THE ENLARGING VIEW OF HEXACARBON NEUROTOXICITY*

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I. HISTORY AND OVERVIEW

The neurotoxic property of certain hexacarbon** solvents was discovered in Japan in the early 1960s when workers exposed to hexanes*** in the laminating industry developed a sensorimotor polyneuropathy. Similar outbreaks of neuropathy were subsequently reported in Japan, Europe, and the U.S. Japanese physicians also identified the first cases of neurological disease in individuals inhaling solvent vapors for their euphoric properties, a practice which subsequently led to the discovery of "glue-sniffers" or "huffers" neuropathy in other countries.

The common thread linking the industrial and glue-sniffers neuropathies was repetitive exposure to normal hexane (*n*-hexane). Although the solvent was repeatedly implicated as the neurotoxic agent, the disease being reproducible in experimental animals exposed to *n*-hexane, this idea was resisted in some quarters because the compound had enjoyed wide usage and was generally regarded as having a low toxicity potential. This view was reinforced by some experimental studies which failed to produce neuropathy in exposed animals, but was finally overthrown when unequivocal neuropathological damage was demonstrated in exposed rats similar to that reported from examination of nerve biopsies from patients with industrial or glue-sniffers' neuropathy.

Another complicating factor was the variety of agents other than *n*-hexane in the exposure history of individuals developing neuropathy, e.g., hexane isomers,** methyl iso-butyl ketone (MiBK), methyl ethyl ketone (MEK), and acetone (all now known to be unable to produce neuropathy in animals), ethyl acetate, xylene, methanol (able to produce retinal and optic nerve damage), toluene (reportedly unable to produce neuropathy in man), isopropyl alcohol, *n*-heptane (once claimed to produce experimental neuropathy), and *n*-octane. The possible role of these compounds has only begun to be investigated, MEK being of principal current interest because of its ability to potentiate the neurotoxic effects of both *n*-hexane and methyl *n*-butyl ketone (M*n*BK). It now seems clear *n*-hexane is the least active of the neurotoxic hexacarbon solvents, and its neurotoxic properties are significantly potentiated by concurrent exposure to MEK (see Section III.D). Despite the publication of a few reports specifically implicat-

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** Compounds containing six carbon atoms.

*** Hexanes have the formula C_6H_{14} and include *n*-hexane, 2-methylpentane, 3-methylpentane, 2,2-dimethylbutane, 2,3-dimethylbutane, and *cyclo*hexane, and 3-methylpentane (once reported[245] to have produced experimental neuropathy, but found in another study[61] to lack this property).

ing MEK in outbreaks of human neuropathy, there is overwhelming evidence to the contrary from animal studies. MEK also appears able to induce neuropathy in humans exposed to subneurotoxic levels of *n*-hexane. Because it would not be surprising if other compounds displayed the ability to potentiate *n*-hexane neurotoxicity, it would appear prudent to minimize human exposure to mixed solvents containing any neurotoxic hexacarbon compounds.

The discovery of 2-hexanone (methyl *n*-butyl ketone, M*n*BK) neuropathy opened another chapter in the enlarging view of hexacarbon neurotoxicity. In the same way as *n*-hexane neuropathy was discovered, the neurotoxic properties of M*n*BK only came to light through industrial accident. Methyl *n*-butyl ketone was first implicated in 1973 after an outbreak of peripheral neuropathy at a fabric factory in the U.S. The compound recently had been introduced as a substitute for MiBK, used with MEK in the printing-ink solvents, in compliance with new air pollution regulations. M*n*BK was subsequently implicated in other neuropathies affecting spray painters and furniture finishers, these times in the absence of MEK. These outbreaks provided the impetus for a large number of experimental studies which confirmed the neurotoxic property of M*n*BK and demonstrated its enhanced neurotoxicity in the presence of MEK.

The structural similarity of *n*-hexane and M*n*BK suggested the possibility of a common neurotoxic mechanism. Experimental studies on the metabolism of M*n*BK and *n*-hexane revealed six interrelated metabolites which showed a gradient of neurotoxic potency correlating with peak serum levels of the common metabolite 2,5-hexanedione (2,5-HD). This compound also produced the earliest nerve damage in organotypic nerve tissue cultures. Closely related compounds, in which the carbonyl groups occupied different relative positions in the six-carbon chain, proved unable to induce neuropathy, suggesting that the neurotoxic property of 2,5-HD was associated with the 1,4 (γ) spacing of the carbonyl groups. This idea received strong support when 2,5-heptanedione and 3,6-octanedione were shown to induce the same type of experimental neuropathy. If this principle holds true for longer chain aliphatic compounds, it may be appropriate to adopt the eponym *γ-diketone* to describe this type of neurotoxicity and thereby discard the prefix *hexacarbon*.

Another major facet of this unfolding story concerns the mechanism of nerve fiber degeneration. This problem engaged the attention of neurological scientists because hexacarbon neuropathy proved to be a useful experimental model of human neurological diseases of the dying-back type. Dying-back diseases — now known as *central-peripheral distal axonopathies* — are characterized by distal and retrograde axonal degeneration occurring in long and large fiber tracts in the central, as well as the peripheral, nervous systems. This pattern of neuropathology probably underlies a number of inherited and acquired conditions of obscure etiologies. The neurotoxic hexacarbons were initially helpful for the study of dying-back disease because the characteristic pathological changes of giant, swollen axons could be readily traced in time and space. It became clear that experimental hexacarbon neuropathy was a disease of brain and spinal cord (CNS), as well as peripheral nerves (PNS), an observation which illuminated some puzzling clinical features of the human disease. Close study of the spatial-temporal pattern of experimental disease in vivo and in culture showed that swellings initially developed on proximal sides of nodes of Ranvier in the distal part of the nerve fiber, later causing fiber breakdown distal to the swelling. Nerves showing these pathological features were associated with changes in their ability to convey materials along their length by axonal transport, transported materials becoming blocked above nodes of Ranvier and presumably contributing to the swelling process. These observations revolutionized our understanding of the central-peripheral distal axonopathy produced by neurotoxic hexacarbons and focused attention on the nerve fiber itself as the likely locus of toxic action, an idea that has recently received experimental verification. Biochemical studies have demonstrated the ability of neurotoxic vs. nonneurotoxic aliphatic hexacarbon diketones to inhibit glycolytic enzymes and bind to other proteins, thereby raising the possibility that axonal transport dysfunction and nerve fiber degeneration are associated with these properties.

II. SCOPE AND DATA BASE

This review synthesizes reports on human hexacarbon neurotoxicity with experimental evidence on the metabolism and pharmacokinetic fate of hexacarbon compounds, and behavioral, morphological, physiological, and biochemical data.

The term *hexacarbon neurotoxicity* was introduced[225] to describe the property of *n*-hexane and methyl *n*-butyl ketone to produce structural damage to the central and peripheral nervous system. This effect should be distinguished from the so-called acute central nervous system (CNS) effect of these compounds which refers to the narcotizing properties of high concentrations of these solvents. The acute CNS effect with high doses of *n*-hexane is a reversible pharmacological event, probably occurring without structural breakdown of the nervous system. By contrast, prolonged exposure to relatively low levels of *n*-hexane or M*n*BK produces a specific pattern of nervous system degeneration which is reversible to the extent that damaged nerve fibers can regenerate and restore functional connections with target organs. This review includes reference to the acute as well as the subchronic effects (i.e., non-lifetime exposure) of hexacarbon compounds. Although *n*-hexane and M*n*BK are the principal subjects of this paper, other six-carbon and related seven- and eight-carbon compounds are also mentioned (Table 1). Reference is also made to refined petroleum solvents in which hexanes are present.

The reader is referred elsewhere[43,120,142-144,201] for full treatment of the acute, reversible CNS effects of *n*-hexane and methyl *n*-butyl ketone, the occupational hazard associated with their use as solvents, their environmental contamination potential and general toxicology, and the problem of human solvent abuse.

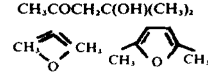
The data base for this review included the foregoing references, the resources of the Neurotoxicology Information Repository (N.I.R.)* at the Institute of Neurotoxicology of the Albert Einstein College of Medicine, and papers, abstracts, and communications submitted in response to personal invitation.

III. HUMAN HEXACARBON TOXICITY

The only hexacarbons convincingly demonstrated to be neurotoxic following prolonged exposure to humans are *n*-hexane and M*n*BK. A few cases of neuropathy have been attributed to MiBK[20,158] and to *cyclo*hexane,[73] but these compounds have failed to produce neuropathy in experimental animals (see Sections VI.B.3 and 4). *n*-Hexane and M*n*BK produce similar neurological syndromes in man[194] and a morphologically identical central-peripheral distal axonopathy in experimental animals.[226] Therefore, throughout the majority of this section, little attempt is made to discriminate between the human neurotoxicity of these two solvents. This policy reflects the current body of *clinical* reports on these two compounds, although there is *experimental* evidence showing that the two substances have substantially different neurotoxic potencies. (See Section VI.B.5.a.)

* Information submitted to N.I.R. prior to January 1980.

Table 1
HEXACARBONS AND OTHER COMPOUNDS

Group	C	Compound name(s)	Abbreviation	Structure	Neurotoxic	Ref.
Alkanes	6	*n*-Hexane		$CH_3CH_2CH_2CH_2CH_2CH_3$	+	192
	6	2-Methyl pentane		$CH_3(CH_2)_2CH(CH_3)_2$	–	62
	6	3-Methyl pentane		$CH_3CH_2CH(CH_3)CH_2CH_3$	–	62,75,245
	6	2,3-Dimethyl butane		$C_2H_5C(CH_3)_3$		62,75
	6	*Cyclo*hexane		$[(CH_2)_6]$	–	62,71,75
	6	Methyl *cyclo*pentane		CH_3–$CHCH_2CH_2CH_2CH_2$ (ring)	–	62
Ketones	3	Acetone		CH_3COCH_3	–	216
	4	Methyl ethyl ketone	MEK	$CH_3COCH_2CH_3$	–	62,222
	5	Methyl *n*-propyl ketone		$CH_3COCH_2CH_2CH_3$	–	117
	6	Methyl *n*-butyl ketone (2-hexanone)	M*n*BK	$CH_3COCH_2CH_2CH_2CH_3$	+	104,138,222
	6	Methyl isobutyl ketone	MiBK	$CH_3COCH_2CH(CH_3)_2$	–	222
	6	Methyl tert-butyl ketone		$CH_3COC(CH_3)_3$	–	96
	6	5-Hydroxy-2-hexanone	5-OH-2-H	$CH_3COCH_2CH_2CHOHCH_3$	+	116
	6	4-Hydroxy-methyl pentane		$CH_3COCH_2C(OH)(CH_3)_2$		
	6	2,3-Hexanedione		$CH_3COCOCH_2CH_2CH_3$	–	216
	6	2,4-Hexanedione		$CH_3COCH_2COCH_2CH_3$	–	216
	6	2,5-Hexanedione	2,5-HD	$CH_3COCH_2CH_2COCH_3$	+	157,219
	6	*Cyclo*hexanone		$[(CH_2)_5CO]$		72,152
	6	Mesityl oxide		$CH_3COCH{=}C(CH_3)_2$		
	7	Methyl *n*-amyl ketone (2-heptanone)	MAK	$CH_3COCH_2CH_2CH_2CH_2CH_3$	–	104,216
	7	Ethyl *n*-butyl ketone (3-heptanone)	E*n*BK	$CH_3CH_2COCH_2CH_2CH_2CH_3$	Probably	108
	7	4-Heptanone		$CH_3CH_2CH_2COCH_2CH_2CH_3$		
	7	2,5-Heptanedione		$CH_3COCH_2CH_2COCH_2CH_3$	+	154
	7	3,5-Heptanedione		$CH_3Ch_2COCH_2COCH_2CH_3$	–	216
	7	2,6-Heptanedione		$CH_3COCH_2CH_2CH_2COCH_3$	–	154
	8	3,6-Octanedione		$CH_3CH_2COCH_2CH_2COCH_2CH_3$	+	155
Alcohols	4	1,4-Butanediol		$CH_2OHCH_2CH_2CH_2OH$	–	216
	6	1-Hexanol		$HOCH_2CH_2CH_2CH_2CH_3$	–	168
	6	2-Hexanol		$CH_3CHOHCH_2CH_2CH_2CH_3$	+	168
	6	3-Hexanol		$CH_3CH_2CHOHCH_2CH_2CH_3$		
	6	2,5-Hexanediol	2,5-HDiol	$CH_3CHOH(CH_2)_2CHOHCH_3$	+	216
	6	1,6-Hexanediol		$HOCH_2CH_2CH_2CH_2CH_2CH_2OH$	–	216
	6	Diacetone alcohol		$CH_3COCH_2C(OH)(CH_3)_2$	–	
Furan	5	2,5-Dimethylfuran			–	116
Lactone	5	γ-Valerolactone		$O\overset{O}{C}CH_2CH_2CHCH_3$ (ring)	–	116

A. Usual Sources of Exposure

1. Industry

Serious human exposures during manufacturing or packaging of these substances have not been reported, probably because these processes are usually totally enclosed. Hexacarbon solvents are commonly mixed with other solvents such as MEK, MiBK, acetone, benzene, and toluene. Such solvent mixtures are rarely labeled *neurotoxic*, and they are used by small factories where protective devices (masks and gloves), worker education, and ventilation are frequently of a low standard. The lack of an organized industrial hygiene program and poor monitoring of the workers' health played a role in recent outbreaks of neurotoxicity. In a small factory with no central medical facility, each affected individual is likely to seek out his own physician as symptoms appear and, unless a single physician sees several affected workers, the problem may reach epidemic proportions before it is detected. This combination of factors has been responsible for industrial outbreaks of hexacarbon neurotoxicity.

Hexacarbon neurotoxicity is a world-wide problem occurring in Italy,[1,22,30,51,52,181,182,191,198] Japan,[70,98-100,139,149,159,212,255-257] Morocco,[18,42] France,[18,79,122] and the U.S.[4,6,7,23,48,94,128,163,190,241] In most instances, the presence of *n*-hexane or M*n*BK has been established by chemical analysis but, on occasion,[42,79,173,188] these hexacarbons have been implicated on circumstantial grounds.

In Italy and Japan, where shoe-making is sometimes a household industry,* workers may spend 8 hr or longer a day confined to small, poorly ventilated rooms with air levels of *n*-hexane as high as 2500 ppm.[257] Inoue and colleagues[99] have suggested *n*-hexane neuropathy develops in 3 to 7 months after daily 8-hr exposure to 500 to 1000 ppm. Individuals who spend long periods of time in an atmosphere with high levels of *n*-hexane gradually accommodate to the odor and the mild euphoric effects.[163] However, a visitor to such a plant is usually immediately aware of the fumes which may be intoxicating.[163] Most reports have emphasized the dangers of inhalation of the vapor (see Section IV.A.1.a). *n*-Hexane and M*n*BK are also readily absorbed through the skin (see Section IV.A.1.b), and a frequent source of worker exposure in the laminating,[256] printing,[23,161] shoe-making,[181,257] or furniture-finishing[94] industries is dermal contact through the handling of solvent-soaked rags. An additional, well-documented source of exposure is eating in the work area.[23] For these reasons, air samples which indicate low levels of *n*-hexane or M*n*BK in the atmosphere may give an erroneous impression that these substances are not responsible for an industrial neurotoxic outbreak.[23]

One of the most carefully investigated incidents of hexacarbon neuropathy occurred in a fabric factory in Ohio. In mid-August of 1973, a 22-year old man who had been employed in the print department for 2.5 years, was discovered to have a severe sensorimotor neuropathy.[5-7,23,47,81] Since he was otherwise healthy, the disease was thought to be associated with exposure to a toxic chemical. The individual indicated that others in the printing shop had had similar symptoms, and an investigation was begun to identify the extent of the outbreak and which of the 275 chemicals in use was the causative agent. Plant workers underwent electrodiagnostic tests, including electromyogram (EMG) and nerve conduction studies. Of the 1157 workers examined, 19 had abnormalities which could be associated with peripheral neuropathy. After clinical evaluation and numerical rating of signs, symptoms, and results of electrodiagnoses, 81 workers were considered within normal limits. Twenty-seven were found to have other neurologic diseases, and 86 were identified as having toxic peripheral neuropathy. Of the latter group, 38 were from the print shop and the remainder from other

* An entire symposium was devoted to the problem of neuropathy in the "shoe-upper" making industry in 1974. A collection[170] of published papers was prepared in association with this meeting.

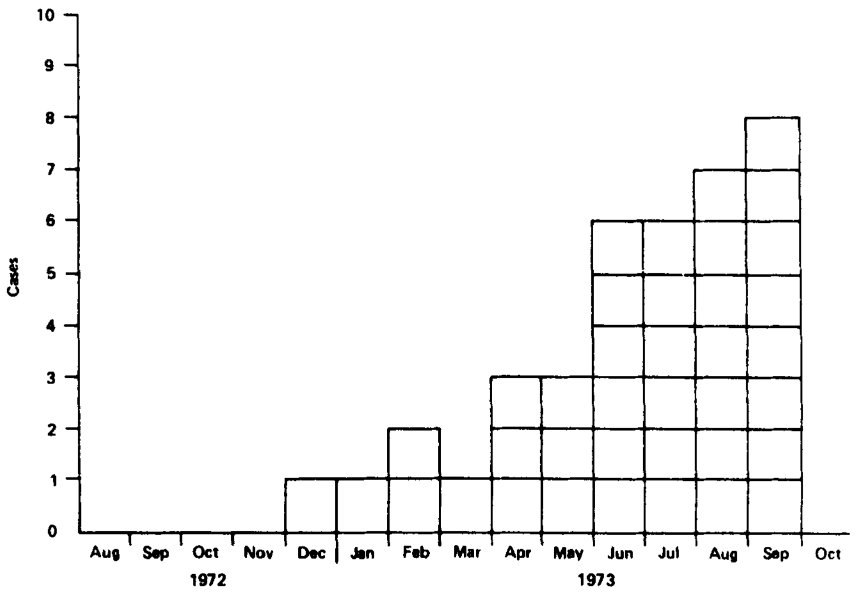


FIGURE 1. Month of onset for the 38 cases of peripheral neuropathy among workers exposed to M*n*BK and MEK in a factory in Ohio. M*n*BK had been introduced into the factory between August 1972 and January 1973. (Reprinted from the Center for Disease Control, *Morbidity Mortality Weekly Report*, 23, 9, 1974.)

departments. Of the neuropathic workers in the printing department, 27 operated the printing machines, 10 were helpers, and one washed ink pans by hand. Symptoms of parasthesiae in arms and legs, commonly followed by weakness of hands and legs, were first noticed between December 1972 and September 1973, most frequently starting in the summer months and improving progressively once the individual was removed from the work environment (Figures 1 and 2).

Information on work practices and production at the plant led to the identification of M*n*BK as the likely causative agent. The substitution of M*n*BK for MiBK as a cosolvent with MEK was the only major process change which had occurred in the past 7 years; the substitution had begun in August 1972 and was complete by January 1973. Workers in the printing department were exposed to solvents by skin contact and to solvent vapors by inhalation. They had not worn respirators or gloves, had eaten in the work area, washed their hands in the solvent, and used rags soaked with solvent to clean equipment and machinery. Airborne levels of solvents at the printing machines were 85 to 763 ppm for MEK, and 2.3 to 156 ppm for M*n*BK. The recent introduction of M*n*BK into this plant and the absence of neuropathy at a similar plant which used MEK but not M*n*BK, led to the conclusion that M*n*BK was the likely neurotoxic agent. Subsequent experimental studies demonstrated M*n*BK produced neuropathy in animals,[59,60,136,229] and that concurrent exposure to MEK accelerated its development.[188]

2. *Abuse*

The most common cause of hexacarbon neuropathy has been deliberate inhalation of the vapors from solvents, lacquers, glue, or glue thinners. "Glue sniffers"[112] or "huffers"[175] are usually underprivileged young males.[44] The technique is to pour the solvent into a rag, balloon, plastic bag, or can, and then inhale. The inhaled solvent may produce a mild euphoria or, more commonly, narcosis. In some cases of chronic

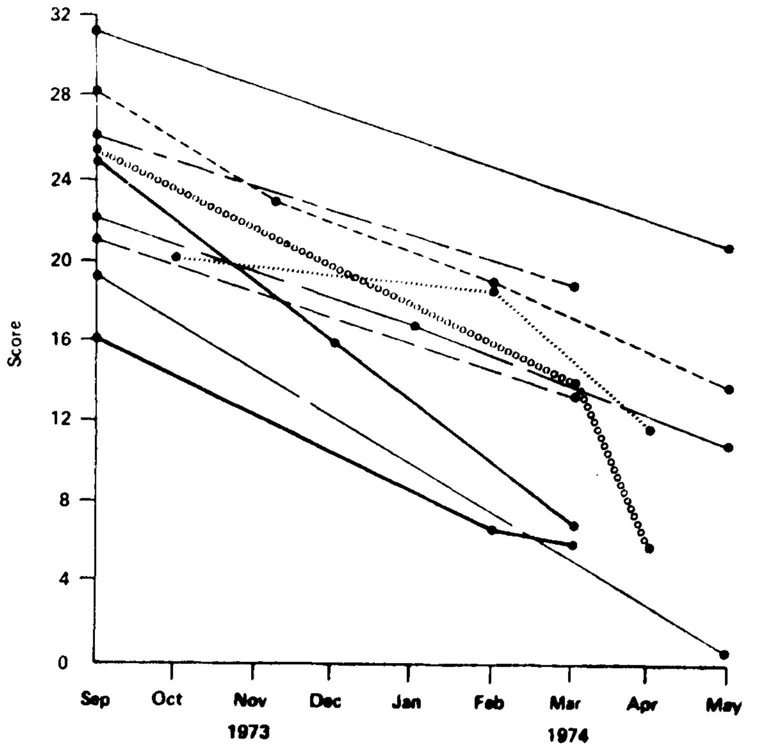


FIGURE 2. Rates of recovery for Ohio factory workers with neuropathies after exposure to MnBK had ceased. Points represent total scores of clinical and EMG findings in individual cases. (Modified from Allen, N., Mendell, J. R., Billmaier, D. J., Fontaine, R. E., and O'Neill, J., *Arch. Neurol.*, 32, 209, 1975. With permission of the American Medical Association.)

abuse, the inhalation periods may last for 10 to 12 hr with a total daily consumption of half a liter. Exposure periods of 5 to 7 years have occurred in some instances.

With one exception,[158] every documented instance of neurotoxicity associated with glue sniffing has occurred in an individual exposed to a substance containing *n*-hexane.[9,84,85,95,112,173,175,204,235,241] The toxic inhalant rarely contains solely *n*-hexane; rather, there is commonly a mixture of *n*-hexane with toluene or other substances such as hexanes, acetone, ethyl acetate, xylene, MEK, nitropropane or 2-heptanone. In addition, commercial grades of *n*-hexane contain varying amounts of other hydrocarbons as impurities. Thus some investigators initially doubted *n*-hexane was responsible. However, nerve biopsies from cases of glue-sniffing neuropathy revealed axonal swelling[9,112,241] and neurofilament accumulation[95] similar to the nerve fiber changes found in animals exposed to pure *n*-hexane.[192] Furthermore, there are several well-documented instances in which individuals had been "sniffing" solvent mixtures without ill effects, but developed peripheral neuropathy within months of increasing the *n*-hexane content of the mixture.[204,241] On the other hand, in two epidemics of glue-sniffing neuropathy characterized by extreme neurological deficits,[9,175] *n*-hexane was a minor component. These two reports, one from Tampa, Fla. and the other from Berlin, suggest the effects of *n*-hexane in humans may be potentiated by the presence of other substances in the solvent mixture (see Section III.D.). The Tampa outbreak is remarkable because only 0.5% *n*-hexane was demonstrated in the lacquer thinner.[173-175]

In general, individuals abusively inhaling glue vapors experience a more rapid onset of clinical signs than those exposed in industrial settings, and weakness is often a prominent feature of glue-sniffing neuropathy. The combination of a predominantly motor neuropathy with subacute onset may produce a clinical picture superficially similar to the Guillian-Barré syndrome.* In severe cases, proximal weakness, respiratory insufficiency, cranial nerve palsy, and markedly slowed nerve conduction velocities (NCV's) strengthen this analogy. The fulminant course of the illness in some individuals may derive from the higher levels of exposure sustained by glue sniffers in contrast to those which could possibly exist in industry. One study of glue sniffing estimates that air levels of *n*-hexane in the plastic bag containing the solvent reach levels of 44,000 ppm![84]

There are several proposals for eliminating glue sniffing as a source of neurotoxicity.[201] One major manufacturer of model airplane cement adds a small amount of synthetic oil of mustard to their preparation. Another claims to have developed a model airplane glue which is nonintoxicating when inhaled.[44] However, since *n*-hexane is a ubiquitous, low-cost, efficient component of adhesives, it is unlikely to disappear from the list of abused drugs.

B. Effects of Acute Exposure (Table 2)

1. n-Hexane Alone

The reports of short-term human exposure to *n*-hexane alone are all controlled studies. It is stated that exposure to an atmosphere containing 500 ppm of *n*-hexane produces no effects in humans after 3 to 10 min.[12,145] Brief exposure to 1400 to 2000 ppm elicits nausea, headache, and eye and throat irritation[12,80], while levels of 5000 ppm for 10 min result in giddiness[162] and mild narcosis.[68] There are no reports of controlled dermal exposure to *n*-hexane.

2. Mixtures Containing n-Hexane

There are several controlled studies of human exposure to hexacarbon mixtures, usually in petroleum naphtha fractions and gasoline. Although CNS effects of a nonspecific nature are reported from all of these studies, it is impossible to be certain if *n*-hexane had any role. Exposure to a petroleum-naphtha fraction produced headache and muscle twitching,[93] while inhalation of a 0.25% concentration of gasoline for 1 hr resulted in mild narcosis. Increasing the gasoline concentration in the air to lethal levels of 1.0% produced "drunken behavior" after 5 min.

