelin staining); +, mild to moderate degeneration of axons and myelin in peripheral nerve or within spinal cord (these lesions may contain nests of phagocytic cells); and ++, severe degeneration of axons and myelin. Lesions are termed severe where there is almost complete destruction of axons and myelin in a tract, such as the anterior columns, or within extensive areas of peripheral nerve.

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

doses became paralyzed and thus could not be kept alive as long as those given 100 ppm MBK. Scattered degenerated axons are seen in the section of the tibial nerve shown in Fig. 7. Axonal and myelin debris can be identified in macrophages. Figure 8 shows axonal degeneration and macrophages containing debris in sections of peripheral nerve from hen 205. A longitudinal section of the peripheral nerve from hen 204 shows markedly enlarged axons (Fig. 9). On either side of the point where the swellings begin at the node of Ranvier, there is retraction of myelin.

FIGURE 4. Thoracic spinal cord from hen 204 (exposed to 100 ppm MBK for 90 d). This longitudinal section of ventral column shows an axon (coursing from left to right) which has become swollen (center) before passing out of the plane of the section. The swollen area of the axon continues to bear a myelin sheath. H&E-LFB, X80.

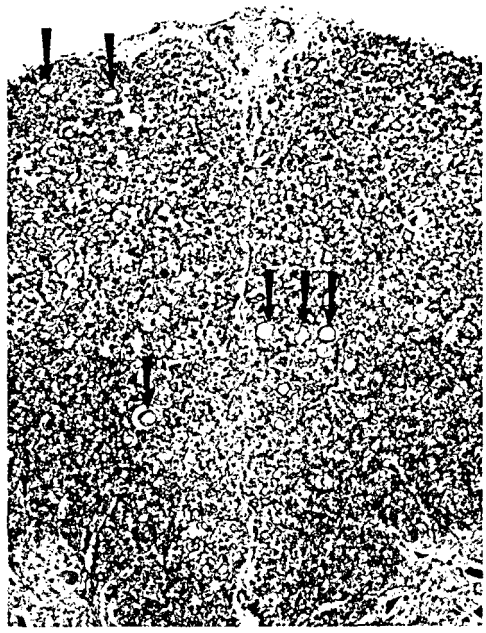

FIGURE 5. Thoracic spinal cord from hen 208 (exposed to 100 pm MBK for 90 d). This cross section of ventral column shows many axons greatly enlarged. Fewer swollen axons were observed in the lateral and dorsal columns. Ventral horn cells (upper corners) are unremarkable except for mild autolysis. H&E-LFB, X75.