Glue sniffers report light-headedness, mild euphoria, and narcosis after prolonged inhalation of mixtures containing *n*-hexane. These individuals may be exposed to exceedingly high levels of ambient *n*-hexane and, despite the underlying psychopathology of the chronic drug abuser, such reports probably constitute the best available data on the acute neurotoxicity of *n*-hexane. The CNS depression and the absence of seizures, delerium, or other signs of toxic encephalopathy in these individuals is striking. Only one report[84] describes hallucinations: the authors conclude these may reflect a preexisting emotional disorder and emphasize that hallucinations are common among certain susceptible glue sniffers regardless of the solvent type or the intensity of exposure. In this respect, the human reaction to acute high-level exposure to *n*-hexane contrasts sharply with the response to certain other industrial neurotoxins which produce a similar pattern of distal axonopathy on prolonged exposure but, on single, high-level exposure, appear to have a CNS excitant effect. For example, exposure to high concentrations of acrylamide monomer causes a severe toxic encephalopathy, while prolonged low-level exposure to this agent results in a distal axonopathy.

* The underlying primary *myelin* pathology of the Guillian-Barré syndrome differs markedly from the primary *axonal* pathology of hexacarbon neuropathy (but see also Griffin et al.[90])

Table 2
EFFECTS ON MAN OF BRIEF EXPOSURE TO HEXACARBON COMPOUNDS AND REFINED PETROLEUM SOLVENTS CONTAINING HEXACARBON COMPOUNDS

Compound[a]	Route	Dose	Duration	Effects	Ref.
Hexane	Respiratory	5000 ppm	10 min	Dizziness, giddiness	162
	Respiratory	500 ppm	3—5 min	Innocuous	145
	Respiratory	5000 ppm	10 min	Dizziness, narcosis	68
	Respiratory	1500 ppm		Nausea, headache, eye, and throat irritation	12
	Respiratory	500 ppm		No irritation	12
M*n*BK	Respiratory	1000 ppm		Irritating to eyes and nose	197
MiBK	Respiratory	100 ppm		Headache, nausea	63
	Respiratory	>100 ppm		Odor detection threshold	202
	Respiratory	>200 ppm		Objectionable	205
2,5-HD		NS		Irritation of mucous membranes	28
Diacetone alcohol	Respiratory	100 ppm		Objectionable	205
Mesityl oxide	Respiratory	25 ppm		Eye irritation	205
	Respiratory	50 ppm		Objectionable	205
*Cyclo*hexanone	Respiratory	50 ppm		Eye irritation	145
	Respiratory	75 ppm		Objectionable	145
Gasoline	Respiratory	0.1%—0.25%	1 hr	Dizziness, throat and eye irritation	58
	Respiratory	5,000—16,000 ppm	5 min		184
Petroleum ether	Respiratory			Severe, clonic convulsions	111
Rubber solvent	Respiratory	430—2000 ppm	15 min	Olfactory fatigue; eye, nose, and throat irritation	37
Naphtha	Respiratory	140—880 ppm	15 min	Olfactory fatigue; eye and throat irritation	36
Mineral spirits	Respiratory	500 mg/m^3		Nausea, vertigo	19
	Respiratory	2500 mg/m^3	2 hr	No effects on psychological testing	78
	Respiratory	4000 mg/m^3	50 min	Prolonged reaction time, impaired short-term memory	78
140° Flash solvent	Respiratory	17—49 ppm	15 min/day, 2 days	Olfactory fatigue and eye irritation	38

[a] Gasoline: mixture of C_4-C_{12} hydrocarbons containing paraffins (1 to 2% *n*-hexane), olefins, naphthenes, and aromatics; petroleum ether: 80% pentane, 20% isohexane, and less than 0.5 vol% of *n*-hexane; rubber solvent: C_5-C_7 compounds consisting of paraffins, mono*cyclo*paraffins, monoolefins, benzene, and alkyl benzene; naphtha (varnish makers' and painters' naphtha): C_5-C_{11} compounds containing paraffins (0.3% *n*-hexane), mono*cyclo*paraffins, di*cyclo*paraffins, benzene, alkylbenzenes, indans, and tetralins; mineral spirits: saturated hydrocarbons, olefins, and aromatics. 140° flash solvent: C_8-C_{12} compounds containing paraffins (1.2% *n*-hexane), mono*cyclo*paraffins, di*cyclo*paraffins, alkyl benzene, benzene, indans, and tetralins.

Modified from references 36 and 143.

3. MnBK

M*n*BK is irritating to the eyes and nose at 1000 ppm.[197] No ill effects were found to occur on exposure to an atmosphere of 100 ppm for 4 or 10 hr, or 50 ppm for 7½ hr.[54]

4. MiBK

Controlled studies of individuals exposed to MiBK for 3 to 5 min describe levels of 100 ppm as tolerable, while 200 ppm evoked eye, nose, and throat irritation.[205] One report of workers repeatedly subjected to short-term, high-level exposure to an acetone-MiBK mixture described vague CNS (insomnia and headache) GI (nausea and colitis), and dermatological effects.[125] It is not clear which symptoms were due to acetone and which to MiBK.

5. Other Hexacarbon Compounds

Browning[28] reported irritation of mucous membranes as an effect of exposure to 2,5-HD. Eye irritation is reported with *cyclo*hexanone[145] and mesityl oxide.[205]

C. Effects of Subacute and Subchronic Exposure to Substances Containing Either *n*-Hexane or M*n*BK

This section analyzes the clinical findings and laboratory data from reported cases of hexacarbon neuropathy, the most carefully documented of which appear in Table 3. The cases represent individuals exposed in industry and through solvent abuse. Both males and females are affected, and the age of onset ranges from adolescence to late middle-age. Individuals in different countries are exposed to a wide variety of solvent mixtures and, in many instances, the contents and methods of chemical analysis are poorly described. Several reports, especially from Japan, appear to be repeated descriptions of the same cases. The quality of the documentation of neurological, clinical, electrophysical, and laboratory data vary considerably, and long-term follow-up examinations are scarce. With the exception of the thorough clinical study of Altenkirch and colleagues,[9] the Japanese and European reports focus more on working conditions, histopathology, and epidemiology, than on neurology. American reports, while less oriented towards occupational medicine, are frequently anecdotal and lack a critical analysis of the data. Exceptions to this are the lucid description of glue-sniffing neuropathy by Korobkin and colleagues[112] and the thorough study of the Ohio M*n*BK cases.[7,23] The Ohio study stands as a paradigm of the correct approach to an outbreak of industrial neurological disease.

1. Clinical Picture

An analysis of the clinical findings indicates that hexacarbon neurotoxicity should be considered in a solvent-exposed worker or glue sniffer with a history of weight loss, gradual onset distal paresthesiae and weakness, and without ataxia or spasticity. Nerve biopsy evidence of paranodal, giant axonal swelling strongly supports a diagnosis of hexacarbon neuropathy.

a. Signs and Symptoms of Nervous System Disease

The most common initial complaint, both in industrial cases and among glue sniffers, is an insidious onset of numbness of the toes and fingers. This type of distal sensory neuropathy is generally the only clinical illness in the least severe industrial cases. The pattern of sensory abnormality is characteristically symmetrical and involves only the hands and feet, rarely extending as high as the knee. A moderate loss of touch, pin, vibration, and thermal sensation is usually prominent and may be ac-

Table 3
REPORTS OF HUMAN NEUROLOGICAL DISEASE FOLLOWING REPEATED EXPOSURE TO NEUROTOXIC HEXACARBONS

Year of report	Substance	Number	Type	Exposure duration	Level	Clinical illness	Electrophysiology	CSF	Clinical lab.	Biopsy	Ref.
1964	*n*-Hexane, 64%; methyl pentane, 16%; methyl *cyclo*pentane, 20%	6	Laminating industry	3—10 months	2500 ppm	Initial complaints of fatigue, weight loss, and poor appetite; distal sensory symptoms in all four extremities followed by weakness of LEs; distal muscle atrophy severe in some cases; blurred vision in one case; recovery incomplete after 1 year in severe cases; deficit progressed for 1 month after hospitalization	EMG—decreased amplitude of resting potentials	Normal in one case		Muscle biopsy: atrophy	159, 253, 255
1967	*n*-Hexane, 95%	11	Pharmaceutical plant	5—6 months	500—1000 ppm						256
1968 — 1970	*n*-Hexane, 70%; toluene	93	Sandal making	ca. 6 months	500—2500 ppm	Gradual onset of distal, symmetrical sensory symptoms in 88%, sensory signs in 100%, distal muscle weakness in 43%, and atrophy in 8% (only in most severe cases) no respiratory paralysis, no incontinence; reflexes hypoactive or absent in 39%; deficit progressed for 1—4 months while in hospital; recovery complete in 91 cases by 18 months; two cases with residual atrophy; blurred vision in [illegible]tic nerve involved	MCV—median and ulnar below 45 msec in 50%; peroneal nerve conduction below 40 msec in 50%; no response in five severe cases	Elevated protein in one of seven cases	LDH elevated in 17 cases	Muscle biopsy in three cases: atrophy; sural nerve biopsy in six cases: loss of myelin and axons; axonal swelling and ballooning in one case	98—100, 212, 213, 258

Table 3 (continued)
REPORTS OF HUMAN NEUROLOGICAL DISEASE FOLLOWING REPEATED EXPOSURE TO NEUROTOXIC HEXACARBONS

Year of report	Substance	Number	Type	Exposure duration	Level	Clinical illness	Electrophysiology	CSF	Clinical lab.	Biopsy	Ref.
1971	*n*-Hexane, 17%; acetone, 68%; methyl pentane, 11%; methyl *cyclo*pentane, 3%	3	Furniture industry	2 to 4 months	650—1300 ppm	Initial complaints of abdominal pain, numbness, and weakness in distal extremities; foot-drop in two cases; moderate impairment of distal sensation and strength; complete recovery in all	EMG — fibrillation potentials in hands and feet; MCV mildly slowed in ulnar nerve, profoundly slowed in peroneal nerve to 28 msec	Normal in one case	Negative	Muscle biopsy in two cases: angulated fibers and target cells; nerves in muscles displayed accumulation of neurofilaments; sural nerve biopsy normal in one case	94 (216)
1972	*n*-Hexane, 80%	1	Glue sniffing	15 months		Gradual onset of fatigue, weight loss, and weakness of lower extremities; moderate distal symmetrical weakness; absent DTR in lower extremities and normal sensation; weakness progressed and moderate sensory loss appeared; almost complete recovery in 6 months	EMG — fibrillations in distal muscles; MCV — moderately diminished in UEs; very slow in LEs (peroneal, 19.9 msec)	Normal	Normal	Muscle biopsy: target fibers; nerve biopsy: normal	84
1974	*n*-Hexane in all but six cases	30	Shoe industry	Weeks to years		Gradual onset of anorexia, nausea, weight loss followed by weakness and paresthesiae in legs; little loss of sensation; recovery complete in some cases; in many cases residual foot-drop	Normal	Normal	52		
1974	*n*-Hexane, 25%; chloroprene, 30%; toluene,	4	Glue sniffing	9 months, 8 months, 3 years, 6		Insidious onset of weakness and sensory loss in distal extremities, very severe in	EMG — fibrillation in distal muscles; MCV prolonged (27 msec)	Normal in two cases	Normal	Muscle biopsy: shrunken fibers and	85,130

	24%; MEK, 20% in 2 cases; other two cases inhaled mixture of toluene and hexane			months	the two individuals with intense exposure; one quadriplegic with widespread atrophy and flaccidity; no cranial neuropathy or respiratory weakness; abnormal speech in one case; recovery excellent in mild cases, and good after 9 months in severe cases; no long-term follow-up	in UE and LE of severe cases			group atrophy; nerve biopsy: axonal swelling	
1974	*n*-Hexane, 0.5%; xylene, 44%; 2-heptanone, 16%; acetone, 13%; iso-butyl acetate, 13%	7	Lacquer sniffing	Uncertain; recent change in lacquer may have had role	Insidious or subacute onset of distal weakness and mild sensory loss; progression to extreme, diffuse motor loss in three cases, bulbar involvement in four, optic neuropathy in two; one death from respiratory complications; weakness and atrophy progressed for 2 months in hospital; recovery was fair in the mild cases, poor in the severe	EMG—denervation in all extremities in proportion to deficit; MCV (median) very slowed in severe cases	Elevated protein in one case	Normal	Nerve biopsy: axonal swelling and accumulations of neurofilaments	132, 174, 175
1974	*n*-Hexane, 53%; toluene, 34%; ethyl acetate, 13%	1	Glue sniffing	1 year	Subacute onset of predominantly distal motor neuropathy with severe atrophy and reflex loss in LEs; decreased thermal and vibration sense in LEs; no cranial nerve or respiratory involvement; recovery not well documented	MCV—markedly reduced in median and tibial nerves	Normal	Normal	Muscle biopsy: target fibers; nerve biopsy: normal	235
1974	*n*-Hexane, 45%; toluene, 55%; other compounds not identified	2	Glue sniffing	2 years; recent change in glue may have had role	One case severely impaired with subacute onset of LE weakness and sensory loss, progressed to near quadriplegia in 3 months; DTRs lost sensory loss less striking than motor; no cranial nerve or respiratory impairment; recovery not documented	Severity of EMG and MCV changes proportional to degree of neuropathy, in general; some disparity in distal motor latency and nerve conduction	Protein elevated in one or four cases	↑ Plasma cholinesterase ↓ RBC cholinesterase	None	204

Table 3 (continued)
REPORTS OF HUMAN NEUROLOGICAL DISEASE FOLLOWING REPEATED EXPOSURE TO NEUROTOXIC HEXACARBONS

Year of report	Substance	Number	Type	Exposure duration	Level	Clinical illness	Electrophysiology	CSF	Clinical lab.	Biopsy	Ref.
1974 — 1975	M*n*BK, MEK	86	Printing process	6 months or longer; majority 7 months; one case within 5 weeks	9 — 36 ppm 331 — 516 ppm	Gradual onset of distal symmetrical sensorimotor polyneuropathy; mild cases predominately sensory with excellent recovery; severe cases: motor and sensory findings with slow recovery to normal in most; relative sparing of reflexes and proprioception; progression of deficit for 3—5 months after removal from exposure, in some	EMG — denervated distal muscles. MCV—severe, persistent slowing	Normal	Normal	None	6,7,23,59,136
1975	*n*-Hexane, 27%; toluene, 44%; ethyl acetate, 10%	1	Glue sniffing	5 years; change in glue several months prior to symtoms may have had role		Gradual onset pain and weakness in LEs then distal UE weakness, and distal paresthesia of all extremities; impotence; transient blurred vision; progression for 3 months after removal from exposure; little loss of DTRs (except AJ), severe position loss and mild vibration loss in LEs with atrophy of distal muscles; gradual improvement, good recovery in UEs; residual foot-drop, spastic catch in LEs, and diminished vibration sense	EMG—denervation of distal muscles, MCV—severe, persistent slowing	Normal	Normal	Nerve biopsy: axonal swellings filled with neurofilaments	17,112
1975	*n*-Hexane, toluene, 2-methyl pentane, 3-methyl pentane, *cyclo*hexane, *n*-heptane, *n*-octane	1	Glue sniffing	8 months		Headache, nausea, vomiting, and a feeling of drunkenness; gradual onset of LE weakness after 10 months, weak hands after 12 months; eventually foot-drop and flaccid pa-	None	None	Aplastic anemia apparent 3 months after discharge	None	95

						ralysis of LEs and hands; atrophy in distal LEs, numbness in all distal extremities; partial recovery on 4-month follow-up					
1975	*n*-Hexane, 12.5%; *n*-heptane, 10%; *n*-pentane, 13%; *n*-octane, 7.5%; benzene, 3%; toluene, 3%; unspecified, 59%	4	Sash cleaning	8—12 + hr/day 5—7 months	*n*-Hexane, 60—240 ppm; *n*-heptane, 20—42 ppm; *n*-pentane, 50—210 ppm; *n*-octane, 13—40 ppm	Insomnia, loss of appetite and weight, constipation, cold sensations in lower extremities, muscle weakness; diminished tendon reflexes, absent patellar and Achilles tendon reflexes; gradual recovery after leaving work	EMG—fibrillations in distal muscles of all extremities	None	Normal	None	239
1976	*n*-Hexane, toluene	2	Glue sniffing	10 and 5 years; recent change may have had role		Insidious onset of distal symmetrical sensorimotor polyneuropathy; one case with marked distal weakness, severe position loss in LEs, weak facial muscles, poor recent memory and calculations — recovery incomplete; other case less impaired and recovered completely	EMG—fibrillations, distal muscles; MCV—diminished in both cases	Protein, 92 mg% in one case	Normal	Nerve biopsies: axonal swelling with accumulations of neurofilaments; muscle biopsy: atrophy, loss of nerve terminals in neuromuscular junctions	241
1976	MEK, MiBK, acetone, toluene, methanol, isopropyl alcohol, ethylene glycol	1	Lacquer thinner	2 years		Gradual onset of severe distal symmetrical weakness and minimal distal sensory loss; DTRs absent in LEs; progression of motor and sensory deficits for 2 months after removal; near total recovery after 2 years	EMG—fibrillation of distal muscles; MCV—delayed motor and sensory conductions	Protein, 53 mg%	Normal	Nerve biopsy: axonal swelling with accumulation of neurofilaments	158
1976	*n*-Hexane and many other substances	8	Printing plant	Unknown	105—4060 mg/m³	Gradual onset of mild distal, symmetrical weakness, absent Achilles reflex, and slight degree of sensory loss; all cases recovered completely	MCV—peroneal conductions moderately reduced	None	Reduced serum cholinesterase	None	163
1976	M*n*BK	3	Spray painters	6—11 months		Gradual onset of moderate distal symmetrical loss of strength and sensation; reflex loss in LEs, footdrop, and paresthesiae; all improved—long-term follow-up not available	EMG—fibrillation in distal muscles; MCV-moderately slowed in median nerve	None		None	128

Table 3 (continued)
REPORTS OF HUMAN NEUROLOGICAL DISEASE FOLLOWING REPEATED EXPOSURE TO NEUROTOXIC HEXACARBONS

Year of report	Substance	Number	Type	Exposure duration	Level	Clinical illness	Electrophysiology	CSF	Clinical lab.	Biopsy	Ref.
1976	M*n*BK, toluene, xylene, acetone, *n*-butyl alcohol	1	Furniture finisher	6 months		Subacute onset of distal symmetrical weakness and paresthesiae; progression to severe, predominantly motor neuropathy with mild sensory loss; all DTRs lost; fair recovery after 3 months — no long-term follow-up	EMG—fibrillation in some distal muscles; MCV—peroneal velocity depressed to 22 m/sec	None	Normal	Two nerve biopsies: the initial (ca. 1 month after first symptom) was normal; the second (ca. 4 months after first symptom) displayed giant axon with accumulation of neurofilaments	48
1976	M*n*BK, lead	1	Printer	10 months		Gradual onset of leg weakness and later, weakness of the upper limbs; dysesthesiae below knee; bilateral foot-drop; weakness of wrist muscles and slight weakness of proximal muscles; reflexes absent at knees and ankles, hypoactive in UEs	EMG—positive waves and fibrillations; MCV—decreased in proportion to weakness	None	Normal	Nerve biopsy; giant axonal change with neurofilaments, regeneration, and remyelination	188
1977	*n*-Hexane, 16%; benzine, 26%; ethyl acetate, 18%; toluene, 29%; MEK, 11%	18	Solvent sniffers	Years of abuse; ca. 3 months after MEK added to solvent		Subacute evolution of severe weakness, predominantly distal, but with quadriplegia in seven cases; loss of DTRs and striking muscle atrophy with stocking-glove sensory loss; no respiratory or cranial nerve palsies; autonomic changes prominent in the extremities; recovery in distal muscles was poor after 8 months	MCV—decreased in proportion to weakness; no peroneal conduction detected in severe cases	Protein elevated in one of ten cases	Normal	Four nerve biopsies: swollen, empty axons or clumping of neurofilaments	8,9,232

1977	*n*-Hexane, toluene	4	Shoe factories	3—6 months		Insidious onset of anorexia, weight loss, nausea, followed by distal weakness and paresthesiae in lower extremities; eventually, mild distal atrophy and foot-drop; flaccid paralysis of the hands; loss of all DTRs; sensory loss much less than motor; recovery excellent after one year in 3 cases	MCV—peroneal nerve conduction at low normal range	None	Mild hypochromic anemia	Four nerve biopsies: giant axonal change	181,182
1977	*n*-Hexane	1	Interior decorators	5—6 years		Insidious onset of distal, predominantly sensory neuropathy				Nerve biopsy: axonal degeneration, loss of large fibers	160

NOTE: LE, lower extremity; UE, upper extremity; DTR, deep tendon reflex; LDH, lactic dehydrogenase; EMG, electromyography; MCV, motor conduction velocity; RBC, red blood cell count; AJ, angle jerks.

companied by loss of the Achilles reflex. In mild cases, there is preservation of position sense and no sensory ataxia, periosteal pain, cranial nerve abnormalities, or autonomic dysfunction, and the other tendon reflexes are spared. In the more severely involved industrial cases, weakness and weight loss occurs, occasionally accompanied by anorexia, abdominal pain, and cramps in the lower extremities. Reflex loss is usually less than that observed in other polyneuropathies and, even in the moderate-to-severe cases, may be confined to the Achilles reflex and finger-jerks. Weakness most commonly involves intrinsic muscles of the hands and long extensors or flexors of the digits. A common complaint in these individuals is difficulty with pinching movements, grasping objects, and stepping over curbs. Cases of pure motor neuropathy are unusual in industrial cases. Vibration and position senses are only mildly impaired, and pinprick- and touch-sensation loss is usually confined to the hands and feet. As the neuropathy becomes more severe, weakness and atrophy dominate the clinical picture and extend to involve proximal extremity muscles. The glue-sniffing cases reported by Altenkirch[9] and colleagues displayed a subacute, distal-to-proximal progression of weakness early in the course of the disease. Rarely in a few glue sniffers with extremely severe involvement are "bulbar" or phrenic nerve paralyses present. Blurred vision is an occasional complaint,[112] but objective evidence of visual loss has not been documented. Optic atrophy has been described in two reports. There is one report of hallucination during glue sniffing and one instance of memory loss. Seizures, toxic delerium, cerebellar ataxia, tremor, or cholinergic symptoms are not documented. No predisposing conditions exist for hexacarbon neurotoxicity, although one study records a high incidence of polyneuropathy in older workers.[30] *A priori,* individuals with diabetes mellitus, renal malfunction, or an alcohol problem would be expected to be most susceptible since distal polyneuropathies commonly occur in all three types of patients.[221] A recent report[30] describes slowed motor nerve conduction in "normal" workers in a factory with documented cases of solvent neuropathy. This strengthens the notion that subclinical and readily reversible nerve damage may be an unrecognized industrial problem.[7]

Autonomic disturbances have been reported among the glue-sniffers, but not in the industrial cases. Prominent among these disturbances is hyperhydrosis of the hands and feet, occasionally followed by anhydrosis. Blue discoloration of the hands and feet, reduced extremity temperature, and Mees lines are sometimes present in these patients.[9] Impotence has occasionally occurred among glue-sniffers with a moderate or severe neuropathy, but its relationship to nervous system dysfunction has not been established.[112]

b. Course

Insidious onset and slow progression are the hallmarks of the industrial cases. In most instances, this is a reflection of low-level, intermittent exposure. In some of the glue sniffers, especially those with excessive abuse, a subacute course develops leading, in the severe cases, to quadriplegia within 2 months of the first symptom. The Guillian-Barré syndrome has been a serious diagnostic consideration in these patients.

A universal feature of hexacarbon neurotoxicity is the continuous progression of disability after removal from toxic exposure. Progression usually lasts for 1 to 4 months and often occurs in the hospital. The degree of recovery in most cases correlates directly with the intensity of the neurologic deficit.[139] Individuals with a mild or moderate sensorimotor neuropathy usually recover completely within 10 months of cessation of exposure. Severely affected industrial cases also improve, but sometimes retain mild-to-moderate residual neuropathy on follow-up examination as long as 3 years after exposure. Such individuals have, on occasion, developed hyperactive knee-jerks. This reflex change may reflect the degeneration in the corticospinal tracts that accompanied the peripheral neuropathy.

Glue-sniffers who sustain an extreme degree of distal atrophy may never recover full strength, and persistent hyperpathia and autonomic dysfunction have been common in these individuals.[9] The rates of recovery from the 1973 outbreak of M*n*BK neuropathy in Ohio are illustrated in Figure 2. Two reports document residual spasticity in the lower extremities of these individuals, probably reflecting damage to the corticospinal tracts.[112,194]

2. Electrodiagnostic Studies

In general, the degree of electrophysiological abnormality, both electromyographic and in nerve conduction, parallels the severity of the clinical illness.

a. Electromyography

The electromyographic abnormalities are usually symmetrical and greater in distal than in proximal muscles. In patients with minimal involvement, positive waves and abnormal motor unit potentials are often the only findings. Nerve conduction times and clinical examinations are usually normal at this stage. Allen and colleagues[7] detected 37 cases in the Ohio M*n*BK epidemic without clinical evidence of nervous system disease, but with the abnormal electrodiagnostic findings mentioned above, they considered these cases to represent subclinical M*n*BK neuropathy. It is difficult to challenge their conclusion, since the authors both eliminated other sources of neurological illness in the workers and also performed the electrodiagnostic studies with great care. This study strongly suggests that electromyography of the distal extremities may be an excellent screening device for populations which risk hexacarbon neurotoxicity. The more severe cases generally display fibrillation potentials, positive waves, and reduced amplitude of muscle action potentials. With recovery or stabilization of the condition, the electromyographic changes disappeared. Residual findings included reduction in numbers of motor unit action potentials and increased amplitudes.

b. Nerve Conduction Velocity (NCV)

In cases with minimal involvement, both motor and sensory nerve conductions are usually normal or at the low normal range of velocity. As the clinical illness intensifies, a progressive slowing of conduction occurs and, in the most extreme cases, distal peroneal nerve conduction cannot be elicited. Allen and colleagues[7] note that in five cases, distal latencies were prolonged in the presence of normal peroneal nerve conduction times. Buiatti and co-workers[30] studied Florentine shoemakers and found both abnormal motor nerve conduction in "normal workers" and a direct correlation between the duration of solvent exposure and the slowing of the motor nerve conduction velocity (MCV). Shirabe and colleagues[204] and Korobkin and colleagues[112] (Table 4) describe in their cases, severe slowing of motor nerve conduction, seemingly out of proportion to the degree of weakness. The latter report, which also incorporated a detailed study of a nerve biopsy from their case, concluded that although such profound slowing is usually associated with primarily demyelinating neuropathies, in *n*-hexane neuropathy it probably reflects the paranodal myelin changes caused by the initial axonal swelling (see Section III.C.4). The same group subsequently reported[27] decrements in nerve conduction in rats subchronically exposed to M*n*BK, but without histological evidence of demyelination. Altenkirch[8] also demonstrated a severe degree of impairment of nerve conduction in the Berlin glue-sniffing cases.