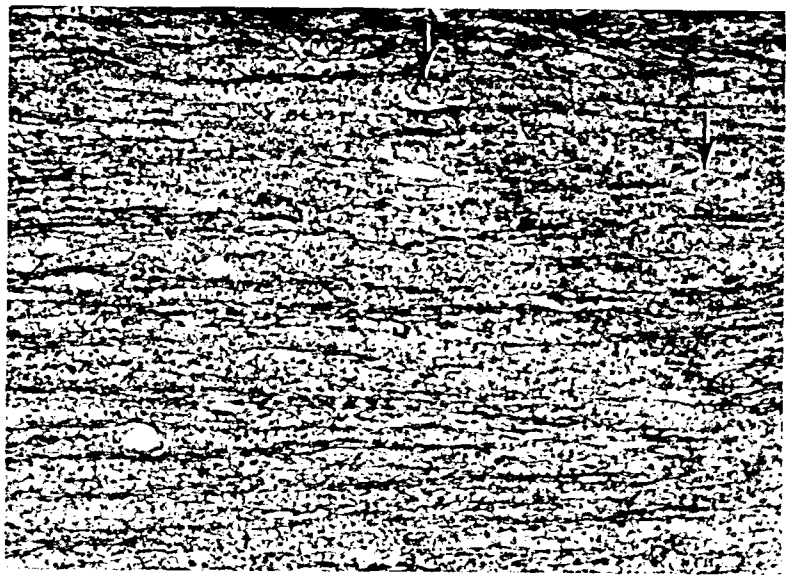

FIGURE 6. Thoracic spinal cord from hen 205 (exposed to 100 ppm MBK for 90 d). This longitudinal section of ventral column contains swollen axons (s). In addition, extensive axonal degeneration with loss of myelin staining and phagocytosis of axonal and myelin debris are evident in the lower 90% of this figure. The normal staining intensity of myelin is shown in the upper 10%, the white matter immediately anterior to the ventral horns. H&E-LFB, X117.

FIGURE 7. Peripheral nerve from hen 205 (exposed to 100 ppm MBK for 90 d). A small branch of the sciatic nerve is seen in longitudinal section. Almost every myelinated axon in this nerve has degenerated, with debris-containing macrophages marking the sites of axonal and myelin degeneration. H&E-LFB, X248.

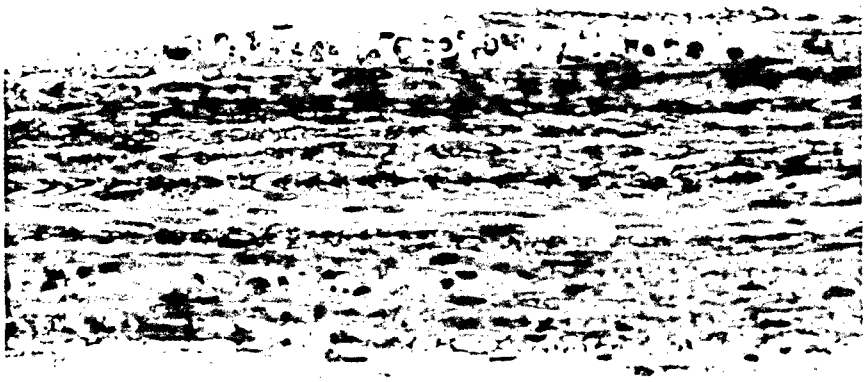

FIGURE 8. *Peripheral nerve from hen 205* (exposed to 100 ppm MBK for 90 d). Most of the myelinated axons of this small sciatic nerve branch are intact. At the top, however, a row of macrophages containing debris with the staining properties of myelin marks the site of axonal degeneration. Less intense phagocytosis is noted in the lower portion of this figure. H&E-LFB, X400.

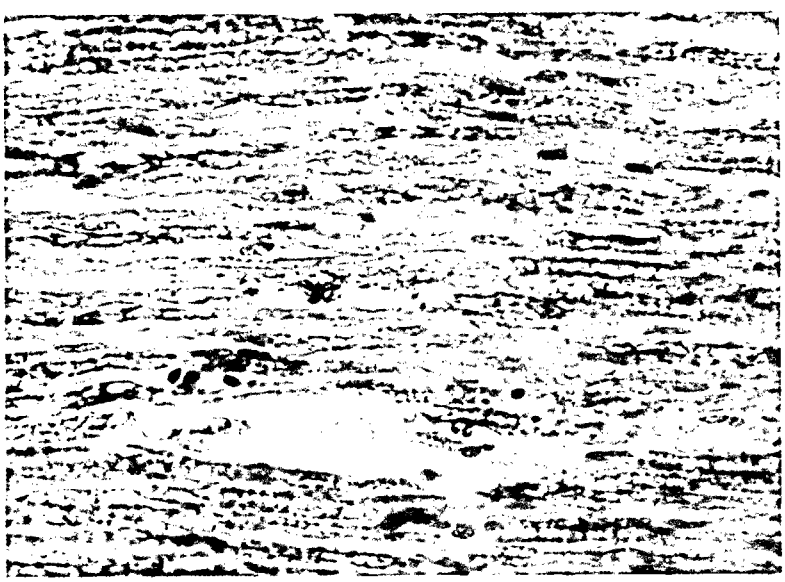

FIGURE 9. Peripheral nerve from hen 204 (exposed to 100 ppm MBK for 90 d). The portion of nerve seen here in longitudinal section is free from axonal degeneration and phagocytosis. In the lower third, running from left to right, an axon is present that has a marked enlargement in its diameter. On either side of the point where swelling begins, apparently a node of Ranvier, there is retraction of myelin. H&E-LFB, X540.