There have been few reports of NCV's in individuals recovering from hexacarbon neuropathy. In the Ohio M*n*BK cases with good clinical recovery, the motor NCV tended to return to normal.[5]

Table 4

MOTOR NERVE CONDUCTION VELOCITIES AND SENSORY NERVE ACTION POTENTIALS IN A 29-YEAR-OLD MAN WHO, FOR A PERIOD OF FIVE YEARS HAD SNIFFED VAPORS FROM A GLUE CONTAINING 27% *N*-HEXANE, 44% TOLUENE, AND 10% ETHYL ACETATE

Motor Conduction Velocities

	Conduction velocity (msec)		Distal latency (msec)	
Location	Patient	Normal values	Patient	Normal values
Right ulnar arm[a]	37	63.4 (SD = 5.3)	—	—
Right ulnar forearm	36	56.2 (SD = 4.6)	6.2	2.9 (SD = 0.49)
Right ulnar forearm[b]	38	55.0 (SD = 4.9)	9.4	3.8 (SD = 0.53)
Right median forearm	33.5	57.2 (SD = 4.2)	7.0	3.8 (SD = 0.5)
Right peroneal	27.5	49.7 (SD = 7.1)	15.6	4.9 (SD = 0.9)
Right sciatic	43	53.8 (SD = 3.3)	8.0	4.7 (SD = 0.6)

Sensory Nerve Action Potentials

	Amplitude (μV)		Latency to peak (msec)	
Location	Patient	Normal values	Patient	Normal values
Right median	8	9—45	5.0	2.5—4.0
Right ulnar	Absent	8—24	—	2.2—3.4
Right sural	Absent	15—62	—	—

[a] Surface electrodes; abductor digiti minimi muscle.
[b] Concentric needle electrode; first dorsal interosseus muscle.

From Korobkin, R., Asbury, A. K., Sumner, A. J., and Nielsen, S. L., *Arch. Neurol. (Chicago)*, 32, 158, 1975. With permission. Copyright American Medical Association.

c. Visual evoked potentials (VEP) and Electroretinogram

A study of VEPs and averaged extraocular retinograms recently has been reported.[200] The amplitude and the latency of the VEP were both abnormal, and were interpreted as consistent with axonal degeneration in the CNS visual system. This finding may correlate with the distal axonal degeneration observed in the optic tracts of animals experimentally intoxicated with 2,5-HD.[193] Abnormalities in the electroretinogram are unexplained, but could reflect macular dysfunction.[176]

d. H-Reflex

Bravaccio and associates[26] reported changes in the H-reflex response and, in particular, the excitability cycle of spinal motoneurons, in cases of *n*-hexane polyneuropathy. About one half of the cases showed a shorter duration of early inhibition phases, an increased late facilitation, and a shorter late inhibition phase.

3. Laboratory Findings

In the majority of cases of hexacarbon neuropathy, the cerebrospinal fluid contains a normal amount of protein, and cells are absent. These findings are compatible with distal axonal neuropathy.[195] The rare case with elevated cerebrospinal fluid protein may reflect nerve fiber degeneration which has ascended to the spinal roots.

Clinical laboratory tests reveal no consistent pattern of hematologic, hepatic, or renal dysfunction, or abnormality of glucose metabolism. The finding, in one study,[7] of low erythrocyte acetylcholinesterase levels accompanied by elevated plasma butyrocholinesterase was not correlated with the presence of neuropathy.[5]. One case report[95] describes aplastic anemia in concert with glue-sniffing neuropathy. This may be coincidental since the hematologic abnormalities did not appear until 3 months after discharge from the hospital; alternatively, it could be related to the toluene present in the glue vapor.

4. Neuropathology Findings

Nerve biopsies from mild cases may be normal, even when examined by electron microscopy. Occasionally, muscle biopsy from such cases may reveal abnormalities of neuromuscular junctions or of intramuscular nerve twigs.[94] The most informative nerve biopsy results are usually obtained from moderate or severely involved individuals; in these cases, teased myelinated nerve fiber preparations have clearly illustrated paranodal giant axonal swellings accompanied by myelin retraction.[9,181,188,241] Electron microscopy reveals the axonal swellings consist of accumulations of 10-nm neurofilaments.[112,182] Giant axonal swellings were also found in the fasciculus gracilis of one case that came to autopsy.[132] Demyelination, remyelination, and normal regeneration are also reported. These pathological features are identical to the abnormalities observed in experimental hexacarbon neuropathy.[225]

D. Potentiation of the Effects of n-Hexane or MnBK by Other Compounds

There are three recent reports of human nervous system damage after exposure to MEK, allegedly in the absence of either *n*-hexane or M*n*BK.[61,67,252] None of these reports of MEK neurotoxicity contains a detailed chemical analysis of the solvent or the atmosphere. Such claims of MEK neurotoxicity should be regarded with skepticism until further confirmation, especially since there is no evidence of nervous system damage in animals after prolonged exposure to high levels of MEK.[66,222]

Altenkirch and co-workers[8-10] in Berlin have reported experimental and epidemiological evidence to suggest that MEK may potentiate *n*-hexane neurotoxicity. In their group of glue sniffers, 18 cases of neuropathy occurred several months after the addition of 11% MEK to the glue and a lowering of the amount of *n*-hexane from 31% to

16%. After removing the MEK from the glue, no new cases occurred, except for one individual who continued to sniff the MEK-containing solvent. Two of the present authors (H.H.S. and P.S.S.) examined some of the Berlin cases, reviewed the biopsy and analytical data, and fully concur with the findings of this study. The conclusion that MEK had a role in the Berlin epidemic is strongly supported by the evidence. However, it is curious that the solvent abusers failed to develop neuropathy with the mixture of 31% *n*-hexane, since they consumed large amounts over the 7-year period prior to the addition of MEK, and the concentration of *n*-hexane was the same as was reported to be toxic in the case of Korobkin and co-workers.[112] Two studies[10,188] demonstrate that animals exposed to a combination of *n*-hexane or M*n*BK with MEK will develop neuropathy more rapidly than if exposed to either *n*-hexane or M*n*BK alone. Potentiation of small amounts of *n*-hexane (0.5%) by other solvent components may also have been a factor in the Tampa glue-sniffing neurotoxic outbreak.[174] However, a definitive explanation of the Tampa outbreak will not be forthcoming until all components of the implicated solvent are fully tested in animal experiments.[133,240] There are also reports of peripheral nerve damage after exposure to gasoline[107,239] and to partially analyzed hydrocarbon mixtures. The role of neurotoxic hexacarbons in these cases also remains *sub judice*.

IV. PHARMACOKINETICS AND BIOTRANSFORMATION

This section discusses the absorption, tissue retention, metabolism, and excretion of *n*-hexane and methyl *n*-butyl ketone in man, animals, tissue cultures, and in vitro. *n*-Hexane and M*n*BK are considered together because they are metabolically interrelated and probably produce nerve damage by common mechanisms.[55] Studies in man and experimental animals are juxtaposed, since widely divergent metabolic pathways have not been reported.[53,54] This section also considers the metabolic modifications induced by concurrent exposure to MEK and either hexacarbon solvent, and the effects of pretreatment with other agents that affect liver-oxidation.

A. Man and Experimental Animals

1. Absorption

Exposure to *n*-hexane or M*n*BK by respiratory, subcutaneous, or intraperitoneal routes induces neurological damage in experimental animals. Subclinical neuropathy was reported in chickens exposed percutaneously to a hexane mixture (35.2% *n*-hexane), and significant amounts of M*n*BK will enter dogs and man by this route.[54] M*n*BK applied to uncovered skin of a guinea pig for 5 days a week for 35 weeks failed to produce either signs or histological evidence of neuropathy.[114]

a. Respiratory

Respiratory uptake in man has been studied for a number of organic solvents including *n*-hexane[147] and M*n*BK.[54] Nomiyama and Nomiyama[147] examined this question in a group of Japanese men and women aged 18 to 25 exposed to *n*-hexane (87 to 122 ppm) and other solvents for periods up to 4 hr. Measurement of *n*-hexane in expired air showed a mean respiratory uptake of 27.8 ± 5.3%, the mean respiratory retention being as low as 5.6 ± 6.2%, the lowest values obtained for seven tested solvents. These results for *n*-hexane stand in contrast to those reported for M*n*BK.[54] Experiments with three human volunteers (22 to 53 years old) and four dogs exposed to 10 to 100 ppm M*n*BK revealed a respiratory retention of 75 to 92% and 65 to 68%, respectively. These data, in part, may account for the significantly greater neurotoxic effect of inhaled M*n*BK in man and animals, and the reported failures of *n*-hexane to induce neuropathy in animals.[70,124]

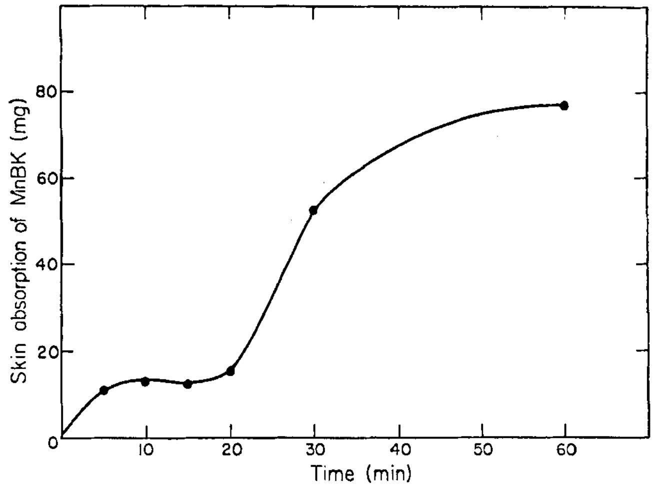


FIGURE 3. Skin absorption of (1-^{14}C)M*n*BK in male beagles. (1-^{14}C)M*n*BK (20 μCi) was diluted with carrier and added to a glass absorption cell (55.6 m^2) secured on the dog's thorax. Hair was clipped 24 hr before testing. (From DiVincenzo, G. D., Hamilton, M. L., Kaplan, C. J., Krasavage, W. J., and O'Donoghue, J. L., *Toxicol. Appl. Pharmacol.*, 44, 593, 1978. With permission.)

b. Percutaneous

We have not encountered controlled studies of skin absorption of *n*-hexane, although cutaneous absorption has been suggested as the major route of entry in man,[147] and was claimed to have caused one outbreak of neuropathy.[148]

Skin absorption of M*n*BK has been examined in man, dogs[54] (Figure 3), and rabbits,[210] and found to be potentially a very significant route of entry. The shaven skin of dogs absorbed 77 mg in one hour, and human skin absorbed 16 to 27 mg during the same period.[54] Concurrent skin exposure to MEK and M*n*BK resulted in the absorption of 75 mg of solvent in 30 min by dog skin and 14 to 19 mg by human skin. The absorption rate in man ranged from 4.2 to 8.0 ug/min^{-1}cm^{-2} for 97% pure M*n*BK and for the 9:1 (vol:vol) MEK:M*n*BK mixture.[54]

c. Oral

Man can absorb (1-^{14}C)M*n*BK from the gastrointestinal tract.[54] Serum 2,5-HD is present in rats exposed by gavage to any of the six interrelated hexacarbon metabolites.[156]

2. Metabolism

The metabolism of *n*-hexane and M*n*BK has been determined in experimental species by gas chromatographic separation of serum and urinary metabolites and their analysis by mass spectrometry.[2,3,45,53,55,168,254] It is assumed man metabolizes these compounds comparably, but few data are available: typical metabolites were not detected in urine of workers exposed to *n*-hexane,[168] and $^{14}CO_2$ was found in respiratory air of volunteers exposed to (1-^{14}C)M*n*BK.[54]

Table 5 summarizes experimental studies of the effects of *n*-hexane and M*n*BK on liver enzymes. Couri and co-workers[46] have demonstrated that MEK also activates hepatic microsomal enzyme activities. Hexabarbitol-induced sleep times were reduced in rats inhaling MEK and M*n*BK, and this property was related to MEK and not to M*n*BK. In vitro studies showed in rats subchronically exposed to MEK/M*n*BK that

Table 5
LIVER MICROSOMAL STUDIES IN ANIMALS EXPOSED TO HEXACARBONS

Compound	Species	Route	Dose	Duration (day)	Effect	Ref.
"Hexane"	Rat				Liver microsomal changes similar to hexobarbital	131
	Rat	Incubation with liver microsomes			Hydroxylation occurred at all carbon atoms yielding mixed hexanols	74
	Mouse	Respiratory	2—3%	4	Cytochrome P450, cytochrome b_5, and cytochrome P450 reductase levels increased in liver microsomes — enhanced microsomal monooxygenase system	113
	Rat				UDP-glucuronyltransferase activated slightly by hexane in a liver microsomal suspension	248
	Rat	Intraperitoneal injection	60 mg/kg/day		After 8 days, liver microsomal protein content increased; UDP-glucuronyltransferase activity induced; increased urinary excretion of D-glucuronic acid and D-glucaric acid	150
	Guinea pig				Treatment of guinea-pig liver microsomal preparation with hexane enhanced glucuronidation of *p*-nitrophenol and *o*-aminophenol; parahydroxylation of aniline increased greatly	151
	Rabbit (pretreated with phenobarbitol)	Incubation with liver microsomes			Partial uncoupling; increased hydroxylation in presence of NADH and NADPH	231
Perpluro-*n*-hexane					Formation of enzyme: substrate complex with P-450, triggering reduction and subsequent oxygen binding to cytochrome b_5	231
*Cyclo*hexane					1:1:1 Monooxygenase system stoichiometry for *cyclo*hexanol: NADPH: O_2	231
M*n*BK/MEK	Rat	Respiratory	225/750 ppm		Enhanced hepatic microsomal activity attributed to MEK	46

Expanded from Chemical Industry Institute of Toxicology, Current Status Report, No. 1, *n*- Hexane, 1977. With permission.

hepatic enzyme activities were increased, as judged by the enhanced production of *p*-aminophenol, formaldehyde, *p*-aminobenzoate, and sulfanilamide. These findings indicate that prolonged exposure to MEK may induce significant changes in the metabolism of a variety of chemicals and drugs. This may help to explain why humans and

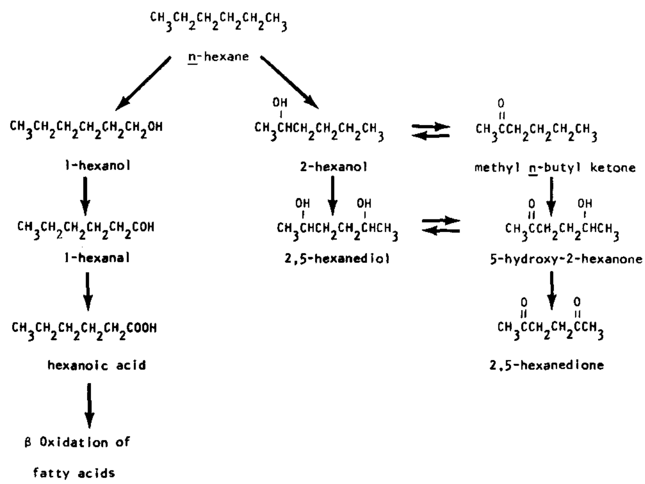


FIGURE 4. *n*-Hexane metabolism and its link with M*n*BK metabolism. (From Perbellini, L., De Grandis, D., Semenzato, F., Rizzuto, N., and Simonati, A., *Toxicol. Appl. Pharmacol.*, 46, 421, 1978. With permission.)

animals exposed to M*n*BK or *n*-hexane, in addition to MEK, develop neuropathy sooner than if exposed to M*n*BK or *n*-hexane alone.

n-Hexane and M*n*BK share the common metabolite, 2-hexanol, which has a neurotoxic potential intermediate between the two parent compounds (Figure 4) (see Section VI.B.5.a). Studies using liver homogenates from animals treated with phenobarbitone show 2-hexanol, 2,5-HD, and M*n*BK appearing as the hydroxylation and oxidation products of *n*-hexane.[45] 2-Hexanol is metabolized to a methyl ketone[106] (presumably M*n*BK) when administered to rabbits. Although 2-hexanol is the principal metabolite of *n*-hexane,[2,74] hydroxylation to 1-hexanol and 3-hexanol also occurs to some degree.[2,74,166,231] 1-Hexanol is successively oxidized to hexanoic acid and follows the pathway for β-oxidation of fatty acids.[97,169] The metabolic fate of 3-hexanol has not been reported.

The metabolism of M*n*BK has been determined only for guinea pigs and rats. DiVincenzo and colleagues[55] found the metabolites of M*n*BK administered to guinea pigs to be 2-hexanol, 5-hydroxy-2-hexanone (5-OH-2-H), 2,5-hexanediol (2,5-HDiol), and 2,5-HD. While the interpretation of metabolic data from serum is complicated, because the concentration of metabolites depends on such factors as clearance, distribution in body water, and further metabolic conversions, the following conclusions were drawn from this study: M*n*BK is hydroxylated to 5-OH-2-H which is then either oxidized to 2,5-HD or reduced to 2,5-HDiol and, to a small extent, may be converted to 2,5-dimethyl-2-3-dihydrofuran. DiVincenzo and co-workers also examined the metabolic fate of MiBK and MEK and established a principle of major importance to an understanding of the neurotoxic potential of alkanes: aliphatic ketones are metabolized both by reduction of the carbonyl group to form a secondary alcohol, and by oxidation of the subterminal (ω-1) carbon atom to form an hydroxylated ketone. In the case of M*n*BK, the hydroxylated ketone is oxidized to 2,5-hexanedione. Couri and co-workers[45] found M*n*BK oxidation occurring in the liver microsomal fraction and reduction in the cytosol fraction (see also Liebman[123]).

DiVincenzo and colleagues[53] further studied the metabolism of M*n*BK by exposing rats by gavage to 20 or 200 mg/kg (1-^{14}C)M*n*BK, with and without pretreatment with 200 mg/kg of the unlabeled compound. Radioactive compounds identified in the

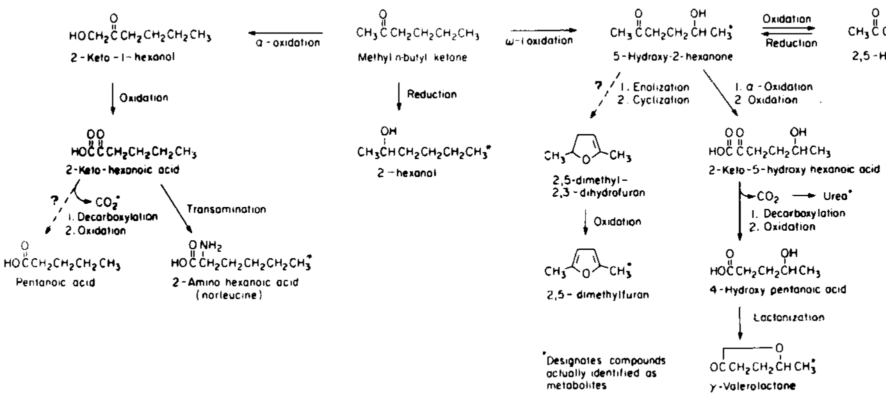


FIGURE 5. Pathway for the metabolism of (1-^{14}C)M*n*BK in the rat. Asterisk (*) designates compounds actually identified in serum. (From DiVincenzo, G. D., Hamilton, M. L., Kaplan, C. J., and Dedinas, J., *Toxicol. Appl. Pharmacol.*, 41, 547, 1977. With permission.)

serum were M*n*BK, 5-OH-2H and 2,5-HD; in the breath, CO_2 and unchanged M*n*BK, and, in urine, 2-hexanol, 5-OH-2-H, 2,5-HD, 2,5-dimethylfuran, γ-valerolactone, urea, and norleucine. Thus in the rat, M*n*BK is handled by a variety of pathways including reduction, alpha-oxidation (detoxification), and ω-1 oxidation (neurotoxic activation). 5-Hydroxy-2-hexanone is a key intermediate in metabolic activation, the neurotoxic compound 2,5-HD and the nonneurotoxic compounds 2,5-dimethylfuran and delta-valerolactone being formed from this intermediate. Although 5-OH-2-H and 2,5-HD are interconvertible, the reaction (in the guinea pig) favors the formation of the neurotoxic γ-diketone 2,5-HD[55] (Figure 5).

Prior to renal excretion, some ketonic solvents may undergo conjugation reactions with glucuronic acid, sulfate, or glutathione. Glucuronic acid conjugation may proceed via either ketonic reduction to the corresponding secondary alcohol or ketone oxidation to a carboxylic acid derivative. Oral administration of M*n*BK to rabbits increases the amount of glucuronic acid in the urine.[146] The general outline of ketone conjugation reactions is summarized in Figure 6. Rats, guinea pigs, and rabbits exposed to M*n*BK vapor excrete 2-hexanol in urine principally as *O*-glucuronides.[2,53] *Cyclo*hexanone administered to rabbits is conjugated and excreted as glucuronide, sulfate ester, mercapturic acid, and other sulfur-containing metabolites.[65,103,242]

3. Blood and Serum Clearance

Studies on the clearance of *n*-hexane, M*n*BK, 5-OH-2-H, and 2,5-HD from blood, serum, and tissues have been conducted in man (serum only), and in guinea pigs and rats.[2,31-33,45,53-55] Humans exposed by inhalation to 100 ppm M*n*BK show serum concentrations of M*n*BK; the metabolite 2,5-HD appears following exposure.[54]

Couri and colleagues[45] demonstrated in guinea pigs exposed to *n*-hexane (660 mg/kg single dose) that a peak blood concentration of 200 μg/mℓ was reached in 30 min. *n*-Hexane was lost from blood in two phases: an initial (*alpha*) rapid elimination with a half-life (T ½) of about 36 min and a slower (*beta*) phase of elimination having a 4-hr half-life (Figure 7). These authors also studied the elimination of M*n*BK from rat blood after a single dose of approximately 480 mg/kg M*n*BK. A peak blood level of 650 μg/mℓ was reached at 30 min, elimination following an initial *alpha* phase with a T ½ of 10 min, followed by a *beta* phase with a T ½ of 7 hr (Figure 8). A comparable excretion time (6 hr) for M*n*BK occurs in serum of rats fed 200 μg/kg of M*n*BK, although the peak serum concentration in this study was only 38 μg/mℓ. The M*n*BK metabolites of 5-OH-2-H and 2,5-HD increase with time, the former peaking with

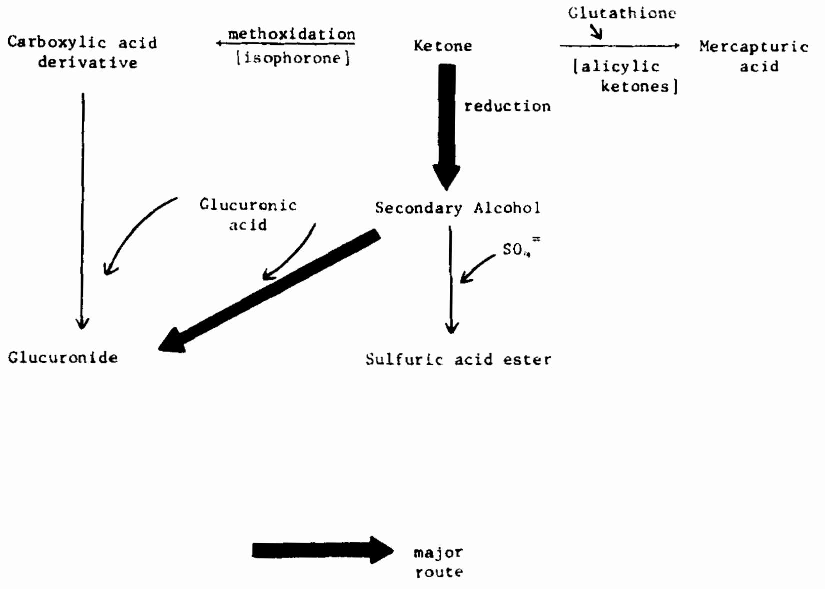


FIGURE 6. Overview of ketone conjugation reactions.

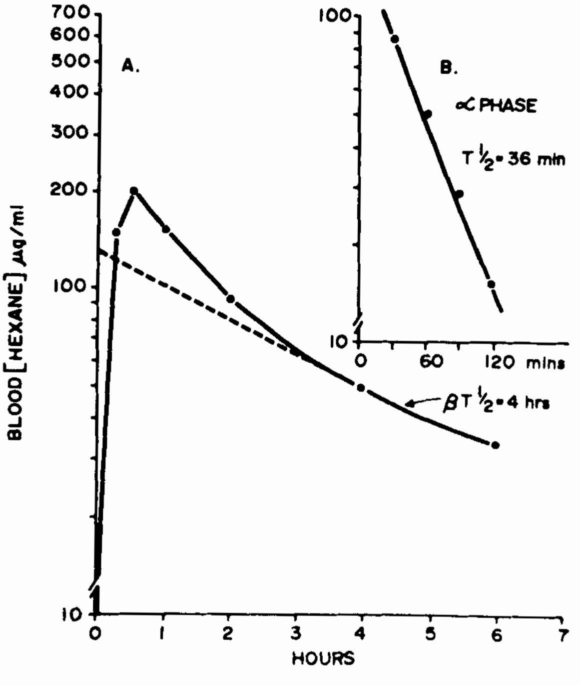


FIGURE 7. Time course for *n*-hexane elimination from guinea pig blood. (From Couri, D., Abdel-Rahman, M. S., and Hetland, L. B., *Am. Ind. Hyg. Assoc. J.*, 39, 295, 1978. With permission.)

M*n*BK at 2 hr and disappearing by 6 hr, the latter peaking at this time, and disappearing at 16 hr[53] (Figure 9). Because 2,5-HD seems to be a primary neurotoxin, its pro-

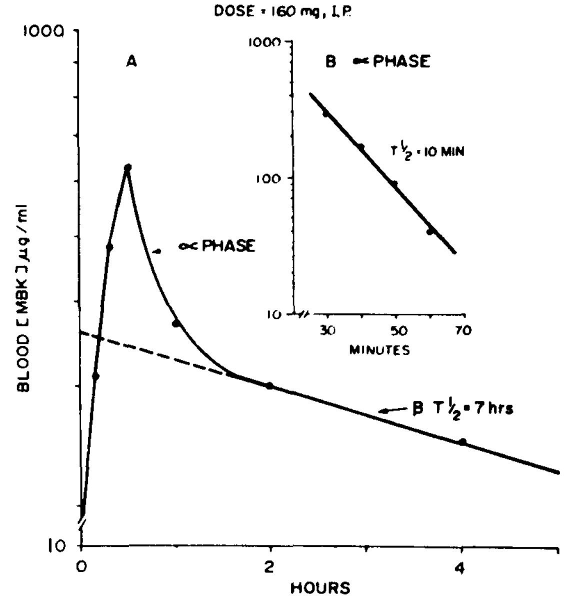


FIGURE 8. Time course for M*n*BK elimination from rat blood. (From Abdel-Rahman, M., Hetland, L., and Couri, D., *Am. Ind. Hyg. Assoc. J.*, 37, 95, 1976. With the permission of the American Industrial Hygiene Association Journal.)

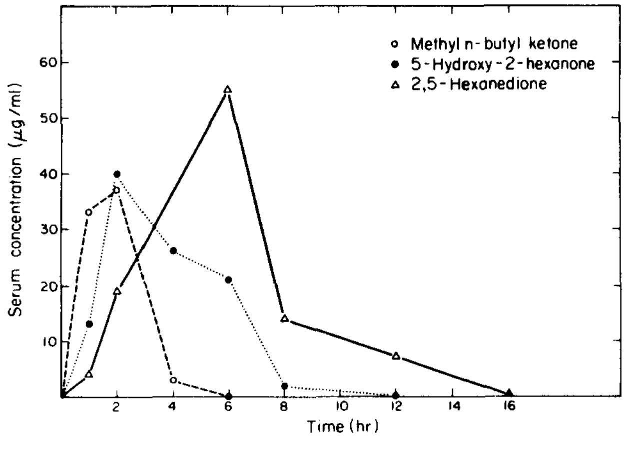


FIGURE 9. Concentration of M*n*BK and its metabolites 5-OH-2-H and 2,5-HD in rat serum after oral administration of 200 mg/kg of M*n*BK (From DiVincenzo, G. D., Hamilton, M. L., Kaplan, C. J., and Dedinas, J., *Toxicol. Appl. Pharmacol.*, 41, 547, 1977. With permission.)

longed residence time in rat and guinea-pig serum may be an important contributory factor in the development of neurological damage from exposure to M*n*BK and *n*-hexane.[33,55] Animals treated with phenobarbitone to activate the microsomal oxidation system before exposure to *n*-hexane or M*n*BK show quantitative changes in blood/serum metabolites[2,53] and (rats) a *reduced* susceptibility to neuropathy.[2] This has not

Table 6
EFFECTS OF PHENOBARBITAL PRETREATMENT[a] ON APPEARANCE IN SERUM OF MNBK AND METABOLITES FOLLOWING SINGLE INJECTION OF MNBK[b]

		Phenobarbital + M*n*BK				Saline + M*n*BK			
Species	Metabolites	50	100	180		50	100	180	
Guinea pig	M*n*BK	1.0 mg	0.4 mg	0.2 mg		1.4 mg	0.75 mg	0.65 mg	
	2-Hexanol	0.12 mg	1.4 mg	0.1 mg		0.1 mg	0.19 mg	0.07 mg	
	2,5-HD	1.3 mg	1.1 mg	1.0 mg		< 20.0 ng	0.14 mg	0.25 mg	
				160	200			160	200
Rat	M*n*BK	0.44 mg	0.3 mg	0.05 mg	0.009 mg	1.8 mg	1.0 mg	0.6 mg	0.23 mg
	2-Hexanol	0.01 mg	0.01 mg	0.01 mg	0.09 mg	<20.0 ng	0.03 mg	0.03 mg	0.05 mg
	2,5-HD	0.06 mg	0.06 mg	0.90 mg	0.47 mg	<20.0 ng	<20.0 ng	0.07 mg	0.06 mg

[a] 50 mg/kg i.p. for guinea pigs and 100 mg/kg i.p. for rats, daily for 3 days.
[b] i.p. injection of 160 mg for guinea pigs and 64 mg for rats.

From Abdel-Rahman, M., Hetland, L., and Couri, D., *Am. Ind. Hyg. Assoc. J.*, 37, 95, 1976. With permission.

been fully explained but may be related to the increased urinary clearance of 2,5-HD in these animals.[2] Table 6 summarizes the effects of phenobarbital on the blood content of M*n*BK and metabolites in guinea pigs and rats exposed to M*n*BK.

The metabolic basis of the reported[188] MEK enhancement of M*n*BK neurotoxicity has been examined in part. From measurements of blood concentrations of M*n*BK in rats exposed to M*n*BK alone or to MEK/M*n*BK combinations, it is evident that either of these solvents may significantly alter the pharmacokinetics of the second agent.[2] Continuous exposure of rats to 440 ppm M*n*BK alone for 1 week or 2 months resulted in undetectable M*n*BK blood levels; however, when rats were continuously exposed to 225 ppm M*n*BK in combination with 750 ppm MEK for one week, M*n*BK concentration in blood increased to 9.5 mg%.[2] This also raised the concentration of 2,5-HD. Potentiation of *n*-hexane neurotoxicity by concurrent exposure to MEK has been established,[10,232] but the metabolic basis has yet to be demonstrated.

Suzuki and co-workers[235] reported *n*-hexane metabolism to be unaffected by concurrent exposure to toluene.

4. *Tissue Pharmacokinetics*

Bohlen and co-workers[24] exposed rats to 170 g/m^3 hexane (*n*-hexane?) vapor for 2 to 10 hr and determined the distribution in various tissues. The uptake of hexane into blood, brain, kidney, and spleen followed an exponential function as predicted by the mathematical methods of Kety[109] and Mapleson.[129] In liver, hexane concentration increased linearly and did not reach saturation. Comparison of the *n*-hexane partition coefficient with the lipid content of brain, adrenal, kidney, and spleen indicated a direct relationship between hexane saturation and lipid content of the organ. However, blood contained hexane in excess of amounts predicted on the basis of the partition coefficient of *n*-hexane, or by the lipid content of blood. *n*-Hexane is cleared from male Fischer-344 rat tissues 4 to 8 hr after single or repeated exposures to 1000 ppm.[31] In pregnant F-344 rats, a single exposure of 1000 ppm *n*-hexane for 6 hr gives on day 20 of gestation, a maximum tissue concentration of *n*-hexane and M*n*BK immediately after exposure. Significant levels of *n*-hexane, M*n*BK, and 2,5-HD appear in the fetus and elsewhere[32] (Table 7). Repeated exposure to *n*-hexane (6 hr/day on days 15 to 18 of gestation) resulted in a more rapid appearance of the peak blood level of 2,5-HD and a more rapid clearance of this metabolite.[33]

Although comparable pharmacokinetic data are unavailable for M*n*BK, the tissue distribution of 2,5-HD has been recently studied by Angelo.[13] Two groups of rats re-

Table 7
DISTRIBUTION OF *n*-HEXANE AND METABOLITES IN ORGANS AND FETUS OF PREGNANT RATS[a]

	Maximum concentration (mg/mℓ)		
Site	*n*-Hexane	M*n*BK	2,5-HD
Blood	0.45	0.70	1.73
Liver	0.84	0.16	1.30
Kidney	0.53	1.04	1.37
Brain	0.04	0.69	3.61
Fetus	0.61	0.51	1.67

[a] Administered 1000 ppm *n*-hexane for 6 hr on day 20 of gestation. Levels of *n*-hexane and M*n*BK were maximal immediately after exposure and less than 10% of peak values after 4 hr. 2,5-Hexanedione was maximal at 4 hr, detectable at 8 hr, and absent after 24 hr. Calculated halftimes of 2,5-HD in blood and fetus were ∿ 3 to 4 times greater than those of *n*-hexane and M*n*BK.

Adapted from Bus, J. S., White, E. L., Heck, H. d'A., and Gibson, J. E., *Teratol.*, 17, 42A, 1978, and Bus, J. S., White, E. L., Tye, R. W., and Barlow, C. S., *Toxicol. Appl. Pharmacol.*, 51, in press, 1979.

ceived 2,5-HD containing (^{14}C)2,5-HD at a dose of 0.8 and 8 mg/kg and the tissue distribution of label determined at 11 intervals between 15 min and 3 days (Figure 10). At 24 hr, label concentration was higher ($P < 0.05$) at the 8.0 mg/kg dose in blood, liver, kidney, muscle, fat, lung, and skin, whereas concentrations in sciatic nerve were similar ($P < 0.05$) at the two dose levels. These observations are in accord with those reported by Bus et al.[31] who found that 2,5-HD was selectively retained by sciatic nerve tissue in rats exposed to single or repeated doses of *n*-hexane, *n*-hexane and its metabolite M*n*BK disappearing from the nerve by 4 to 8 hr after exposure (Table 8). These data have been interpreted as indicating that 2,5-HD has a special affinity for peripheral nerve tissue. This would be surprising since areas of the brain and spinal cord are equal to sciatic nerve in their vulnerability to neurotoxic hexacarbons. It will be important to explain the apparent discrepancy between residency data for sciatic nerve and spinal cord,[13] and brain.[31]

5. Subcellular Distribution

DiVincenzo and colleagues[53] reported that the subcellular distribution of radioactivity from (1-^{14}C) M*n*BK was similar for liver, kidney, and brain tissue. Incorporation of radioactivity into lipids and protein reached a peak at 8 hr and remained unchanged or decreased at 24 hr (Table 9). Unfortunately, these data offer few clues as to the site(s) of neurotoxic action.

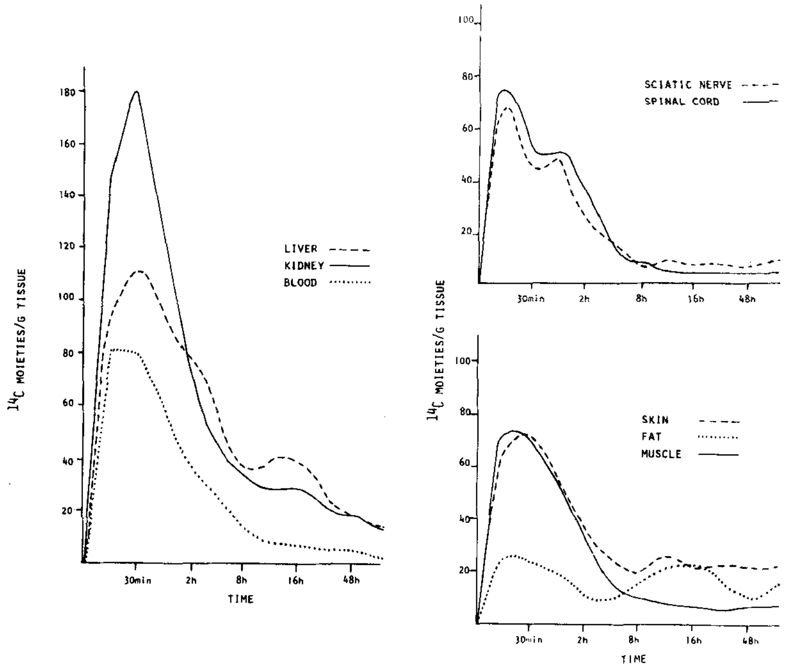


FIGURE 10. Concentration of label in different organs after i.p. administration of (^{14}C)2,5-HD to rats. Muscle was removed from abdomen and thigh, skin was from the distal region of the ear, and fat was taken from the lumbar region. (Courtesy of Mr. M. Angelo.)

Table 8
DISTRIBUTION OF 2,5-HEXANEDIONE IN MALE FISCHER-344 RATS[a]

	2,5-Hexanedione μg/g	
	12 hr	24 hr
Blood	—	<0.05
Brain	—	<0.05
Liver	—	<0.05
Kidney	—	<0.05
Nerve	0.63, 0.56	0.48, 0.46

[a] After exposure to 1000 ppm *n*-hexane 6 hr/day for 1 or 5 days.

6. Excretion

a. Normal and Diabetic Subjects

Many ketone solvents are normal constituents of human urine. This was first reported by Tsao and Pfeiffer,[246] who found traces of MEK in the urine of healthy adults. Zlatkis and co-workers[259] subsequently found a variety of acyclic ketones in normal urine, along with a few additional ketones and *cyclo*hexanone in the urine of individuals with diabetes mellitus. M*n*BK and ethyl *n*-butyl ketone, a seven-carbon compound probably capable of producing neuropathy,[108] is present in nanogram quan-

Table 9
SUBCELLULAR DISTRIBUTION OF RADIOACTIVITY IN THE LIVER, KIDNEY, AND BRAIN OF MALE CHARLES RIVER CD,® COBS® RATS[a]

Tissue	Time (hr)	Tissue fractions[b] (dpm × 10^3/g tissue wet weight) O.H	ASF	CLF	RNA	DNA	Protein	Recovery (%)
Liver	2	118	87	10.3	0	1.8	4.9	88
	4	206	155	13.1	0	4.6	13.7	91
	8	195	112	26	0	4.5	30	89
	24	92	28	23	2	5.5	30	97
Kidney	2	82	55	5.7	1.1	0.4	3.8	80
	4	145	102	5.7	0	4.3	10.9	88
	8	134	74	15.9	0	3.6	23.3	87
	24	64	20.2	15.4	0	2.6	20.8	92
Brain	2	48	30	2.7	1.3	0	1.4	74
	4	94	66	3.4	0	1.8	3.0	79
	8	81	50	9.7	0	1.8	6.2	84
	24	28	12.1	9.9	0	0.8	5.3	100

[a] Treated orally with 200 mg/kg of (1-^{14}C) M*n*BK.
[b] O.H., original homogenate; ASF, acid-soluble fraction; CLF, crude lipid fraction; RNA, ribonucleic acid; DNA, deoxyribonucleic acid. Values are expressed as the average for two rats.

tities in sera of treated and untreated diabetics with and without neuropathy, and in similar concentrations in normal individuals.[260]

b. Exposed Subjects

Controlled studies show that M*n*BK and *n*-hexane are each excreted in the breath as the parent compound or as carbon dioxide, and as the parent compound and other metabolites in urine. Few excretion data are available for humans exposed to hexacarbon solvents.

Shoe-factory workers exposed to "commercial hexane"* excreted metabolites of *n*-hexane in urine: 2,5-HD (36% of total), 2,5-dimethylfuran (32%), γ-valerolactone (30%), and 2-hexanol (2%).[165,166] Higher levels of exposure increased the concentration of all metabolites except γ-valerolactone. Perbellini and co-workers[165] pointed out that, in their hands, the excretion pattern was different in man and animals: in rats (exposed to pure *n*-hexane), 2-hexanol contributed 33 to 55% and 2,5-HD 10 to 20% of the total urinary metabolites.[75]

Controlled studies with (1-^{14}C)M*n*BK examined the fate of label after oral administration to two human volunteers.[54] Expired breath was collected for 5 min/hr for 14 hr and at intervals thereafter (Figure 11), and urine samples at 4-hr intervals for 16 hr and thereafter (Figure 12). Feces were not examined. Volunteers given 0.1 mg/kg (1-^{14}C)M*n*BK excreted $^{14}CO_2$ which peaked within 4 hr and decreased slowly over 3 to 5 days. Most of the radioactivity in urine was found within 48 hr and was completely eliminated by 8 days. By this time, breath elimination of $^{14}CO_2$ averaged 39.5% of the total dose and the excretion of radioactivity in urine 26.3%. Overall recovery of ^{14}C was 65.8%; the remainder was presumed to have been incorporated into intermediary

* n-hexane (20 to 60%); a methylpentane (4 to 18%); 3-methylperthane (3 to 16%); *cyclo*hexane (5 to 42%); methylcyclopentane (1 to 15%).

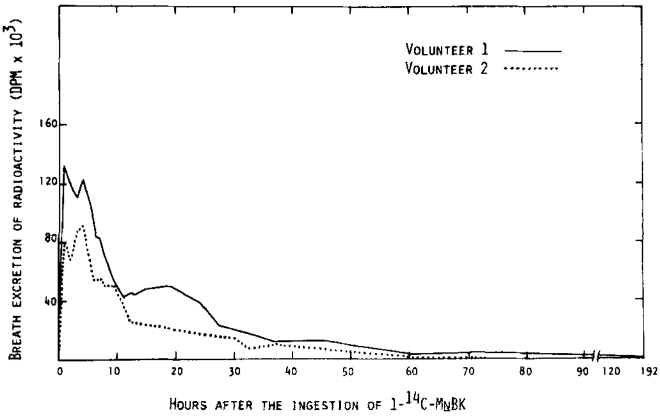


FIGURE 11. Excretion of radioactivity in the breath by two volunteers following the ingestion of M*n*BK. Two microcuries of (1-^{14}C)M*n*BK were mixed with corn oil, placed in a gelatin capsule, and taken with water. The dose was 0.1 mg/kg of body weight. (From DiVincenzo, G. D., Hamilton, M. L., Kaplan, C. J., Krasavage, W. J., and O'Donoghue, J. L., *Toxicol. Appl. Pharmacol.*, 44, 593, 1978. With permission.)

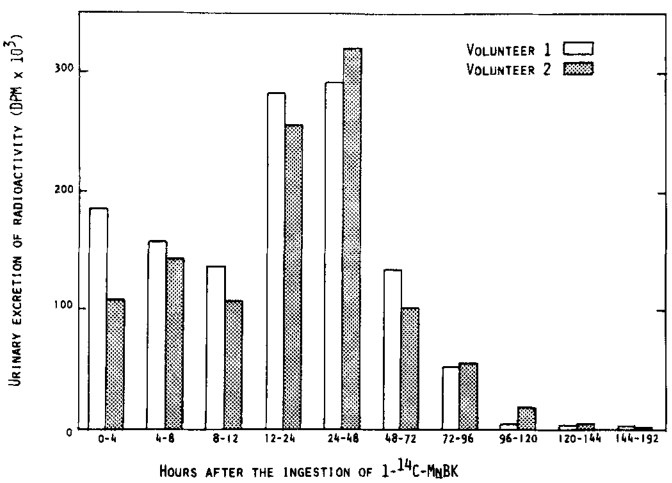


FIGURE 12. Excretion of radioactivity in urine by two volunteers following the ingestion of 2 μCi of (1-^{14}C)M*n*BK. (From DiVincenzo, G. D., Hamilton, M. L., Kaplan, C. J., Krasavage, W. J., and O'Donoghue, J. L., *Toxicol. Appl. Pharmacol.*, 44, 593, 1978. With permission.)

metabolism and/or stored in tissue or fat depots. These data demonstrate that radioactivity derived from (1-^{14}C) M*n*BK is excreted more slowly by man than by the rat.

c. Experimental Animals

When *n*-hexane is administered to rats, the following compounds appear in the urine: *n*-hexane,[45] 2,5-HD, 2,5-dimethylfuran,[75] 2-hexanol,[2,75,168] and 3-hexanol,[166] the amount of 2-hexanol representing 0.5 to 1.2% of the given dose. Phenobarbital pretreatment affects 3-hexanol and 2-hexanol[45] excretion.

Rats inhaling 2-methylpentane or 3-methylpentane, excrete 2-methyl-2-pentanol and

Table 10
EXCRETION OF RADIOACTIVITY (UNITS NOT STATED) IN RATS DOSED WITH MNBK[a]

Collection period (hr)	Urine	Expired air	Feces	Total
0—6	10.3			
6—12	8.9			
12—18	10.0			
18—24	2.6			
0—24	31.8	12.7	2.0	46.5
24—48	4.8	0.05	2.7	7.6
48—72	3.5	0.02	3.2	6.7
0—72	40.1	12.8	7.9	60.8

Effect on Rats Exposed to M*n*BK of Pretreatment With Phenobarbital[b]

	Phenobarbital + M*n*BK		Saline + M*n*BK	
Treatment	Hydrolyzed	Nonhydrolyzed	Hydrolyzed	Nonhydrolyzed
M*n*BK	0.5 mg	<30 ng	1.4 mg	<30 ng
2-Hexanol	1.7 mg	<20 ng	2.7 mg	<20 ng
2,5-HD	2.4 mg	<20 ng	0.3 mg	0.3 mg

[a] Dosed i.p. with 12 μCi ^{3}H-M*n*BK

[b] 100 mg/kg i.p. daily for 3 days — on excretion of hexacarbons following i.p. injection of 64 mg M*n*BK

From Abdel-Rahman, M., Hetland, L., and Couri, D., *Am. Ind. Hyg. Assoc. J.*, 37, 95, 1976. With permission.

3-methyl-2-pentanol, respectively. None of these compounds was found in control rats.[75]

M*n*BK elimination by experimental species is summarized in Table 10 from the work of Abdel-Rahman and colleagues.[2] These investigators recovered in a 3-day period 61% of an administered dose of tritiated M*n*BK; pulmonary excretion accounted for 12.8%, with 40.1% and 7.9% appearing in the urine and feces, respectively. Pretreatment of rats with phenobarbital halved the urinary concentration of M*n*BK and 2-hexanol, and (in rats) increased the amount of 2,5-HD 8-fold. In comparably treated guinea pigs, M*n*BK excretion was increased by 3-fold, 2-hexanol by 2-fold, and 2,5-HD by 22-fold.[2] Angelo[13] found that rats injected intraperitoneally with ^{14}C-labeled 2,5-HD excreted approximately equal amounts of label in air and urine (approximately 40%) and only 1.45% in the feces. Urinary components in M*n*BK-exposed guinea pigs include M*n*BK, 2-hexanol, and 2,5-HD[245] and in male Charles River rats, 2-hexanol (22%), 5-OH-2-H (21%), 2,5-HD (14%) 2,5-dimethylfuran (6%), γ-valerolactone (5%), amino acids, and urea.[53]

d. Tissue Cultures

Spencer and co-workers[228] studied 2,5-HDiol and 2,5-HD in tissue culture models of the nervous system (see Section VI.B.5.h.(i)).The test agents were each introduced into the nutrient fluid maintaining the culture and demonstrated to produce a similar pattern of slowly evolving nerve fiber degeneration comparable to that found in humans and animals with hexacarbon neuropathy. Similar results were obtained by Veronesi and colleagues[250,251] with all six of the metabolically interrelated hexacarbon

Table 11
ORGANOTYPIC TISSUE CULTURE STUDY OF NEUROTOXIC HEXACARBONS[a]

Test hexacarbon	Metabolites: *n*-Hexane	2-Hexanol	2,5-HDiol	M*n*BK	5-OH-2-H	2,5-HD	Neurotoxic in vitro	Neurotoxic in vivo
n-Hexane	× +		+			+	+	+
2-Hexanol		× +			+	+	+	+
2,5-HDiol			×			× +	+	+
M*n*BK		+	+	× +	+[b]	+[b]	+	+
5-OH-2-H			+		× +	+	+	+
2,5-HD					+	+	+	+

Note: Analyses were performed by Dr. G.D. DiVincenzo.

[a] 2.85m*M* of each test compound was administered in the nutrient fluid to cultures twice weekly for 5 weeks. Hexacarbon compounds in nutrient fluid before (×) and after (+) exposure to tissue cultures are indicated.
[b] Traces found in tissue, not in nutrient fluid.

metabolites. By comparing the composition of fresh and exhausted nutrient fluid using gas chromatography-mass spectrometry (GC-MS), it was possible to determine the metabolic capacity of nervous tissue in vitro. The data shown in Table 11 are consistent with the thesis that 2,5-HD is a *common* neurotoxic agent.[16]

V. THE REGULATION OF HUMAN EXPOSURE TO NEUROTOXIC HEXACARBONS

A. Occupational Exposure Potential

1. Uses of Hexacarbon

n-Hexane is widely used as a solvent and is a natural component of gasoline. U.S. production of *n*-hexane and other C_6 hydrocarbons totaled approximately 387 million lb in 1973.[247]

Methyl *n*-butyl ketone was used as a solvent substitute for MiBK until its neurotoxic properties were discovered in 1973.[144] MEK is a widely used ketonic solvent; demand in 1978 was expected to reach 622 million lb in the U.S. *Cyclo*hexanone (see Section VI.B.4), used in the nylon industry, was produced at the rate of 638 million lb. U.S. sales for diacetone alcohol in 1973 were 51 million lb.[120] Occupations with potential exposure to alkanes and ketones are listed in Table 12. Uses of ketones are listed in Table 13.

2. Exposure Limits

Occupational exposure limits for some hexacarbon compounds and MEK are listed by country in Table 14.