DISCUSSION

The neurotoxic effect of subchronic (90 d) continuous inhalation of technical MBK in the chicken was concentration-dependent. Exposure to 200 and 400 ppm MBK caused ataxia that progressed to paralysis and death in some hens, 100 ppm MBK produced severe ataxia, 50 ppm MBK caused gross ataxia, and 10 ppm had no effect. Although a mixture of M*n*BK and MiBK (7:3) was used in this study, the neurotoxic effect is believed to be a result of M*n*BK and not MiBK. This conclusion is based on the report that exposure to commercial grades of methyl ethyl ketone (MEK) and MiBK (9:1) did not cause neurotoxicity in cats (Spencer and Schaumburg, 1976). Nevertheless, it is possible that MiBK pontentiates the neurotoxic effect of M*n*BK, as reported for the potentiating effect of MEK on M*n*BK (Abdel-Rahmen et al., 1976; Saida et al., 1976) and *n*-hexane (Altenkirch et al., 1977). This possibility is being investigated.

Following the identification of M*n*BK as the causative agent in the 1973 outbreak of peripheral neuropathy in a fabric plant in Ohio (Billmaier et al., 1974; Mendell et al., 1974), M*n*BK neurotoxicity was produced in dogs, cats, rats, guinea pigs, primates, and chickens (Spencer et al., 1980). When chickens, rats, and cats were exposed to 400 ppm M*n*BK by inhalation, chickens were the most sensitive, followed by cats; rats were the least sensitive (Mendell et al., 1974). Relative species susceptibility to M*n*BK is: chicken $>$ cat $\sim$ dog $>$ primate $>$ rat. Nevertheless, the rat has been the animal most used in studying M*n*BK neurotoxicity.

In the present study we chose the chicken for the following reasons. (1) In toxicological studies it is assumed that humans are at least as sensitive to a particular toxic effect as the most sensitive species; the chicken fulfills this requirement. (2) The chicken is sensitive to neurotoxicity produced by either *n*-hexane-related solvents or organophosphorus esters, allowing the study of both syndromes in one species. (3) The chicken is ideal for studying species selectivity, a common phenomenon in neurotoxicity of chemicals.

Neurotoxicity of MBK was produced by several exposure routes in the chicken. Subchronic (90 d) oral administration of MBK and related compounds in the hen showed that the relative neurotoxicity of the test compounds in increasing order of potency was: *n*-hexane $<$ M*n*BK $<$ 2,5-HDOH $<$ 2,5-HD (Makkawy et al., 1981). Similar results were obtained following subchronic (90 d) ip injection of these chemicals in the hen (M. B. Abou-Donia, unpublished data) and the rat (Spencer et al., 1978; Egan et al., 1980; Krasavage et al., 1980). *n*-Hexane undergoes oxidation by microsomal enzymes to form alcohols, principally 2-hexanol (Frommer et al., 1974). 2-Hexanol is then further oxidized to 2,5-HDOH, which is oxidized to 2,5-HD. The latter metabolite also results from M*n*BK hydroxylation and oxidation (Kramer et al., 1974; DiVincenzo et al.,

1976; Abdel-Rahman et al., 1976) (Fig. 1). These studies postulated that 2,5-HD may be the active neurotoxic metabolite for *n*-hexane, M*n*BK, and 2,5-HDOH. The low sensitivity of the hen, as opposed to the rat, to the neurotoxicity of *n*-hexane may be explained by the low metabolizing activity of xenobiotics in the chicken. In harmony with this is our recent finding that the specific content of cytochrome P-450 in the rat liver is 3.6-fold greater than that in the chicken liver (Lasker et al., 1981).