3. n-Hexane and M*n*BK Relative Neurotoxicity

n-Hexane administered by inhalation is considerably less neurotoxic than equivalent exposure to M*n*BK. This is related to two factors: (1) the respiratory retention of *n*-hexane in man is 5.6 ± 5.7%,[147] whereas that of M*n*BK is 75 to 92%[54] (i.e., 6 to 16 times), and (2) *n*-hexane is probably not a primary neurotoxic agent, the neurotoxic properties of *n*-hexane and M*n*BK being attributable to their common metabolite 2,5-HD, of which M*n*BK by gavage produces in the serum of single-dosed rats a concentration 4.6 times larger than that produced by an equivalent dose of *n*-hexane.[156] The neurotoxicity index (see Section VI.B.5.a) of M*n*BK in the Charles River rat has been estimated to be 12.5 times that of *n*-hexane.[156]

The present recommended U.S. (N.I.O.S.H.) Time Weighted Average Concentration Limit for exposure to M*n*BK is 1 ppm[144] and 100 ppm for *n*-hexane.[142] This level

Table 12
OCCUPATIONS WITH POTENTIAL EXPOSURE TO KETONES, AND TO ALKANES INCLUDING *N*-HEXANE[142,144]

Alkanes	Ketones	
Adhesive workers	Acetic acid makers	Methyl *n*-butyl ketone makers
Automobile fuel handlers	Acetic anhydride makers	Methyl methacrylate workers
Aviation fuel handlers	Acetone workers	Nylon makers
Cabinet finishers	Acetylene cylinder fillers	Oil processors
Degreasing workers	Adhesive makers	Organic chemical synthesizers
Farm-fuel handlers	Adipic acid makers	Painters
Furniture makers	Benzene workers	Paintmakers
Glue-fabrication workers	Bronzers	Paint remover workers
Gluing machine operators	Butanone workers	Paraffin processors
Laboratory workers, chemical	Celluloid makers	Pentanone workers
Lacquerers	Cellulose acetate makers	Perfume makers
Lacquer makers	Cellulose cement makers	Pesticide makers
Laminators	Chloroform makers	Petroleum refinery workers
Leather cementers	Cleaning compound makers	Photographic film makers
Metal degreasers	Colorless synthetic resin makers	Printers
Petrochemical process workers	Cosmetic makers	Raincoat makers
Petroleum distillation workers	Dewaxers	Resin makers
Petroleum extraction workers	Diacetone alcohol makers	Rubber cement workers
Petroleum refinery workers	Dope workers	Rubber workers
Plastics manufacturing workers	Drug makers	Shoemakers
Polyethylene laminating workers	Dyemakers	Smokeless powder makers
Printers	Electronic equipment cleaners	Solvent workers
Printing ink production workers	Electronic equipment dryers	Stain makers
Resin makers	Explosive makers	Textile makers
Rubber-cement workers	Fungicide makers	Varnish makers
Shoe-factory workers	Garage mechanics	Varnish remover workers
Solvent workers	Glycol makers	Vinyl raincoat makers
Spray painters	Iodoform makers	Wax makers
Stainers	Ketone manufacturers	
Stain makers	Lacquerers	
Synthetic chemical production workers	Lacquer makers	
Synthetic rubber workers	Lacquer remover workers	
Thermometer makers, low temperature	Leather workers, artificial	
Varnish makers	Lubricating oil dewaxers	
Vegetable oil extraction workers	Mesityl oxide makers	
Vinyl production workers	Metal cleaners	

Modified from References 142 and 144.

of *n*-hexane produces no morphological or functional evidence of neuropathy in rats exposed for the equivalent of a human working day for over 6 months.[124]

At the time of going to press, the American Conference of Government Industrial Hygienists has established a Threshold Limit Values (T.L.V.) for M*n*BK of 5 ppm and recommended a T.L.V. for *n*-hexane of 25 ppm.[64] The basis for setting a 5 to 1 ratio for the T.L.V. for *n*-hexane and M*n*BK is not apparent from the above data; indeed, it seems clear that a scientific rationale for comparison will only be obtained when the

Table 13
MAJOR AND MINOR USES OF KETONES

Major uses	Ketone(s)	Minor Uses	Ketone(s)
Application solvent		Intermediate in the synthesis of perfume ingredients and other materials	2,5-HD
Coatings			
Cellulosic, vinylic, and acrylic resins	All aliphatic ketones and acetophenone	Preparation of chelates	
		Tanning agent	
		Solvent for inks	
		Solvent for wood stains	
		Gasoline additive	
Coatings			
Alkyl, epoxy, and other natural and synthetic resins	All aliphatic ketones and acetophenone	Solvent for inks, pesticides, and for cleaning and degreasing metals and leathers	*Cyclo*hexanone
Adhesives	MEK, MiBK	In paint removers as a spotting and relustering agent	
Selective extraction		Solvent for textile dying	
		Sludge solvent in oil for piston-type aircraft lubrication	
Lube oil/wax refining	MEK, MiBK diisobutyl ketone	Component in castor oil-based hydraulic fluids	Diacetone alcohol
Rare metal refining	MiBK, diisobutyl ketone, mesityl oxide	Solvent for inks, pesticides, and wood stains, paint and rust removers	
Chemical intermediate		Viscosity index improver for lube oils	
Product			
ε-caprolactam Adipic acid	*Cyclo*hexanone		

Modified from References 120 and 144.

peak serum levels of 2,5-HD generated from orally administered equimolar concentrations of the two solvents are established for man. It will also be important to establish the degree of potentiation of neurotoxicity during concurrent exposure to M*n*BK or *n*-hexane and to other chemicals or drugs. MEK has this property and has been used as a solvent in conjunction with *n*-hexane and methyl *n*-butyl ketone. Experimental studies indicate that (1) 2,5-HD levels increase with concurrent exposure to MEK and M*n*BK vs. M*n*BK alone, and (2) neuropathy appears almost 3 times more rapidly in rats exposed to a mixture composed of 83% MEK and 17% M*n*BK compared to an equivalent concentration of 100% M*n*BK.[175] With *n*-hexane, neuropathy appears 1.6 times more rapidly in rats concurrently exposed to a mixture of 10% MEK and 90% *n*-hexane, compared to 100% *n*-hexane alone.[10] These figures are based on the appearance of weakness and should be more accurately assessed in the rat by morphological and/or nerve conduction studies.

Table 14

NATIONAL LIMITS (MG/M³) FOR HUMAN EXPOSURE TO N-HEXANE AND SOME KETONES

	Hexane	MnBK	MiBK	MEK	Cyclohexanone	Diacetone alcohol
Australia	—	410	410	590	200	240
Belgium	—	410	410	590	200	240
Bulgaria	—	—	—	200	10	—
Finland	1800[a]	410	410	590	200	240
D.D.R.	—	—	—	300	—	—
F.D.R.	—	410	400	590	200	240
Hungary	—	—	—	200	—	—
Italy	—	—	300	400	200	—
Japan	360[a]	—	410	590	100	—
Netherlands	—	100	410	590	200	240
Poland	400	200	200	200	20	20
Romania	1500[a]	200	200	200	100	150
Sweden	—	—	210	440	—	—
Switzerland	—	410	410	590	200	240
U.S.S.R.	—	—	—	200	10	—
Yugoslavia	1800[a]	410	410	200	200	240
U.S.		100				
1978 Federal Standards (29 CFR 1910.1000)	—	410	410	590	200	240
NIOSH Recommended	100	4	200	590	100	240

[a] Values for straight chain isomers.

Modified from References 142 and 144.

Another important factor not accounted for by a Threshold Limit Value for workroom air is exposure to these solvents by skin contact. There is strong evidence that significant amounts of M*n*BK can be absorbed through the skin of man,[54] and one report[148] suggested the same may be true for *n*-hexane. DiVincenzo and colleagues[54] combined their observations on respiratory and skin absorption of M*n*BK to calculate the relative importance of concurrent skin and respiratory exposure to this solvent: the total dose of M*n*BK absorbed during 1 hr by an individual breathing 25 ppm M*n*BK and working with hands immersed in the solvent, would be 314 mg, of which only 30% would be derived from respiratory absorption and 70% percutaneously.

4. 2,5-Hexanedione

Water-soluble 2,5-HD enjoys a minor use in industry (Table 13) compared to the more volatile solvents. The majority of experimental evidence is compatible with the idea that 2,5-HD is a *primary* neurotoxic compound.

B. Environmental Exposure

Man is exposed to small amounts of *n*-hexane in automobile exhaust[69] (Table 15), and to residual *n*-hexane and ketones in foodstuffs (Table 16). Worker exposure to these compounds is considered in Table 12.

Table 15
PREDOMINANT HYDROCARBONS IN AUTO EXHAUST

Hydrocarbon	Fraction of total exhaust hydrocarbons, vol % 62-Car survey	15-Fuel study	Engine-variable study
Methane	16.7	18	13.8
Ethylene	14.5	17	19.0
Acetylene	14.1	12	7.8
Propylene	6.3	7	9.1
n-Butane	5.3	4	2.3
Isopentane	3.7	4	2.4
Toluene	3.1	5	7.9
Benzene	2.4	—	—
n-Pentane	2.5	—	—
m- and *p*-Xylene	1.9	—	2.5
1-Butene	1.8	3	6.0
Ethane	1.8	—	2.3
2-Methylpentane	1.5	—	—
n-Hexane	1.2	—	—
Isooctane	1.0	—	—
All others	22.2	30	26.9

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VI. EXPERIMENTAL TOXICOLOGY

A. Effects of Brief Exposure to Hexacarbons

1. Hexanes

The effects of brief exposure to large concentrations of "hexane" are listed in Table 17. Narcotization is a well-demonstrated effect. *Cyclo*hexane is reported[110] to be more toxic in 14-day-old rats than in young adult animals. Further details on the acute effects of hexane can be found in the N.I.O.S.H. Criterion Document for Alkanes.[142]

2. Ketones

The reader is referred to the U.S. Environmental Protection Agency document on ketonic solvents[120] for detailed information on the acute toxicity of ketones. The primary response to the inhalation of ketone vapors is progressive narcosis with loss of corneal, auditory, and equilibratory reflexes, as well as a general depression of vital signs such as heart rate, respiratory rate, and body temperature. These qualitative similarities were noted by Specht and co-workers,[214,215] who exposed guinea pigs to a number of ketones including M*n*BK, MiBK, 2,5-HD, and *cyclo*hexanone. Behavioral changes seen in rats exposed to M*n*BK are probably associated with its narcotizing property.[104] Information on the acute inhalation toxicity of M*n*BK gives no indication of its chronic neurotoxic property; MiBK and 2-heptanone which have failed to produce neuropathy in animals,[187,222,240] are more narcotizing to rats upon single exposure to high concentrations.

3. Refined Petroleum Solvents

The effects of acute exposure to large concentrations of refined petroleum solvents containing hexacarbon compounds are listed in Table 17. Further details are available in the N.I.O.S.H. Criterion Document on Refined Petroleum Solvents.[143]

Table 16
RESIDUAL HEXANE IN VEGETABLE OILS, AND KETONES FOUND IN FOODSTUFFS

Compound	Foodstuff	Concentration (ppm)
Hexane	Crude soybean oil	550—3500
	Crude cotton-seed oil	35—5000
	Crude peanut oil	20
	Refined soybean oil	150—170
	Refined cotton-seed oil	5—80
M*n*BK	White bread	
	Toasted oats	
	Milk	0.007—0.011
	Cream	0.017—0.018
MiBK	Oranges	
MEK	Cheeses	0.3
	Milk	0.077—0.079
	Cream	0.154—0.177
	Milk fat	8
	Roasted barley	
	Bread	
	Honey	
	Chicken	
	Corn silage	
	Oranges	
	Black tea	
	Rum	
	Tobacco	

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B. Effects of Repetitive or Continuous Exposure to Hexacarbons (Table 17)

Several studies have examined the subacute and subchronic (neuro)toxicity of hexacarbon compounds (Table 18). The majority has been concerned with the effects of *n*-hexane and methyl *n*-butyl ketone, and the interactive effect of MEK on adult animals. It is apparent from these studies that the duration of respiratory exposure to these solvents has a marked effect on the incidence and speed of onset of neuropathy. For example, whereas rats continuously inhaling 400 to 600 ppm of *n*-hexane develop pathological evidence of neuropathy after 7 weeks,[192] no changes are found in rats exposed intermittently to 500 ppm for 30 weeks; giant axonal swellings do appear in rats exposed intermittently to 5000 ppm for 14 weeks.[75]

1. Hexane Isomers

Egan and co-workers[62] examined the nervous system of Sprague-Dawley rats exposed continuously for up to 6 months to a 500 ppm mixture of hexane isomers* largely free of neurotoxic *n*-hexane. A detailed neuropathological examination failed to reveal structural damage; by contrast, littermates inhaling 500 ppm of M*n*BK developed severe neuropathy and central and peripheral nervous system damage.

2. Refined Petroleum Solvents

Carpenter and colleagues[36] found no significant changes in rats or dogs breathing naphtha intermittently for 64 weeks or 8 to 37 ppm 140° aliphatic flash solvent inter-

* 2,3-Dimethylbutane (60 ppm), 2-methylpentane (618 ppm), 3-methylpentane (525 ppm), methyl cyclopentane (431 ppm), cyclohexanone (109 ppm), and *n*-hexane (5.3 ppm).

Table 17
EFFECTS ON ANIMALS OF SHORT-TERM EXPOSURE TO HEXACARBON COMPOUNDS AND REFINED PETROLEUM SOLVENTS CONTAINING HEXACARBONS

Compound	Species	Route	Dose	Duration	Effects	Ref.
Hexane	Mouse	Respiratory	64,000 ppm	5 min	Respiratory arrest by 2.5—4.5 min	236
	Mouse	Respiratory	51,120 ppm	9 min	Death after spasms	76
	Mouse	Respiratory	42,600 ppm	127 min	Loss of reflexes and death	76
	Mouse	Respiratory	42,600—34,080 ppm	2 hr	Death	121
	Mouse	Respiratory	36,920 ppm	127 min	Death	76
	Mouse	Respiratory	34,080 ppm	123 min	Light narcosis	76
	Mouse	Respiratory	32,000 ppm	5 min	Deep anesthesia	236
	Mouse	Respiratory	28,400 ppm	2 hr	"Lying down"	121
	Mouse	Respiratory	16,000 ppm	5 min	No anesthesia	236
	Mouse	Respiratory	8,000 ppm	5 min	No anesthesia	236
	Rat	Oral	1—49 mℓ/kg		LD_{50} studies	110
M*n*BK	Rat	Respiratory	8,000 ppm	4 hr	Death	210
	Rat	Intraperitoneal	10.914 g/kg		LD_{10}	177
	Rat	Respiratory	<5,000 ppm	>0.5 hr	Lethal to some animals	210
	Rat	Oral	2.59 g/kg		14-day LD_{50}	210
	Rabbit	Dermal	4.85 g/kg		14-day LD_{50}	210
	Rat	Inhalation	8,000 ppm	4 hr	Lethal to all animals	210
	Guinea pig	Inhalation	6,000 ppm	6.6 hr	Lethal to 2/10, and 10/10 by 72 hr of exposure	214
	Guinea pig	Inhalation	2,300 ppm	13.5 hr	No deaths	197
MiBK	Rat	Respiratory	4,000 ppm	4 hr	Death	135
	Rat	Respiratory	Saturated vapor	0.5 hr	Death	209
	Mouse	Respiratory	20,000 ppm	0.5 hr	Death	202
	Guinea pig	Respiratory	10,000 ppm 30,000 ppm	4 hr	Lethal to most animals	214
2,5-HD	Rat	Oral	2.7 g/kg		14-day LD_{50}	208
	Guinea pig	Dermal	6.4 g/kg		14-day LD_{50}	208
Diacetone alcohol	Rabbit	Dermal	13.6 g/kg		14-day LD_{50}	207
	Rat	Oral	4.0 g/kg		14-day LD_{50}	207
Mesityl oxide	Rat	Oral	1.12 g/kg		14-day LD_{50}	134
	Guinea pig	Oral	1.0 g/kg		Lethal to six animals within 14 days	35
	Rat	Respiratory	500—2,500 ppm	8 hr	30—100% mortality	211
	Guinea pig	Respiratory	13,000 ppm	0.17—1 hr	0—100% mortality	211
	Guinea pig	Respiratory	5,000 ppm	5.4 hr	Coma and death	215
	Guinea pig	Dermal	1.9 g/kg		14-day LD_{50}	35
	Guinea pig	Dermal	0.43 g/kg		Death after 3—9 hr	203
*Cyclo*hexanone	Rat	Respiratory	2,000—4,000 ppm	4 hr	Anesthesia after 1—5 hr	153

Table 17 (Continued)

EFFECTS ON ANIMALS OF SHORT-TERM EXPOSURE TO HEXACARBON COMPOUNDS AND REFINED PETROLEUM SOLVENTS CONTAINING HEXACARBONS

Compound	Species	Route	Dose	Duration	Effects	Ref.
*Cyclo*hexa-none	Guinea pig	Respiratory	4,000 ppm	7.6 hr	3/10 died after 4 hr	153
	Rat	Oral	1.54 g/kg		14-day LD_{50}	153
	Mouse	Oral	1.4 g/kg		LD_{50}	152
	Rabbit	Oral	1.6—1.9 g/kg		LD_{100}	242
	Mouse	Oral	2.78 g/kg		24 hr LD_{50}	39
Naphtha	Rat	Respiratory	25,000 ppm	4 hr	Death	36
	Rat	Respiratory	15,000 ppm		Loss of motor coordination, death	36
	Mouse	Respiratory	7,700 ppm	1 min	Decreased respiration	36
	Cat	Respiratory	4,100 ppm	4 hr	CNS depressant effect	36
	Dog	Respiratory	3,400 ppm	2 hr	Eye irritation, tremor, poor coordination	36
	Dog	Respiratory	1,700 ppm	4 hr	No significant effect	36
140 Flash Solvent	Rat	Respiratory	1,620 ppm	340 min	Sluggish movement, tremor, death	38
	Rat	Respiratory	461 ppm	8 hr	Loss of coordination and death in some	38
	Mouse	Respiratory	56—1906 ppm		No respiratory tract irritation	38
	Cat	Respiratory	1,588 ppm		No CNS depressant effect	38
	Dog	Respiratory	33—280 ppm	8 hr	Transitory lacrimation	38
Rubber solvent	Rat	Respiratory	180,000 ppm	2—8 min	Convulsions, death	37
	Rat	Respiratory	9,800—24,000 ppm	4 hr	Loss of motor coordination and death at high level	37
	Mouse	Respiratory	63,000 ppm	1 min	Respiratory tract irritation	37
	Cat	Respiratory	12,400 ppm	4 hr	CNS depressant effect	37
	Dog	Respiratory	3,300—6,300 ppm	4 hr	Loss of motor coordination	37

Gasoline: mixture of C_4-C_{12} hydrocarbons containing paraffins (1 to 2% *n*-hexane), olefins, naphthenes, and aromatics; petroleum ether: 80% pentane, 20% isohexane, and less than 0.5 vol% of *n*-hexane; rubber solvent C_5-C_7 compounds consisting of paraffins, monocycloparaffins, monoolefins, benzene, and alkyl benzene; naphtha (varnish makers' and painters' naphtha): C_5-C_{11} compounds containing paraffins (0.3% *n*-hexane), monocycloparaffins, dicycloparaffins, benzene, alkylbenzenes, indans, and tetralins; mineral spirits: saturated hydrocarbons, olefins, and aromatics; 140° flash solvent: C_8-C_{12} compounds containing paraffins (1.2% *n*-hexane), monocycloparaffins, dicycloparaffins, alkyl benzene, benzene, indans, and tetralins.

Table 18

EFFECTS ON ANIMALS OF SUBACUTE OR SUBCHRONIC EXPOSURE TO HEXACARBON COMPOUNDS

Compound	Species	Route	Dose	Duration	Effects	Ref.
n-Hexane	Pigeon	Respiratory	3,000 ppm	5 hr/day, 5 days/week for 17 weeks	No functional, electrophysiological, or pathological change	70
n-Hexane mixture	Chicken	Dermal	1 g/kg/day	65 days	Histological damage to nerves	71
n-Hexane	Mouse	Respiratory	1,000—2,000 ppm	6 days/week for 1 year	Muscular atrophy and muscle fibrillation potentials	140
	Mouse	Respiratory	250—2,000 ppm	6 days/week for 1 year	Higher strength duration curves with higher doses	140
	Mouse	Respiratory	500 ppm	6 days/week for 1 year	Abnormal posture and muscle atrophy	140
	Mouse	Respiratory	100—2000 ppm	6 days/week for 1 year	No neuropathy at 100 ppm, mild at 250 ppm; muscle atrophy at 500 ppm	140
n-Hexane/ *n*-Pentane 1:1	Mouse	Respiratory	3—30 ppm	N.S.	Changes in motor chronaxy and structural changes in cerebral cortex	25
n-Hexane	Rat (Crl: COBS,® CD®/ (SD) BR)	Respiratory	125 ppm	21 hr/day for 6 months	No morphological changes in central or peripheral nervous system	124
n-Hexane	Rat	Respiratory	500 ppm, 1500 ppm	9hr/day, 5 days/week for 30 weeks (500 ppm) or 14 weeks (1500 ppm)	No morphological changes in sections or teased fibers fixed by systemic perfusion with glutaraldehyde	75
n-Hexane	Rat	Respiratory	5000 ppm	9 hr/day, 5 days/week for 14 days	Giant axonal degeneration	75
n-Hexane	Rat	Respiratory	10,000 ppm	8 hr/day, 7 days/week for 19 weeks	Hindlimb weakness after 8 weeks	10
n-Hexane/MEK 9:1	Rat	Respiratory	10,000 ppm total	8 hr/day, 7 days/week for 19 weeks	Hindlimb weakness after 5 weeks	10
n-Hexane	Rat	Respiratory	2,060 ppm	5 hr/day, 5 days/week for 6 months	Reduced nerve conduction velocity	244
	Rat	Respiratory	3,000 ppm	12 hr/day, 16 weeks	"Disturbed" nerve conduction velocity and residual latency of tail nerve	238
	Rat	Respiratory	1,500—3,000 ppm	10 months	Axonal neuropathy: distal nerves more damaged than proximal	245
	Rat	Respiratory	500 ppm	6 months	Absence of central or peripheral nervous system damage	124

Table 18 (continued)
EFFECTS ON ANIMALS OF SUBACUTE OR SUBCHRONIC EXPOSURE TO HEXACARBON COMPOUNDS

Compound	Species	Route	Dose	Duration	Effects	Ref.
n-Hexane	Rat	Respiratory	2,000 ppm	Intermittently for a few months	Giant axonal changes	57
	Rat	Respiratory	850 ppm	143 days	Sciatic nerve demyelination and degeneration; myoneural junctions intact	118
	Rat	Respiratory	400—600 ppm	2—23 weeks	Axonal swellings after 42 days; neuropathy after 45 to 69 days	192
n-Hexane (0.5%)	Rat	Respiratory	N.S.	900 hr	Neuropathy	133,240
n-Hexane	Rat	Subcutaneous	N.S.	4 months	Dying-back neuropathy (Prefunctional)	101
	Rat	Subcutaneous	650 mg/g/day	5 days/week for 35 weeks	Giant axonal swellings in CNS and PNS	225,226
	Rat	Oral	0.1—0.2 mℓ/day	N.S.	Drop in motor conduction velocity in tail nerve	237
	Rat	Oral	N.S.	10—13 months	Slight weight loss	117
	Guinea pig	Respiratory	150 mg/ℓ	1 hr/day for 30 days	Extinction of electroretinogram	21
Hexane isomers (0.3%) *n*-hexane	Rat	Respiratory	100 ppm	6 months	No CNS or PNS changes	124
2-Methyl pentane, or 3-Methyl pentane	Rat	Respiratory	1500 ppm,	9 hr/day, 5 days/week for 14 weeks	No morphological signs of neuropathy in glutaraldehyde-perfused tissue sections and teased nerve fibers	75
*Cyclo*hexane	Rat	Respiratory	1500 ppm	9 hr/day, 5 days/week for 30 weeks	No morphological signs of neuropathy in glutaraldehyde-perfused tissue sections and teased fibers	75
M*n*BK	Chicken	Respiratory	200—100 ppm	4—5 weeks	Neuropathy after 35 days	138
	Rat	Respiratory	6,500± 1,060 ppm	6 hr/day, 5 days/week for 5—15 weeks	Decrease in MCV after 5 weeks; neuropathy after 10 weeks	27
	Rat	Respiratory	1,300 ppm	6 hr/day, 5 days/week for 7 weeks	Neuropathy after 10—12 weeks; CNS and PNS nerve fiber degeneration	178,229
	Rat	Respiratory	1,050—1,460 ppm	6 hr/day, 5 days/week for 15 weeks	Decrease in MCV by 5 weeks; neuropathy after 11—13 weeks; nerve fiber changes	50
	Rat	Respiratory	976 ppm	6 hr/day, 5 days/week for 6 months	Hindlimb dragging; reduction in nerve conduction velocity	104
	Rat	Respiratory	700 ppm	11 weeks	Neuropathy	108
	Rat	Respiratory	400—600 ppm	5—8 weeks	Neuropathy in 60 days	138
	Rat	Respiratory	600 ppm	9 days	Pathological changes in CNS and PNS	225,226

Table 18 (continued)
EFFECTS ON ANIMALS OF SUBACUTE OR SUBCHRONIC EXPOSURE TO HEXACARBON COMPOUNDS

Compound	Species	Route	Dose	Duration	Effects	Ref.
MnBK	Rat	Respiratory	330 ppm	72 weeks	Functional and histological evidence of neuropathy after 30 weeks	115
	Rat	Respiratory	225 ppm	42 days	Paralysis	188
	Rat	Respiratory	200 ppm	8 hr/day, 5 days/week	Functional and histological signs of neuropathy	60
MnBK/MEK 1:11	Rat	Respiratory	200 ppm + 2,000 ppm	5—8 weeks	Functional and histological evidence of neuropathy	60
MnBK	Rat	Respiratory	100 ppm	6 hr/day, 5 days/week for 5 months	No neuropathy	129
	Rat	Respiratory	100 ppm	4 weeks	No evidence of neuropathy	50
	Rat	Respiratory	100 ppm	72 weeks	No histological or functional signs of neuropathy	115
	Rat	Respiratory	100 ppm	10 months	Reduced nerve conduction velocity	104
	Rat	Respiratory	60 ppm	6 weeks	No evidence of neuropathy	50
	Rat	Respiratory	50 ppm	6 months	Nerve conduction velocity reduced by 10 m/s	234
	Rat	Oral	(1,000 ppm for 4 days) 68—406 mg/kg	1 day/week for 6 months	Reduced fixed-interval performance; no neuropathy	14
	Rat	Oral	0.1—0.2 mℓ/d	N.S.	Decreased conduction velocity in rat tail nerve	237
	Rat	Oral	0.25—1%	13 months	Functional and morphological signs of neuropathy	117
	Rat	Oral	1 g/kg/day		Peripheral neuropathy	96
	Cat	Respiratory	600—400 ppm	60 days	Neuropathy after 33 days	138
	Cat	Respiratory	400 ppm	7 wk + 4½-month recovery	Paralyzed after exposure; able to walk on recovery	188
	Cat	Respiratory	100 and 330 ppm	6 hr/day, 5 days/week for 4—5 months	No functional or histological signs of neuropathy	179
	Cat	Subcutaneous	150 mg/kg/day	8.5 months	Peripheral neuropathy with CNS and PNS damage	222
MnBK/MEK 1:9	Cat	Subcutaneous	150 mg/kg/day (total)	8.5 months	No functional signs of neuropathy; minor PNS changes	222
MnBK	Dog	Subcutaneous	150 mg/kg/2 days	2 times daily, 5 weeks	Neuropathy	179
	Monkey	Respiratory	1,000 ppm	6 hr/day, 5 days/week up to 6 months	Decreased conduction velocity	104
	Monkey	Respiratory	100ppm	6 hr/day, 5 days/week up to 10 months	Decreased conduction velocity	104
MiBK	Mouse	Respiratory	20,000 ppm	0.33 hr/day for 15 days	10/14 died	202

Table 18 (continued)
EFFECTS ON ANIMALS OF SUBACUTE OR SUBCHRONIC EXPOSURE TO HEXACARBON COMPOUNDS

Compound	Species	Route	Dose	Duration	Effects	Ref.
MiBK	Rat	Respiratory	1,500 ppm	6 hr/day, 5 days/week for 5 months	No neuropathy signs	229
	Various	Respiratory	100—200 ppm	14 days	No neurological signs; kidney changes noted	126,127, 249
2,5-HD	Mouse	Oral	1%	13—23 weeks	Neuropathy after few weeks	172
	Rat	Oral	1%	13—23 weeks	Neuropathy after few weeks	172
	Rat	Oral	520 mg/kg/day	2 months	Neuropathy	179
	Rat	Oral	0.5%, 1%		Neuropathy	141
	Rat	Oral	0.05 mℓ, 0.1 mℓ		Decreased nerve conduction velocity	237
	Rat	Oral	0.5%	12 weeks	Neuropathy with CNS and PNS degeneration	219,220, 225,226, 230
	Rat	Oral and subcutaneous	230—520 mg/kg/day	6—19 weeks	Neuropathy with CNS and PNS degeneration	225,226
	Rat	Subcutaneous	340 mg/kg/day	5 days/week for 23 weeks	Peripheral neuropathy of giant axonal type	179
	Cat	Oral	0.5%	136 days	Neuropathy and CNS damage	193
2,4-Hexanedione	Rat	Oral	0.5%	7 weeks	No functional changes	216,224
2,3-Hexanedione	Rat	Oral	0.5%	7 weeks	No functional changes	216,224
Cyclohexanone	Rat	Intubation	0.05—5.0 mg/kg/day	6 months	Changes in conditioned reflexes; degenerative changes in nervous system and other organs claimed	152
	Mouse	Intubation	280 mg/kg/day	25 days	Decline in work capacity	152
	Rabbit	Respiratory	190—3082 ppm	6 hr/day, 5 days/week for 3—10 weeks	Slight narcosis at high levels	243
	Monkey	Respiratory	605 ppm	6 hr/day, 5 days/week for 10 weeks	Conjunctival congestion	243
Mesityl oxide	Rat	Respiratory	50—500ppm	8 hr/day, 10—30 exposures	Death at 500 ppm	206,211
	Rat	Respiratory	300 ppm	2 hr/day, 30 days	Leucocytosis; hypertrophy of liver, spleen, kidney	102
	Guinea pig	Respiratory	50—500 ppm	8 hr/day, 10—42 exposures	2/10 died at 500 ppm	102,211
1-Hexanol	Rat	Intraperitoneal	102.5 mg/kg/day	6 days/week for 30 weeks	Decrease in sensory nerve conduction only	168

Table 18 (continued)
EFFECTS ON ANIMALS OF SUBACUTE OR SUBCHRONIC EXPOSURE TO HEXACARBON COMPOUNDS

Compound	Species	Route	Dose	Duration	Effects	Ref.
2-Hexanol	Rat	Intraperitoneal	101.2 mg/kg/day, 151 mg/kg/day	6 days/week for 20 weeks, (2 for 14 weeks only)	Motor nerve conduction velocity decreased; giant axonal neuropathy	168
1,6-Hexanediol	Rat	Oral	0.5%	12 weeks	No functional changes	216,224
2,5-Hexanediol	Rat	Oral	0.5%	14 weeks, 29 weeks	Neuropathy with CNS and PNS degeneration	216, 224

Note: Respiratory exposures were continuous unless otherwise stated. N.S. means not stated.

mittently for 73 weeks.[38] Neuropathological examination, which can detect subclinical damage, was not performed in these studies.

3. MiBK

Prolonged exposure to MiBK has not produced neurological damage in Sprague-Dawley rats. Spencer and co-workers[229] found glycogen-filled axonal mitochondria and Schwann cell sequestration in tibial and ulnar nerves without fiber breakdown in rats exposed to 1500 ppm MiBK intermittently for 6 months. These subclinical changes may have been related to the small (0.9%) contamination of M*n*BK in the MiBK solvent, or to nerve changes which normally occur in aging rats.[92]

4. Cyclohexanone and Cyclohexane

According to one source,[120] "marked changes in conditioned reflex activity accompanied by degenerative morphological changes of nervous system, liver, stomach and spleen" were reported[152]* in rats inhaling 5 mg/kg/day of *cyclo*hexanone.

According to another source,[164] both *cyclo*hexanone and *cyclo*hexane have been reported[72]* to "cause experimental neuropathies". However, in a detailed investigation, Frontali and co-workers[75] failed to find morphological evidence of neuropathy in rats subchronically exposed to *cyclo*hexane.

5. n-Hexane, MnBK, 2,5-HD, 2,5-HDiol, 5-OH-2-H, and 2-Hexanol

a. General

Peripheral neuropathy (Figure 13) develops in a number of species upon subchronic exposure to any of these six metabolically related neurotoxic hexacarbons. The relative neurotoxicity of these compounds in Charles River CD® COBS® rats (2,5-HD > 5-OH-2-H > 2,5-HDiol > M*n*BK > 2-hexanol > *n*-hexane) has been attributed to differential serum levels of their common metabolite 2,5-HD.[116] The *neurotoxic index* (N.I.), calculated by dividing the time-to-severe-neuropathy** for M*n*BK into that for each of the other for compounds, is directly related to the peak serum concentrations of 2,5-HD***: *viz.* $N.I._{2,5\text{-}HD} = 3.3$ $N.I._{5\text{-}OH\text{-}2H} = 2.6$; $N.I._{2,5\text{-}HDiol} = 1.9$; $N.I._{MnBK} = 1.0$; $N.I._{2\text{-}hexanol} = < 0.7$; $N.I._{n\text{-}hexane} = < 0.08$[156] The relatively low neurotoxicity of *n*-hexane

* Not available for review.

** Calculated as mean duration of intoxication for onset of hindlimb paralysis with dragging of one or both hindfeet in a group of 5 or more animals.

*** Calculated as mean peak serum levels in 3 to 5 animals repetitively sampled over 24-hr-period after a single oral dose of test compound.

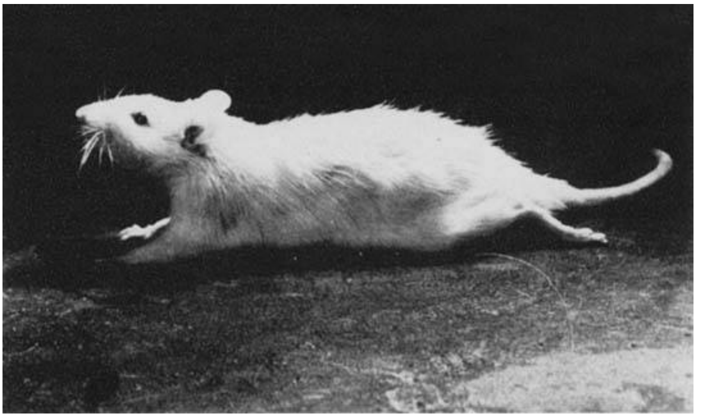


FIGURE 13. Rat with severe hexacarbon neuropathy displaying a pronounced hindlimb footdrop. (From Spencer, P. S. and Schaumburg, H. H., *J. Neuropathol. Exp. Neurol.*, 36, 300, 1977. With permission.)

ane accounts for some studies[70,71] which failed to produce neuropathy by subchronic exposure.

Hexacarbon neuropathy has been produced by various routes (except dermal[114]) in the pigeon, chicken, mouse, guinea pig, rat, cat, dog, and monkey (Table 18). Pigeons failed to show any evidence of neuropathy after subchronic intermittent exposure to 3000 ppm of *n*-hexane.[70] Chickens may be somewhat more susceptible to neuropathy than other species,[138] and giant axonal changes have been reported following dermal exposure to a solvent mixture containing *n*-hexane.[71]

Sprague-Dawley rats are more severely affected by 2,5-HD than the Wistar variety or mice.[172] Guinea pigs die after 8 weeks of drinking 0.5% 2,5-HD[3], whereas rats tolerate this dose well for at least 14 weeks.[216] The basis for this differential sensitivity has not been determined. Differences in response with respect to age or sex have not been reported.

b. Body Weight and Other Changes

Weight loss or failure to gain weight normally has been reported to precede or accompany hexacarbon neuropathy. An *increased* rate of body weight gain during exposure to M*n*BK, 2-hexanol, or 2,5-HD was found by Abdel-Rahman and colleagues.[3] Testicular atrophy may also precede the onset of 2,5-HD neuropathy.[157]

One study[33] has examined *n*-hexane for perinatal toxicity. With the exception of a delayed postnatal growth, no significant changes were noted in Fischer-344 rats exposed to 1000 ppm of *n*-hexane (6 hr/day, on days 8 to 16 of gestation). The nervous system was not examined histologically, so that it may be premature to conclude that the tested levels of *n*-hexane are free of teratogenic effects.

c. Behavioral Changes

Short-term administration of neurotoxic M*n*BK (oral) and nonneurotoxic methyl amyl ketone (i.p.) showed that both solvents caused a reduction in the response rate of rats trained on a multiple schedule of reinforcement.[15,104,105] This was attributed to CNS depression in the form of a mild narcosis.[104] Narcosis and excessive salivation was also noted in cats injected subcutaneously with M*n*BK.[222]

d. Evoked Response

Johnson and colleagues[104] recorded visual evoked potentials from monkeys exposed

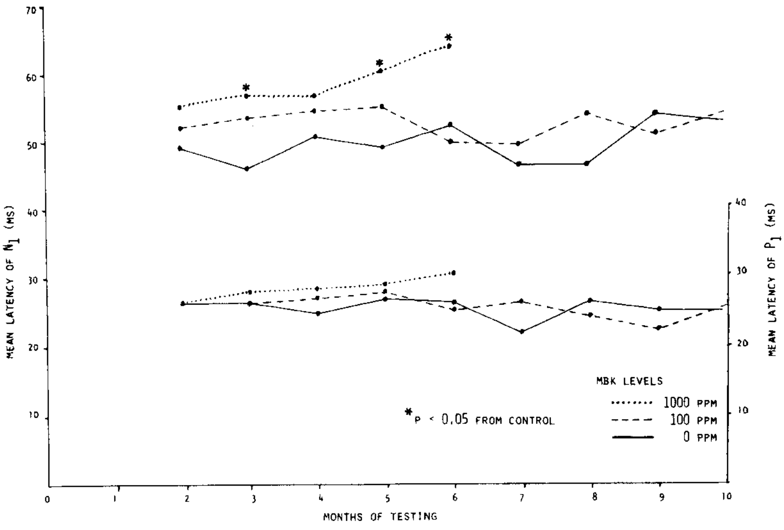


FIGURE 14. Effect of M*n*BK (MBK) on the latency of components N_1 (upper) and P_1 (lower) averaged visual evoked potential. (From Johnson, B. L., Anger, W. K., Setzer, J. V., Lynch, D. W., and Lewis, T. R., *Proc. 1st Ann. NIOSH Scientific Symp., Cincinnati,* Pathotox Publ., Ill. 1978. With permission.)

to 100 ppm and 1000 ppm M*n*BK and found that latencies for component waves were increased after 4 months (Figure 14). They suggested these findings represented the electrophysiological consequences of terminal giant axonal changes found in the optic tract of animals with hexacarbon neuropathy.[193]

Abdel-Rahman and co-workers[3] reported a decrease in the pupillary response of guinea pigs receiving M*n*BK, 2,5-HD, or 2-hexanol in their drinking water for periods up to 214 weeks. Proptosis and a misshapen cornea were also found in the 2-hexanol group. The origin of these changes is unknown.

e. Electrodiagnostic Studies

(i.) Nerve Conduction Velocity (NCV)

Several experimental studies[104,167,168,234,245] have reported early (preclinical) and progressive reduction in conduction velocities of sciatic and ulnar nerves in animals receiving neurotoxic hexacarbons. These changes have been ascribed to focal demyelination accompanying giant axonal swelling, although one recent report[27] has emphasized that nerve conduction changes precede demyelination and may be associated with an early, slight attenuation of large-diameter nerve fibers. Streletz and co-workers[234] found slowing (77% of normal) of the sciatic-tibial NCV of Wistar rats exposed to 50 ppm of M*n*BK, 5 days a week for 6 months.

The NCV of the tail nerve of the rat is reduced following subchronic exposure to *n*-hexane, but remains normal in animals inhaling *n*-pentane or *n*-heptane.[238] Perbellini and co-workers[168] exposed rats to intraperitoneal injection of 2-hexanol or 1-hexanol and determined weekly the conduction velocity of the tail nerves. Sensory nerve conduction was reduced in rats with neuropathy after 8 months of treatment with 1-hexanol, although clinical neuropathy was absent. Both motor and sensory NCVs were reduced in rats treated with 2-hexanol. The amplitude of the sensory action potential was also reduced in both groups, while distal motor latency was reduced only in the 2-hexanol group.

Johnson and co-workers[104] sequentially examined motor nerve conduction velocity in the ulnar and sciatic tibial nerves, and the amplitude of evoked muscle potentials

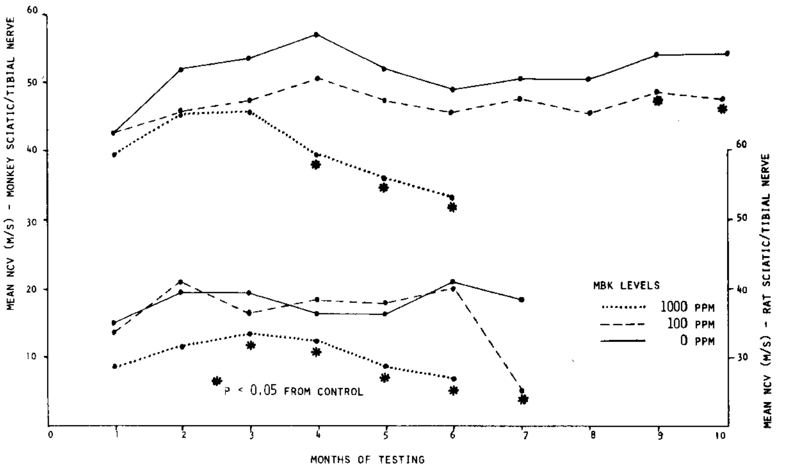


FIGURE 15. Effect of M*n*BK (MBK) on the maximum motor NCV (m/sec, of sciatic-tibial nerves in monkeys (upper) and rats (lower). (From Johnson, B.L., Anger, W.K., Setzer, J.V., Lynch, D.W., and Lewis, T.R., *Proc. 1st Ann. NIOSH Scientific Symp., Cincinnati,* Pathotox Publ., Ill. 1978. With permission.)

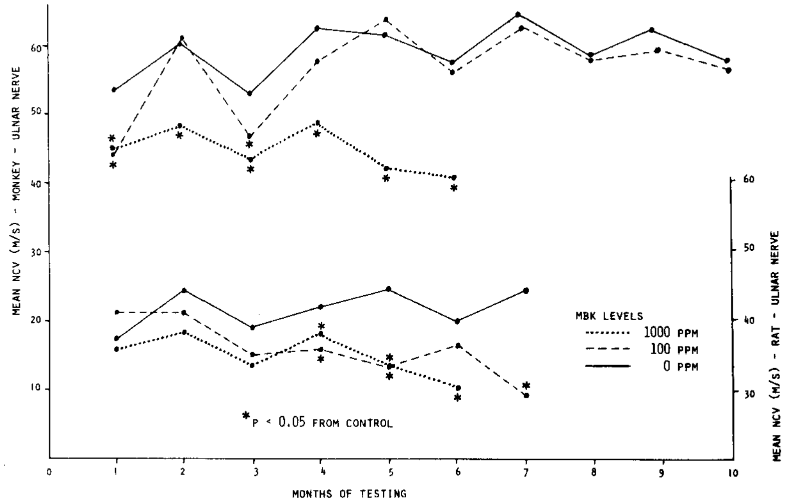


FIGURE 16. Effect of M*n*BK (MBK) on the maximum motor NCV (m/sec of the ulnar nerve in monkeys (upper) and rats (lower). (From Johnson, B.O., Anger, W.K., Setzer, J.V., Lynch, D.W., and Lewis, T.R., *Proc. 1st Ann. NIOSH Scientific Symp., Cincinnati,* Pathotox Publ., Ill. 1978. With permission.)

(MAP) in monkeys and rats inhaling 100 ppm and 1000 ppm of M*n*BK 6 hr/ day, 5 days/week for periods up to 1 year (Figures 15 and 16). The NCV of high-dosed primates showed a progressive decrease beginning at 3 months, a value of 63% of normal (33 m/s) being obtained at 9 months. Decrements began in the 100 ppm groups at 9 months, and were significantly ($P<0.025$) reduced by 12% after 1 year. Ulnar NCV diminished after 4 months, and was 64% of normal after 6 months in the high-dose group.

(ii.) Muscle Action Potentials (MAP)

Animals exposed to 1000 ppm M*n*BK showed a continuous decrease in MAP amplitude which became significant statistically after 6 months of exposure (Figure 17).

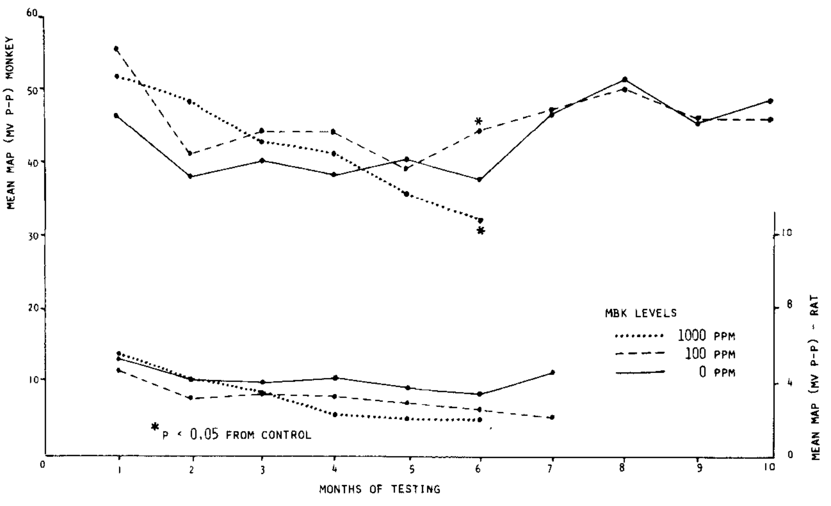


FIGURE 17. Effect of M*n*BK (MBK) on the peak-to-peak (P—P) amplitude in mv of evoked muscle action potentials (MAP) from stimulation of the sciatic nerves of monkeys (upper) and rat (lower). (From Johnson, B. O., Anger, W. K., Setzer, J. V., Lynch, D. W., and Lewis, T. R., *Proc. 1st Ann. NIOSH Scientific Symp., Cincinnati,* Pathotox Publ., Ill. 1978. With permission.)

Hexanol, in common with other aliphatic alcohols, increased the amplitude and duration of miniature end-plate potentials at the toad neuromuscular junction.[77] There was a prolongation of the decay phase of miniature end-plate currents, perhaps due to a change in the dielectric constant of the post-synaptic membrane. Various *n*-alkanols are able to block nerve conduction, possibly by interacting with target proteins.[180] Motor nerve terminal function has also been examined in rats treated with 2,5-HD.[34]

(iii.). Electroencephalography

No effects were found in monkeys exposed to 1000 ppm M*n*BK.[104] *n*-Hexane-induced Stage 2 narcosis in rats decreased the frequency and amplitude of rapid waves in common with other solvents.[56]

f. Functional Signs

Experimental animals exposed to neurotoxic hexacarbons initially show no structural changes in the nervous system. With continuous or repeated intermittent exposure, animals develop progressive structural changes concurrently in the central and peripheral nervous systems.[104,221,223,226] The rate of onset of damage and the amount of degeneration found are both a function of the duration and degree of exposure. Experimental neuropathy has been studied most thoroughly in the rat — the species of choice for most studies of hexacarbon neurotoxicity.

Chickens develop neuropathy after 4 to 5 weeks of continuous exposure to 100 to 200 ppm of M*n*BK. Sprague-Dawley rats exposed continuously to 400 to 600 ppm of *n*-hexane display morphological damage after 42 days, with weakness appearing at 8 to 10 weeks of exposure. Neuropathy fails to develop in Wistar rats exposed to 700 ppm of *n*-hexane 8 hr/day, 7 days/week, for 20 weeks.[8] Comparable data have been reported by Frontali and Colliagner.[75] 2,5-Hexanedione induces neuropathy in Charles River rats 3.3 times more rapidly than M*n*BK and 4 times faster than *n*-hexane; 6.6 m*M*/kg of 2,5-HD administered by gavage daily, 5 days/week, will produce neuropathy in 2 to 3 weeks.[156]

Peripheral nerve damage is associated with impairment of nerve conduction, both of which antedate onset of functional weakness by weeks or months. Weakness always develops symmetrically and, in the rat, first affects the hindlimbs and then the forelimbs.[223] Signs of increasingly severe murine neuropathy include: (a) placing of the surface of the distal hindfeet flat on the ground and, later, eversion of the feet and the development of a waddling gait, (b) inability to extend the hindlimbs when the animal is held off the ground by the tail, (c) muscular atrophy and hindlimb footdrop, and (d) weakness of the upper extremities. Rats exposed to 2,5-HD develop an orange-brown discoloration of the skin and hair,* rough fur, muscle-wasting of hindquarters and abdominal musculature, impaired respiration, and increased lacrimation.[3,157,219] Cutaneous discoloration also occurs in rats inhaling *n*-hexane.[8]

Cats developing M*n*BK neuropathy display weakness of the hindquarters which results in a crouching, unsteady gait, later progressing to a severe hindlimb footdrop.[222] Eventually, cats became unable to walk and drag themselves with weakened forelimbs. Cessation of intoxication may be associated with a slight functional deterioration, and then a slowly progressive recovery. Peripheral nerves are able to regenerate and reinnervate the musculature, strength is regained, and the animals can walk. If the disease has been severe, the gait of recovered animals may show an ataxic or spastic component, which is probably associated with permanent damage to the cerebellum and descending motor tracts of the spinal cord, respectively.[192]

g. Neuropathological Findings

(i.) General

The pattern of structural damage underlying hexacarbon neuropathy falls into the category of a central-peripheral distal axonopathy.[221] These conditions are so-named because distal portions of vulnerable axons in the central and peripheral nervous system undergo retrograde degeneration (die-back), producing structural and functional disconnection in the neuraxis. Many neurotoxic chemicals produce these diseases, and for this reason they are frequently used to create experimental models of the naturally occurring genetic and metabolic distal ("dying-back") axonopathies such as Friedreich's ataxia[40] and diabetic and uremic polyneuropathies.[218,221] The study of experimental hexacarbon neuropathy revealed new insights into the pattern of nerve fiber degeneration and the distribution of the disease in the peripheral and central nervous systems.[225,226]

(ii.) Distribution of PNS Nerve Damage

Nerve fiber vulnerability in central-peripheral distal axonopathies is proportional to some function of fiber length and axon diameter. Thus long and large nerve fibers in the central and peripheral nervous system commence distal degeneration at similar times; later, shorter and smaller myelinated fibers participate in the degeneration process, and eventually unmyelinated fibers may become involved.[227]

The differential vulnerabilities of nerve fibers causes a stereotyped distribution of nervous system damage to develop.[194,221] In the hindlimb of intoxicated rats, degeneration is seen first in the branches of the tibial nerves supplying the calf muscles, second in plantar nerve branches supplying the flexor digitorum brevis muscle, and later in the sensory plantar nerve branches innervating the digits (Figure 18).[226] With time, the disease process moves up affected nerve fibers, axonal degeneration ascends the plantar and tibial nerves, while the degeneration in the tibial, peroneal, and sural nerves ascends the sciatic nerve. The special vulnerability of the very large-diameter fibers

* Graham[86] attributes the discoloration to the formation of a stable chromogenic Schiff base by 2,5-HD and skin proteins. (See Section VI.B.5.1.)