Although the biochemical mechanism of the neurotoxic action of these chemicals is not known, it has been suggested that 2,5-HD and M*n*BK may inhibit the sulfhydryl enzymes glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphofructokinase (Sabri et al., 1979a, 1979b). Such inhibition would interfere with glycolytic energy production and lead to the interruption of fast axoplasmic transport (Mendell et al., 1977). Our recent results indicated, however, that 2,5-HD is unlikely to inhibit GAPDH through binding to sulfhydryl groups (Graham and Abou-Donia, 1980). We postulated that 2,5-HD neurotoxicity might be related to its high water solubility and its ability to derivatize amino groups of proteins. Evidence for the latter has been presented (Graham, 1980).

The histopathologic results of the present study are similar to those of other reports (Mendell et al., 1974; Spencer et al., 1975; Makkawy et al., 1981). The severity and frequency of the lesions depended on (1) the exposure concentration, (2) the exposure duration, (3) the time between beginning of exposure and termination, (4) the severity of the clinical condition, and (5) the site of tissue sampling.

Since humans have been simultaneously exposed to solvents structurally related to *n*-hexane and neurotoxic organophosphorus esters, it is important to study neurotoxicity produced by both classes of neurotoxicants in a sensitive species such as the chicken. Although axonal swelling was the earliest lesion in both instances, organophosphorus-induced axonal degeneration and M*n*BK neurotoxicity in hens had the following characteristic differences. (1) Axonal swellings in MBK neurotoxicity were larger and more abundant than those in organophosphorus neuropathy (nerve) or myelopathy (spinal cord). (2) The axonal swellings in the hexacarbon lesion were also more intensely stained with eosin, showing a high protein content (i.e., neurofilaments). (3) The swellings in the spinal cord in MBK myelopathy were more common in the ventral columns; in organophosphorus myelopathy, they were most commonly observed in dorsal horns, dorsal columns, and ventral columns. (4) When an MBK-induced swelling was observed in a longitudinal section of the spinal cord and peripheral nerve, its abrupt beginning and proximity to an apparent node of Ranvier suggested strongly that it was paranodal, as described earlier (Mendell et al., 1974; Spencer et al., 1980); axonal swellings in organophosphorus neurotoxicity are internodal (Cavanaugh, 1979). (5) Microscopic examination showed that wallerian degeneration appeared to follow axonal swelling in MBK neurotoxicity and was

identical to that seen in organophosphorus neurotoxicity, except that it was almost exclusively observed in the ventral columns of the lower spinal cord. In the lateral and, less commonly, in the dorsal columns at all levels scattered axonal enlargement was the most severe lesion. Organophosphorus lesions were most severe in the ventral columns, but significant lesions were often observed in the lateral and dorsal columns of the upper cord as well.

The extent and permanence of injury and the progression or improvement of signs of neurotoxicity depended on the dose and duration of exposure. Continuous exposure to high concentrations of MBK (400 and 200 ppm) led to ataxia, paralysis, and death. Most animals exposed to the intermediate concentration, 100 ppm, became ataxic, with no improvement in clinical condition during the 30-d observation period. Some hens continuously exposed to the low concentration of MBK (50 ppm) showed improvement of neurological function during the observation period. Hens treated with the smallest dose (10 ppm) did not show any neurological dysfunction. It is noteworthy that the threshold limit value (TLV) for M*n*BK is 25 ppm (Spencer et al., 1980).

This report demonstrates that neurotoxicity produced by continuous inhalation of MBK is concentration-dependent. This offers hope of recovery to humans exposed to low concentrations of this type of chemical who exhibit only mild neurological dysfunction. However, since continuous exposure leads to permanent spinal cord damage or even death in experimental animals, humans should handle such solvents with great caution.

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