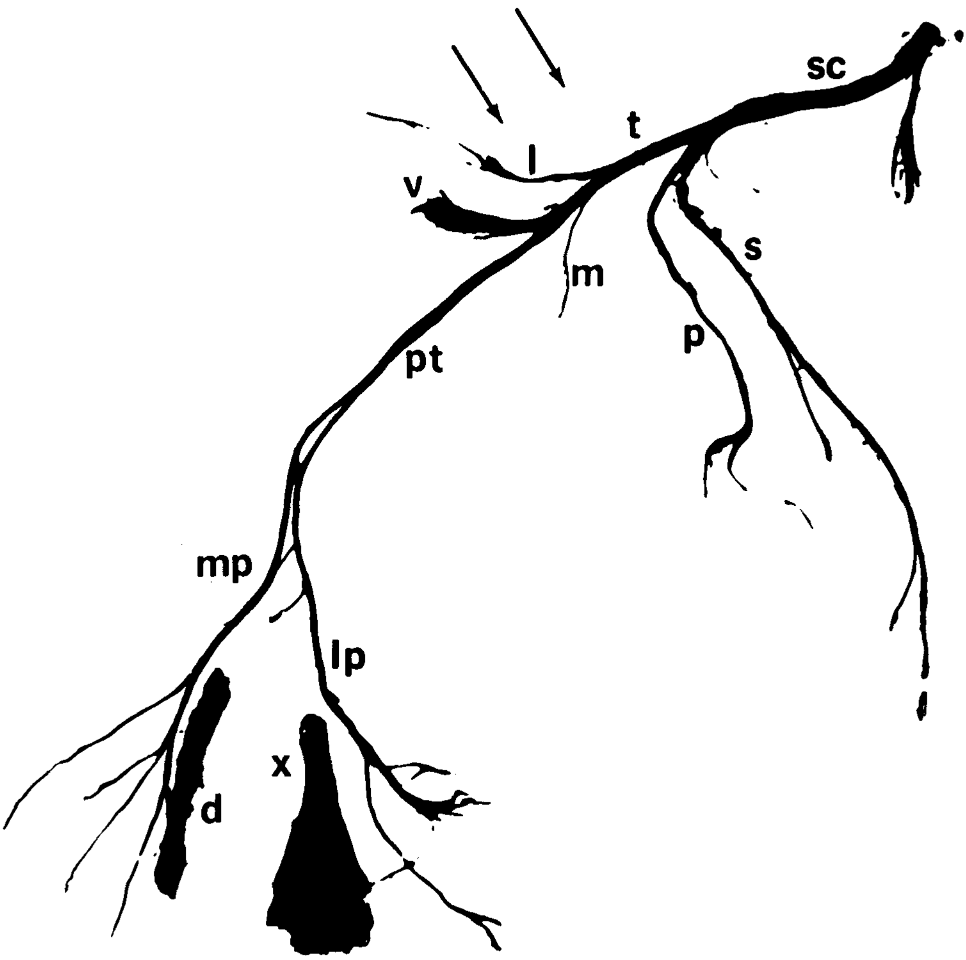


FIGURE 18. Gross dissection of the sciatic/tibial/plantar nerve and some associated intrinsic hindfoot muscles of the rat. The sciatic nerve (sc) branches into the sural (s), peroneal (p), and tibial (t) branches. At the level of the popliteal fossa, the tibial nerve gives a branch to the medial head of the gastrocnemius (m), a branch to the lateral head of the gastrocnemius (i), plantaris and soleus, and a cluster of branches accompanying the posterior tibial blood vessel to flexor hallicus longus, tibialis posterior, and flexor digitorum longus (v). Distally, the posterior tibial nerve (pt) branches into lateral plantar (lp) and medial plantar (mp) nerves. The medial plantar nerve supplies three digits and a branch to the flexor digitorum brevis muscle (d). The lateral plantar nerve supplies two digits and, in addition, supplies the lumbrical muscle (x) with a delicate branch. Degenerative changes in murine hexacarbon neuropathy first appear in *v*, *l*, and *m*, which then ascend to *t* and *sc*. Subsequently, changes appear in *mp* and *lp*, ascend *pt* and later appear in *t* and *sc*. Similar retrograde spread starts in *p* and *s*, and then ascends *sc*. (Fixed in glutaraldehyde and osmium tetroxide, dehydrated and embedded in epoxy resin; Magnification × 1.8.) (From Spencer, P. S. and Schaumburg, H. H., *J. Neuropathol. Exp. Neurol.*, 36, 300, 1977. With permission.)

which leave the tibial nerve at the knee causes degeneration to appear in the sciatic nerve before similar changes appear more distally in the posterior tibial nerve at the ankle — a paradox which led to an early, erroneous claim that hexacarbon neuropathy was not a dying-back type of neuropathy.[188] After prolonged intoxication, these independent, ascending waves of nerve degeneration contribute to the overall pattern of damage in the sciatic nerve. Nerve fiber changes ascend the sciatic nerve and, in severely affected animals, may be found in the dorsal and ventral roots. These areas display a spectacular pattern of demyelination in animals with long-standing axonal

Table 19
PATHOLOGY AND DISTRIBUTION OF PERIPHERAL NERVE DAMAGE IN RATS WITH HEXACARBON NEUROPATHY

Histological features	
Giant axonal swellings	Characteristic
Paranodal demyelination	Common
Fiber corrugation	Common
Intercalated, remyelinated segments	Frequent and late
Chains of myelin ovoids	Frequent and late
Axonal regeneration and myelination during intoxication	Frequent and late
Ultrastructural features	
10-nm neurofilament proliferation in axons	Pronounced focally
Neurotubules grouped together, sometimes forming a core	Frequent
Honeycombed, interdigitated Schwann cell/axon profiles	Very common
Mitochondrial remnants filled with dense particles	Common
Distribution of degeneration	
Early, multifocal, distal distribution	Pronounced
Proximal, temporal spread up affected nerve trunks	Characteristic
Involvement of sensory plantar nerve branches to digits	Late
Involvement of plantar muscle nerves	Early
Involvement of tibial nerve branches to calf muscles	Earliest
Tibial nerve branches to calf muscles most vulnerable	Characteristic
Involvement of dorsal roots and dorsal root ganglia	Minimal
Involvement of ventral roots	Involved late
Involvement of anterior horn cells	Minimal
CNS changes present when first PNS changes detected	Yes

Modified from Spencer, P.S. and Schaumburg, H.H., *J. Neuropathol. Exp. Neurol.*, 36, 300, 1977. With permission.

disease.[90] Dorsal root ganglion neurons and anterior horn cells usually are spared. Sensory and motor nerve terminal degeneration seems to commence after changes are advanced in the distal, nonterminal part of the fiber.[188,225] Atrophy of muscle fibers follows denervation.

(iii.) Distribution of Central Nervous System Damage

The long ascending and descending spinal cord tracts are first affected: degeneration appears in the rostral part of the gracile tract (and subsequently in adjacent parts of the shorter cuneate tract) and the caudal part of lateral, ventrolateral, and ventromedial tracts (Figure 19) (Table 20). As the disease advances, degenerative changes spread retrograde; dorsal column changes appear in the cervical and then the thoracic segments of the spinal cord, whereas, in descending tracts, changes first appear in lumbar segments and later in thoracic regions.[226] Degeneration is also found in the cerebellar white matter and distal optic nerve corresponding to distal breakdown of spinocerebellar and optic tracts, respectively; giant axonal changes have also been described in

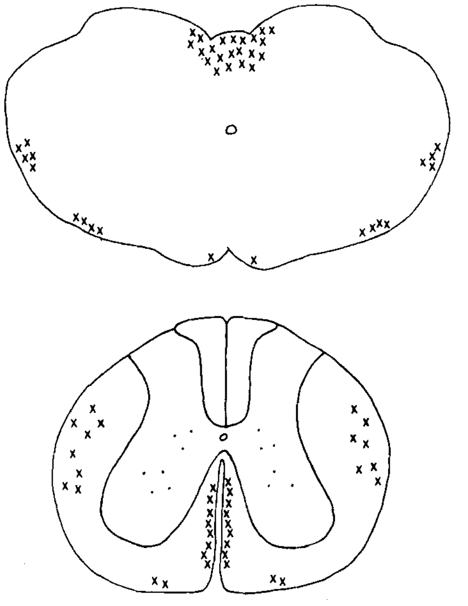


FIGURE 19. Diagrams of cross sections of medulla oblongata (upper) and lumbar spinal cord (lower) showing areas of maximum nerve fiber vulnerability in the gray (•) and white (X) matter.

the mammillary bodies.[193] Spastic gait and impaired vision and memory, respectively, are believed to represent the human correlates of this pathological substrate found in animals.[194,195]

(iv.) Sequence of Nerve Fiber Changes

The evolution of nerve fiber degeneration has been determined by examining single isolated nerve fibers and nerve sections from rats and cats at various stages of intoxication. Axonal degeneration seems to begin by increases in the number of axonal 10 nm neurofilaments[137] which accumulate distally in swellings on the proximal sides of nodes of Ranvier (Figure 20).[225] Paranodal swelling occurs concomitantly with shrinkage and myelin corrugation of the adjacent, more distally located internode. The enlarged nodal and paranodal axons displace the paranodal myelin sheaths, leaving denuded axonal swellings in the vicinity of nodes of Ranvier. Schwann cells appear at the denuded regions, the axon swelling attenuates, and remyelination of the denuded axon then takes place. At some axonal swellings, axonal reconstitution and remyelination fail to occur; instead, the length of nerve fiber between the swelling and nerve terminal undergoes a more-or-less simultaneous breakdown, as if the fiber had been transected at the position of the axonal swelling (Figures 21 and 22).[225]

The hallmark of hexacarbon neuropathy is the presence of giant axonal swellings containing filaments ultrastructurally and immunocytochemically indistinguishable from normal 10 nm neurofilaments (Figures 23-25). This pattern is not a specific response to hexacarbon intoxication; for example, giant axonal swellings are also characteristic of carbon disulfide neuropathy and may be seen in slowly evolving experimental acrylamide neuropathy.[221] Neurofilaments are composed of three major polypeptides (200 daltons, 145 daltons, and 68 daltons) which move constitutively along the nerve in the slowest phase (1mm/day) of axonal transport. Their accumula-

Table 20
TYPE AND DISTRIBUTION OF CENTRAL NERVOUS SYSTEM DAMAGE IN CATS WITH HEXACARBON NEUROPATHY

Histological features	
Scattered giant axonal swellings	Characteristic
Fiber breakdown late	Characteristic
Ultrastructural features	
10-nm neurofilament proliferation in axons	Pronounced
Honeycomb, interdigitated oligodendrocyte/axon profiles	Present
Distribution of pathology	
Distal in long ascending and descending tracts initially	Characteristic
Proximal in long ascending and descending tracts later	Characteristic
Involvement of shorter tracts later	Characteristic
Involvement of cerebellar vermis	Early in white matter
Involvement of mammillary body	Late
Involvement of lateral geniculate body	Late
Involvement of medulla oblongata	
Dorsal spinocerebellar tracts	Characteristic
Ventro-lateral (vestibulospinal?) tracts	Late
Pyramidal tracts	Late
Gracile tracts and nuclei	Early
Cuneate tracts and nuclei	Late
Involvement of spinal cord	
Ascending tracts	
Dorsospinocerebellar tracts	T6 and rostrally
Gracile tracts	Frequent at C1—C6
Cuneate tracts	C1 only
Descending tracts	
Lateral columns (corticospinal and vestibulospinal?)	C4, increasing in severity to L5
Ventrolateral and ventromedial (vestibulospinal and reticulospinal?)	C4, increasing in severity to L5
Spinal gray	
Rexed laminae V through dorsal VII	Preterminal and terminal changes at C4—S3
Rexed laminae ventral VII	C6—L5

Modified from Spencer, P.S. and Schaumburg, H.H., *J. Neuropathol. Exp. Neurol.*, 36, 300, 1977. With permission.

tion proximal to nodes of Ranvier and sparseness distal to these regions is suggestive of a progressively multifocal blockade of the slowest phase of axonal transport. Many other organelles accumulate in degenerating axons including mitochondria, some filled with glycogen granules, decorated particles, and clear-core vesicles of synaptic-vesicle proportions.[225] Many of these "trapped" or effete organelles seem to be sequestered and selectively removed from the axon by invagination of a sheet of the adaxonal

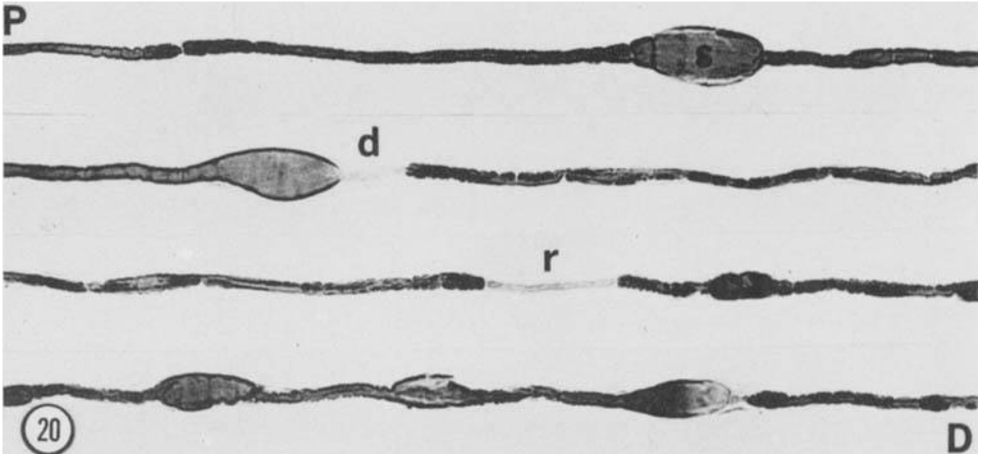


FIGURE 20. Figures 20 to 22 show sequence of PNS nerve fiber degeneration in murine 2,5-HD neuropathy. (P) and (D) mark the proximal and distal positions respectively of each nerve fiber. Bright field micrographs of tissue fixed in glutaraldehyde and osmium tetroxide, dehydrated and embedded in epoxy resin. Figure 20 shows consecutive lengths of a single teased myelinated nerve fiber displaying multifocal giant axonal swellings (s), paranodal demyelination (d), segmental remyelination (r) and myelin corrugation. (Magnification × 150.) (From Spencer, P. S. and Schaumburg, H. H., *J. Neuropathol. Exp. Neurol.*, 36, 276, 1977. With permission.)

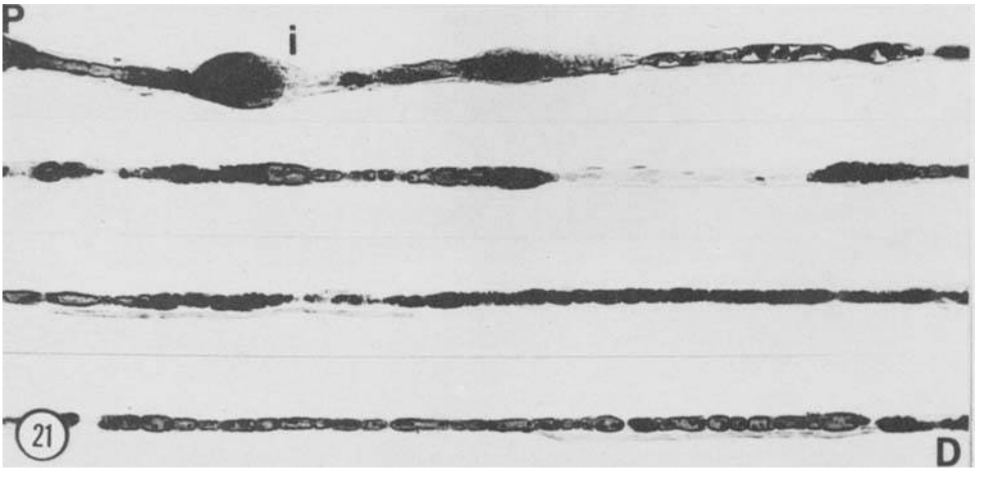


FIGURE 21. Consecutive lengths of single teased fiber undergoing complete distal breakdown. The interface (i) between the distal, degenerating portion and the proximal preserved portion is marked by a massively swollen axon. Early stages of ovoid formation are present distal to the swollen region. (From Spencer, P. S. and Schaumburg, H. H., *J. Neuropathol. Exp. Neurol.*, 36, 276, 1977. With permission.)

Schwann cell (PNS) or oligodendrocyte (CNS) cytoplasm.[188,221] Neurotubules are often segregated with normal mitochondria and endoplasmic reticulum into channels penetrating the mass of neurofilaments (Figure 25).

Schwann cells also show structural changes in hexacarbon neuropathy, and it has been suggested that the changes are not merely reactive.[172] However, proximal and distal parts of individual nerve fibers would have to be examined to distinguish a primary Schwann cell event from a reaction to distal axonal injury. Schwann cell abnor-

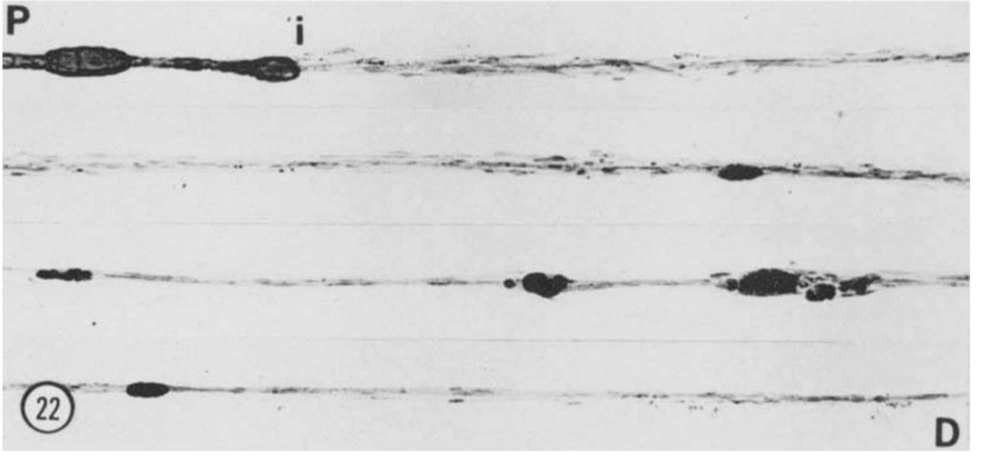


FIGURE 22. Single teased myelinated fiber showing advanced degeneration. Note the interface (i) between the proximal myelinated fiber with multifocal swellings, and the distal, degenerated portion. (From Spencer, P. S. and Schaumburg, H. H., *J. Neuropathol. Exp. Neurol.*, 36, 276, 1977. With permission.)

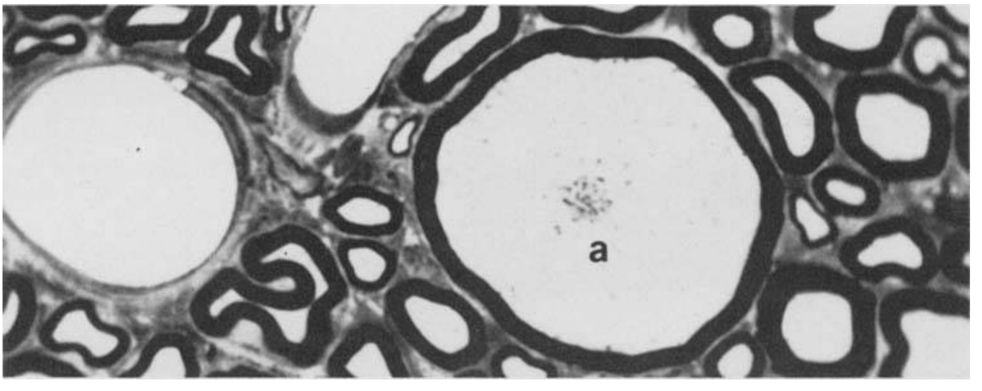


FIGURE 23.. Giant axonal swelling (a) of a myelinated nerve fiber in the sciatic nerve of rat with hexacarbon neuropathy. (One micrometer epoxy cross sections stained with toluidine blue; light micrograph; magnification 1600.)

malities include increased cytoplasm, focal enlargements containing cytoplasmic filaments which lack side arms, vesicular complexes, lamellated bodies, osmiophilic droplets,[172] and filament-filled inner loops of Schwann cells.[172,230]

(v.) Recovery From Nerve Fiber Damage

Three structural mechanisms seem to participate in the recovery process: reconstitution, remyelination, and regeneration. Reconstitution occurs when a neurofilament-filled swollen axon or axon terminal (Figure 24) shrinks to a more normal size and avoids the cycle of breakdown and repair. Reconstitution of a swollen and demyelinated paranodal axon allows the axon to become remyelinated. Regeneration refers to the process of axon sprouting from the end of the intact portion of a degenerated fiber, longitudinal growth, and reestablishment of end-organ connection. All three processes can probably occur during intoxication. Postexposure regeneration of peripheral nerve fibers is responsible for the reestablishment of muscle strength and sensory function in animals and man recovering from hexacarbon neuropathy.

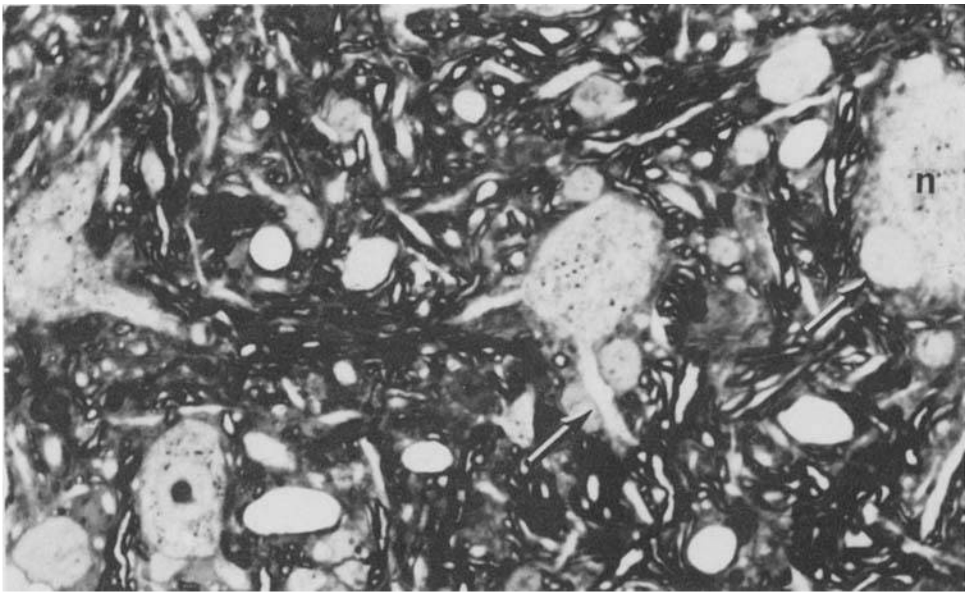


FIGURE 24. Terminal axonal swellings (arrows) abutting neurons (n) and their dendrites in the sacral spinal cord of a cat with severe M*n*BK neuropathy. (One micrometer epoxy cross sections stained with toluidine blue; light micrograph; magnification × 550.)

h. Tissue Culture Studies

(i.) Organotypic

A model of hexacarbon neuropathy has been established using combination tissue cultures[228,250,258] (Table 21). These are composed of spinal cord, dorsal root ganglia, motor and sensory nerves, and striated muscle which develop structural and functional associations in vitro analogous to those which normally occur between these components in vivo. Cultures were maintained in a lying drop of nutrient fluid which was replaced twice a week. After the culture was mature (8 to 12 weeks in vitro), as evidenced by large-diameter myelinated fibers innervating spontaneously twitching muscle fibers, the hexacarbon compound could be introduced into the nutrient fluid. All six neurotoxic hexacarbon metabolites produced distal axonopathy in vitro, but pathological changes were most closely followed in the case of 2,5-HD. The following sequence of events was recorded in living motor nerve fibers during periods of up to 1 year of intoxication and recovery: 1. giant axonal swellings filled with neurofilaments (Figure 25) developing on the proximal sides of distal nodes of Ranvier, 2. associated paranodal myelin retraction, 3. proximal spread of paranodal swelling, 4. distal degeneration and muscle denervation, and 5. remyelination of denuded proximal internodes. These events, summarized in Figure 26, reproduced the pattern and timing of slowly developing hexacarbon neuropathy found in subchronically intoxicated animals.[250]

(ii.) Neuroblastoma Cell Cultures

M*n*BK, *n*-hexane, and 2,5-HD, but not MiBK or MEK, produced a reproducible series of cytotoxic effects in neuroblastoma cells and caused a dose-dependent inhibition of cellular proliferation[199] (Figure 27). Cytotoxic effects included multinucleation, cytoplasmic vacuolation, decrease in the extension or maintenance of cell processes, and fine structural alterations of the rough endoplasmic reticulum. One striking fea-

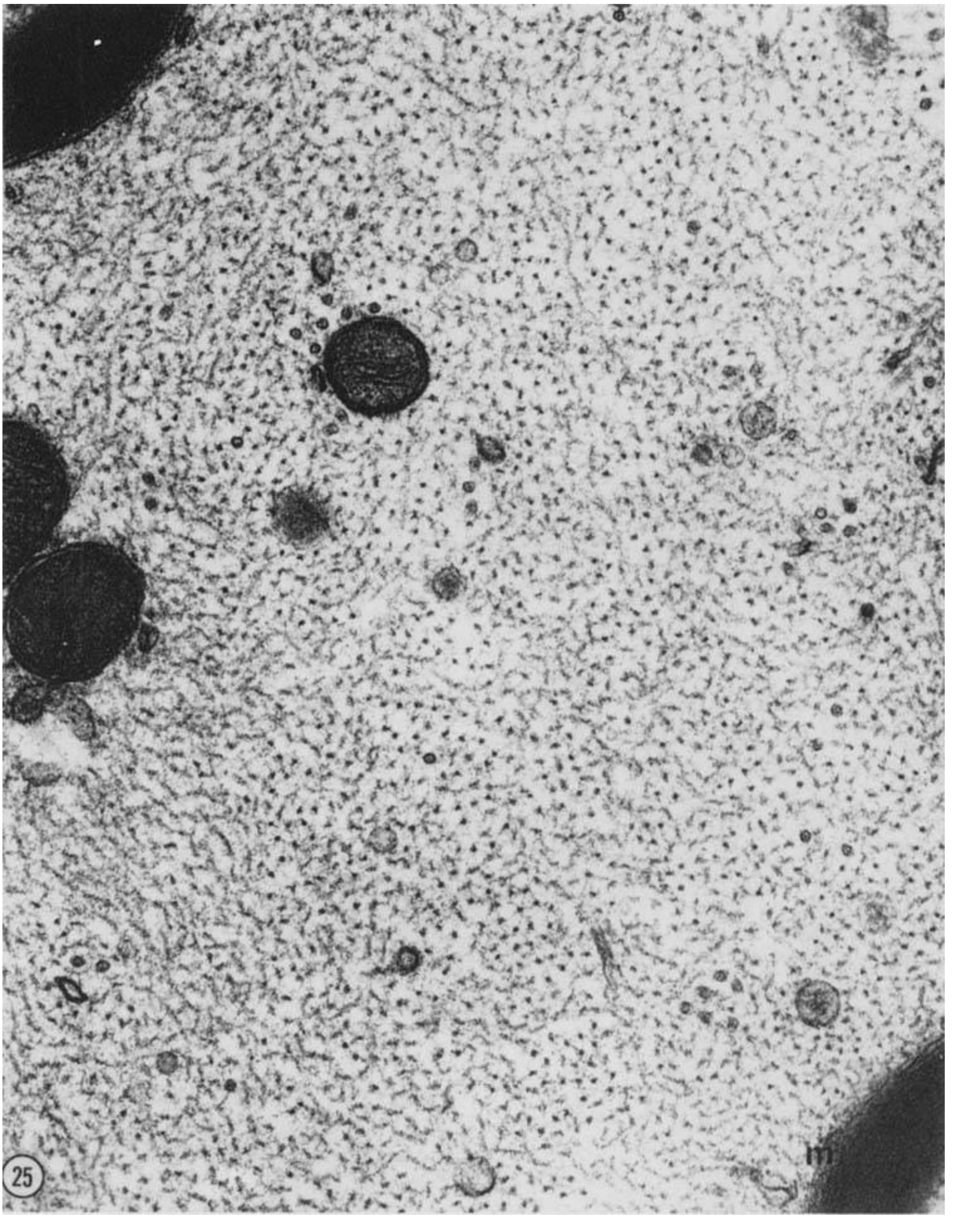


FIGURE 25. Part of a swollen myelinated axon containing a network composed of an abnormally large number of 10 nm neurofilaments. Organotypic cord-ganglion-muscle combination tissue culture chronically exposed to 2,5-HD. (Thin epoxy cross section stained with uranyl acetate and lead citrate; electron micrograph; magnification × 66,000.)

ture was an absence of neurofilament proliferation, which lends indirect support to the idea that neurofilaments accumulate focally in axons[225] rather than proliferate.[137]

i. Axonal Transport Studies

Mendell and co-workers[137] studied fast axoplasmic transport in rats exposed to M*n*BK by injecting lumbar dorsal root ganglia or spinal cord anterior horns with ^{3}H-

Table 21
SUMMARY OF TISSUE CULTURE STUDIES ON EFFECTS OF HEXACARBON COMPOUNDS

Type	Compound	Dose	Duration	Effect	Ref.
CNS-PNS-muscle combinations	2,5-HD	∿ 2.6 m *M*	4 weeks—1 year plus	Duplication of giant axonal distal nerve fiber changes and degeneration, with proximal demyelination and remyelination more proximally	228
CNS-PNS-muscle combinations	*n*-Hexane, M*n*BK, 2,5-HD, 5-OH-2-H, 2,5-HDiol, 2-hexanol	2.8 m*M*	5—6 weeks	Giant axonal changes in all between 2-5 weeks; relative potency: 2,5-Hd ≈ M*n* Bk> 5-OH-2-H = 2,5-HDiol> 2-hexanol ≈ n-hexane	251
Cord-ganglia	M*n*BK		3 months	Unspecified changes	83
	2,5-HDiol	10^{-3}g/m*l*	2-3 days	Focal axonal swellings containing neurofilaments; later, Schwann cell and neuronal degeneration	258
Neuroblastoma	*n*-Hexane	0.1%—2%	Days	Reproducible series of cytotoxic effects and inhibition of proliferation at higher doses; LD_{50} = 2%	199
Neuroblastoma	M*n*BK, 2,5-HD	0.02%—0.16%	Days	Cytopathological and growth inhibiting effects; 2,5-HD 25% more active than M*n*BK	199
Dorsal root ganglia (dissociated)	M*n*BK, 2,5-HD	800—1600 μg/m*l*	2—3 days	Nonspecific cytotoxic effects	83
Dorsal root (dissociated)	M*n*BK, 2,5-HD	200—400 μg/m*l*		Lipid granulation	83
Neuroblastoma Hela cells Glioma cells	M*n*BK, 2,5-HD	200—1200 μg/m*l*	Up to 11 days	Lipid vacuoles in cytoplasm; extension of neurites with 2,5-HD; depression of uridine uptake by 2,5-HD	83
Neuroblastoma	MiBK	0.1%; 0.2%; 0.5%	10 days	No effect on cell structure; inhibition of proliferation; cell death	83

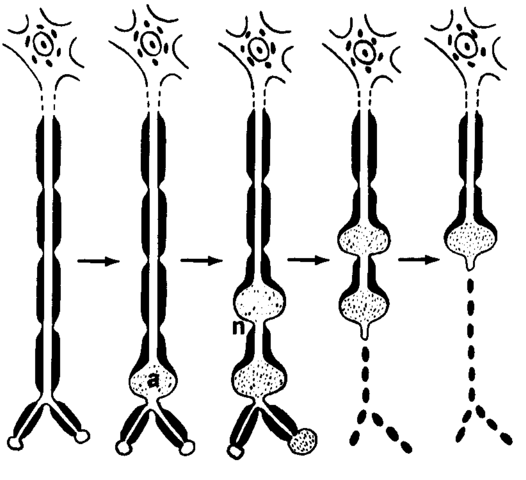


FIGURE 26. Diagram of the process of retrograde nerve fiber degeneration (distal dying-back axonopathy) in myelinated nerve fibers innervating muscle in cord-ganglia-muscle combination tissue cultures. Axonal swellings filled with neurofilaments (a) develop on the proximal sides of nodes of Ranvier (n). Complete fiber breakdown later occurs distal to a swelling (right pair).

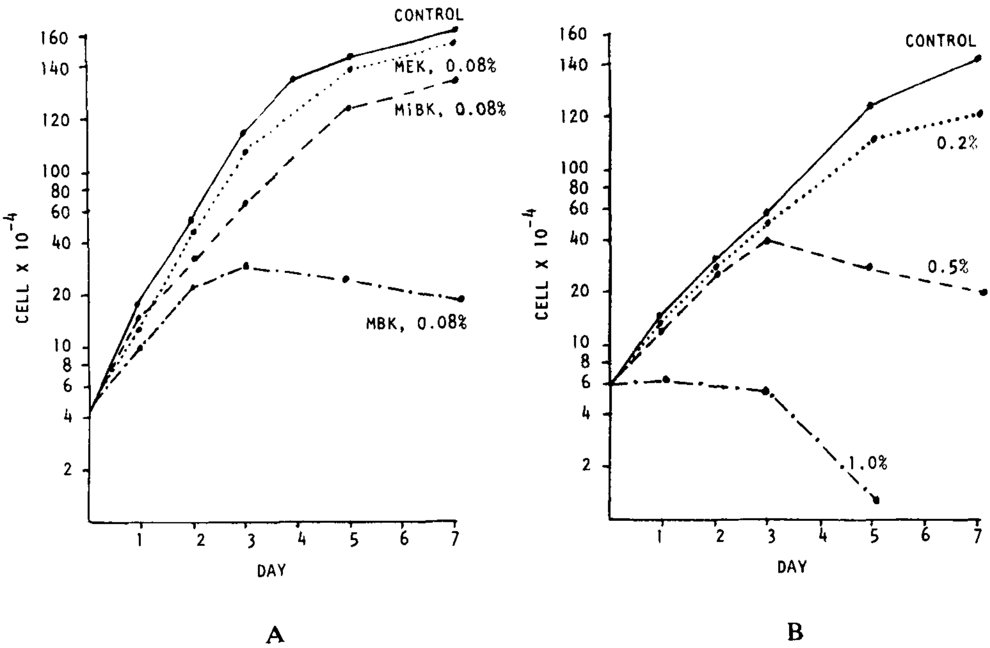


FIGURE 27. (A) Effects of M*n*BK (MBK), MiBK and MEK on cell multiplication in neuroblastoma cultures. (B) Effects of varying concentration of *n*-hexane on cell multiplication in neuroblastoma cultures. Cell numbers are expressed as number of attached cells per 25 cm^2 of the flask. (From Selkoe, D. J., Luckenbill-Edds, L., and Shelanski, M.L., *J. Neuropathol. Exp. Neurol.*, 37, 768, 1978. With permission.)

leucine and following the wave of label during its passage down the sciatic nerve. The mean downflow rate for motor nerve fibers was significantly impaired, and the degree of impairment correlated with the severity of the neuropathy (Figure 28). The rate of fast axonal transport in sensory fibers was also reduced. Griffin and co-workers[91] examined the distribution of fast transported material in serial cross-sections of single nerve fibers and demonstrated a focal blockade in the region of the paranodal axonal swelling (Figure 29). These studies suggest that the impediment of fast axoplasmic transport is related either to the increased numbers of neurofilaments, which might produce areas of axonal blockage,[89] or to a blockade of both rapidly and slowly transported components at the node of Ranvier.[218]

j. Effects on 24 nm Neurotubules

Neurotoxic hexacarbons do not seem to affect neurotubules. Quantitative morphological determinations of the number of neurotubules per unit cross-sectional myelin area of peripheral nerve fibers are comparable in normal and M*n*BK-intoxicated rats.[137] *n*-Hexane, M*n*BK, and 2,5-HD have no effect on the polymerization of tubulin subunits and do not cause microtubule disassembly.[199] ^{3}H-colchicine binding activity of brain and sciatic nerve is also normal.[141]

k. Effects of Direct Application to Nerves

Application of M*n*BK directly to the sciatic nerve results in a circumferential distribution of Wallerian degeneration.[137] Similar results are obtained with 2,5-HD and 2,4-HD although, with the systemically neurotoxic diketone 2,5-HD, enlarged demyelinated and neurofilament-filled axons also appear several days after the application of the toxin.[171] This result suggests that high levels of 2,5-HD can specifically block neurofilament transport, and may offer insight into the mechanism of giant axonal swell-

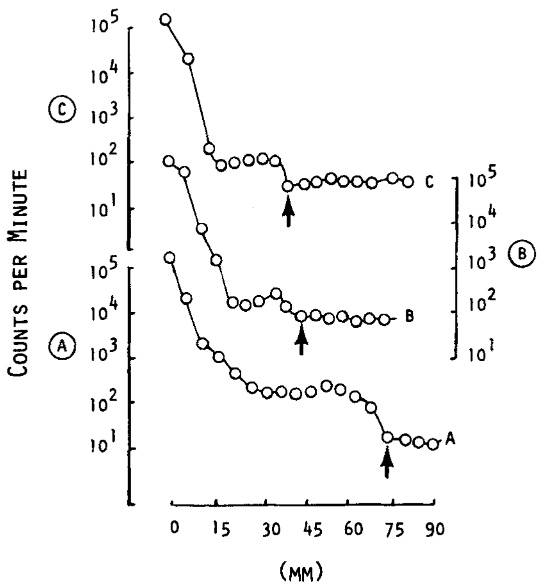


FIGURE 28. Changes in axonal transport of materials in sciatic nerve of rats with hexacarbon neuropathy. Graphs show pattern of down flow of transported label 4 hr after injection of (^{3}H)leucine into the L_4-L_6 segments of the ventral horn of the spinal cord. The activity present in 5 mm (MM) segments of cord, roots, and sciatic nerve is given on the ordinate in logarithmic divisions in cpm. (A) represents a normal control animal, (B) a partially paralyzed rat, and (C) a severely paralyzed rat. (Redrawn from Mendell, J.R., Sahenk, Z., Saida, K., Weiss, H. S., Savage, R., and Couri, D., *Brain Res.*, 133, 107, 1977. With permission.)

ing seen in systemic intoxication. The observed wallerian degeneration appears to be a nonspecific solvent effect on the nerve fibers.

I. Biochemical Studies

(i.) Energy Transformation (Glycolysis)

This area of biochemical investigation[218] was stimulated by neuropathological and axonal transport studies[91,225] which revealed the special vulnerability of nodes of Ranvier, where the dual functions of nerve impulse conduction and axonal transport putatively make these regions large consumers of chemical energy. Sabri and co-workers[186] found that crystalline and endogenous PNS and CNS glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a major regulatory enzyme in glycolysis on which fast axonal transport is known to depend,[187] was inhibited in vitro by M*n*BK and 2,5-HD as a function of hexacarbon concentration and duration of preincubation of toxin and enzyme in vitro. At a hexacarbon concentration of 50 m*M*, with 10 min preincubation at 37°C, M*n*BK was approximately 5 times more active on GAPDH from rat brain (approximately 100% inhibition) than 2,5-HD (approximately 20% inhibition). Graham and Abou-Donia[88] have shown that the inhibition of GAPDH by 2,5-HD is irreversible, proceeding via a reversible enzyme-inhibitor intermediate. Sabri and colleagues[185] subsequently demonstrated hexacarbon inhibition of crystalline and brain phosphofructokinase (PFK), the major control step in glycolysis. Under conditions similar to those used for the assay of GAPDH, 2,5-HD produced a twofold greater inhibition than equimolar concentrations of M*n*BK (Table 22). The effects of

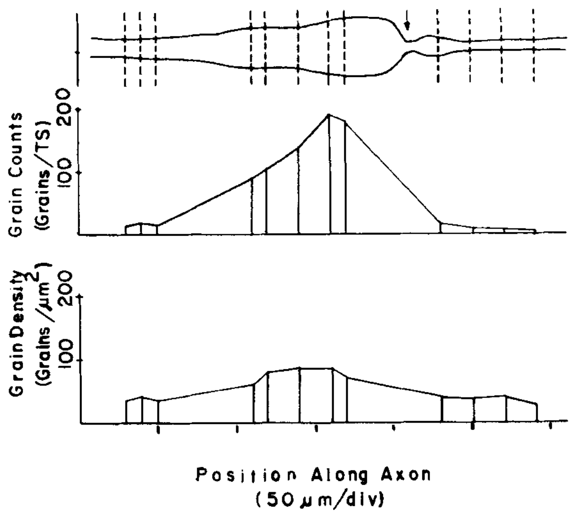


FIGURE 29. Abnormalities in the fast axonal transport of label along (left to right) a single myelinated nerve fiber (top) focally swollen with neurofilaments proximal to a node of Ranvier (arrow). ^{3}H leucine was injected into the L_5 ventral horn of a rat with 2,5-HDiol neuropathy and the incorporated radioactivity transported down the sciatic nerve past the position of the swelling. Electron autoradiographs of skip serial transverse sections (TS) were prepared and the grain number (center) and density per unit area (lower) plotted in relation to each sampled position on the fiber. Grains accumulated in the swollen region as illustrated. (From work described in Griffin, J. W., Price, D. L., and Spencer, P. S., *J. Neuropathol. Exp. Neurol.*, 36, 603, 1977. With permission of Dr. J. Griffin.)

Table 22
EFFECT OF 2,5-HD AND M*n*BK ON THE ENZYME ACTIVITY OF THE CRYSTALLINE GLYCOLYTIC ENZYMES GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE (GAPDH) AND PHOSPHOFRUCTOKINASE (PFK)

Sample	GAPDH[a] (µmol of NADH formed/min/mg of protein)	PFK[b] (µmol of FDP formed/min/mg of protein)
Enzyme + Buffer (Control)	16.77 ± 1.1	144.5 ± 5.6
Enzyme + 2,5-HD	7.17 ± 0.67	32.0 ± 1.2
Enzyme + 2,5-HD + DTT	14.33 ± 1.5	142.5 ± 4.7
Enzyme + M*n*BK	2.87 ± 0.19	126.4 ± 3.7
Enzyme + M*n*BK + DTT	19.64 ± 3.4	154.6 ± 7.3

[a] 50 µℓ of crystalline GAPDH (0.7 mg protein/mℓ) were preincubated with 17 m*M* 2,5-HD or 17 m*M* M*n*BK for 20 min at 37°C with and without the addition of 0.02 M dithiothreitol (DTT).

[b] 100 µℓ of crystalline PFK (1 mg protein/mℓ) and 100 µℓ of 2,5-HD (80 m*M*) or M*n*BK (50 m*M*) were incubated at 37°C for 20 min with and without the addition of DTT. In the control experiment, 2,5-HD or M*n*BK was replaced by an equivalent volume of buffer. Enzyme activity of GAPDH was measured according to the method described by Sabri and Ochs;[187] PFK activity was determined as described by Sabri and co-workers.[185]

Table 23
EFFECT OF *n*-HEXANE AND ITS METABOLITES ON CRYSTALLINE ENZYMES GAPDH, PFK, AND LDH

	μmol of NADH oxidized or reduced/mg of protein		
Hexacarbon	**GAPDH**	**PFK**	**LDH**
Buffer control	9.9 ± 1.3	247.8 ± 10.2	80.6
n-Hexane	9.5 ± 0.4	256.2 ± 13.6	91.4
2-Hexanol	8.5 ± 0.7	246.3 ± 11.6	75.3
2,5-Hexanediol	7.4 ± 0.4	262.8 ± 22.6	88.7
5-Hydroxy-2-hexanone[a]	8.5 ± 0	194.4 ± 16.5	90.5
Methyl *n*-butyl ketone	3.8 ± 0	175.6 ± 21.6	86.0
2,5-Hexanedione	5.4 ± 0	116.7 ± 17.7	80.6

Note: Crystalline GAPDH, PFK and LDH (1 mg/mℓ) were each preincubated for 20 min at 37°C with each solvent prepared in appropriate buffer at a final concentration of 25m*M*. In the control the hexacarbon was replaced by an equivalent amount of buffer. Enzyme activities of GAPDH and PFK were determined as described in the footnotes of Table 22. LDH activity was determined as described by Sabri and coworkers.[186]

[a] Courtesy of Dr. G. D. DiVincenzo.

the six interrelated hexacarbon metabolites are shown in Table 23. While the concentrations of toxin used in these experiments are significantly higher than those likely to be encountered under physiological conditions, much lower concentrations (in the range 1 m*M*) also result in significant (25% for GAPDH) inhibition of enzyme activity, if the period of preincubation of enzyme and toxin is prolonged from minutes to hours (2 hr).[217] Dithiothreitol, a sulfhydryl providing reagent, protected GAPDH and PFK but failed to restore activity once the enzyme was inhibited. The neurotoxic hexacarbons failed to inhibit the enzyme activity of lactic dehydrogenase, succinic dehydrogenase, succinic dehydrogenase, or transketolase.[217]

2,5-HD causes a slight loss of respiratory control in liver mitochondria.[29]

The activity of GAPDH and PFK was unaffected by the neurologically inactive compounds 2,4-hexanedione, 1,6-hexanediol, and acetone.

(ii.) Lipid Metabolism

Gilles and associates[82] found in Fischer 344 rats with advanced neuropathy, a decreased incorporation of (1-^{14}C) acetate into triacylglycerides (32%), total sterols plus diacylglycerides (54%), C-30 hydrocarbons (55%) and digitonin-precipitable sterols (55%). It is not known whether these changes in lipid metabolism are associated with the causation of nerve fiber breakdown, or merely mirror the nerve fiber degeneration.

(iii.) Chomophore Generation

Graham[87] has demonstrated that 2,5-HD forms a stable chromophore in reaction with ε-amino acids of proteins (GAPDH, bovine serum albumin, chicken egg-white ovalbumin) and poly-L-lysine. The absorption spectrum (λ_{max} = 475nm) and fluorescent properties ($\lambda_{excitation}$ = 370 to 380nm; $\lambda_{emission}$ eq 435 and 545 nm) are characteristic of a conjugated imine or Schiff base. An orange chromophore developed in the presence of 2,5-HD but not with acetone. The rate of appearance of the chromophore increased with 2,5-HD concentration, temperature, and incubation time. Rapid reduction was observed after the addition of $NaBH_4$ or $Na_2S_2O_4$.

6. Relationship of Chemical Structure to Neurotoxic Activity

The six metabolically interrelated hexacarbons listed in Section VI.B.5 all produce neuropathy when administered to experimental animals. The hierarchy of neurotoxic potential in the rat, i.e., 2,5-HD > 5-OH-2-H > 2,5-HDiol > M*n*BK > 2-hexanol > *n*-hexane, is in proportion to the peak serum values of 2,5-HD that each compound generates.[156]

The relative neurotoxic activity of the six interrelated hexacarbon compounds was also investigated in a tissue culture model of hexacarbon neuropathy (see Section VI.B.5.h). Veronesi and co-workers[251] traced the fate of the applied neurotoxin by examining the nutrient fluid by GC-MS before and after exposure to the tissue culture. 2,5-HD appeared in the exhausted nutrient fluid with each metabolite, and 2,5-HD and M*n*BK were the most potent agents in vitro.

The specific molecular configuration of hexacarbon compounds that produce peripheral neuropathy has been investigated in experimental animals. Spencer and colleagues[16,223,224] showed that Sprague-Dawley rats exposed to the hexacarbon aliphatic diketones 2,4-hexanedione and 2,3-hexanedione, or to the heptacarbon ketones 3,5-heptanedione and 2-heptanone, failed to develop neuropathy, in contrast to 2,5-HD and 2,5-HDiol. These data supported the suggestion of DiVincenzo and co-workers[55] that 2,5-hexanedione is a primary neurotoxic agent, the property being related to the γ-spacing of the carbonyl groups. Spencer and associates emphasized the importance of examining for neurotoxic properties the commercially unavailable diketone 2,5-heptanedione, the 7-carbon γ-diketone analogue of 2,5-hexanedione, and 2,6-heptanedione, the presumptive β-oxidation product of *n*-heptane (an alkane formerly alleged[245] to possess neurotoxic properties). 2,5-Heptanedione and 2,6-heptanedione were subsequently synthesized by the Rochester group and administered to Charles River rats by gavage.[154] 2,6-Heptanedione given in ascending doses from 500 to 1000 mg/kg/day for 13 weeks did not produce neuropathy, whereas 2,5-heptanedione at doses of 1000 and 2000 mg/kg/day produced neuropathy with neuropathological alterations identical to those produced by 2,5-hexanedione. 3,6-Octanedione also produced an experimental giant axonal neuropathy.[155] Although the type of neurotoxic change appeared identical with the 6-, 7-, and 8-carbon γ-diketones, the neurotoxic potency decreased with increasing length of the carbon chain.

VII. THEORIES OF HEXACARBON NEUROTOXICITY

A. Interruption of Energy Metabolism

Defective energy metabolism is one explanation for the mechanism by which neurotoxic hexacarbons produce nerve fiber degeneration. The idea is attractive because a number of other neurotoxic compounds and vitamin deficiency states which produce peripheral neuropathy may affect various loci in energy transformation pathways, the common net effect being defective synthesis of chemical energy (Figure 29). Two theories point to a defect in energy transformation: one[218] implicates glycolytic enzymes, the second,[196] a coenzyme required for pyruvate metabolism. Another hypothesis[49] suggests that γ-diketones behave as ionophores and thereby divert energy metabolism away from the maintenance of axonal transport and impulse conduction. All three ideas require the toxin to act directly on the nerve fiber (reported in Section VI.B.5.i).

1. Coenzyme Interference

Schoental and Cavanagh[196] suggested that thiamine might be rendered inactive if

2,5-HD were to fit into the available space between a thiol in the apoenzyme and the amino group of the thiamine molecule. Such a firm bridge would destroy thiamine function by inhibiting the reversibility of the enzyme-substrate binding. This would destroy its function as a cofactor for pyruvate decarboxylase which in turn would disrupt energy metabolism.

2. Enzyme Inhibition

Spencer and colleagues[218] suggested that neurotoxic compounds such as carbon disulfide, acrylamide and hexacarbons might bind to enzymes distributed along nerve fibers, causing a dose-dependent inhibition of enzyme activity. Kurita[119] postulated a similar mechanism. The neuronal perikaryon, which is responsible for maintaining enzyme in the normal axon, would be unable to resupply the elevated demand for enzyme in the nerve fiber exposed to neurotoxic hexacarbons. Proximal portions of the nerve fiber would receive an adequate resupply of enzyme, such that supplies to the distal axon would become deficient. An inadequate supply of glycolytic enzymes arriving in the distal portion of the fiber would lead locally to diminished energy supplies, axonal transport abnormalities, and nerve fiber degeneration.

3. Ionophore Hypothesis

Stereological models of aliphatic diketones reveal the neurotoxic 1,4-diketones may have unbonded gaps between adjacent carbonyl groups identical to those displayed by the (ester) carbonyls of the potassium ionophore valinomycin.[49] Nonneurotoxic diones such as 2,4-hexanedione have much longer unbonded gaps (Figure 30). If γ-diketones such as 2,5-HD were to act as ionophores at the nodal axonal membrane, striking abnormalities might develop in ion balance and energy conservation.

The ionophore hypothesis thus explains disruption of nerve fiber integrity by metabolic interference at the level of the surface membrane of the axon. Location of the hexacarbon in nerve membranes might account for the increased membrane fluidity reported[141] to occur after systemic treatment with 2,5-HD.

B. Neurofilament Binding

The hallmark of neuropathies produced by neurotoxic hexacarbons, as well as by acrylamide and carbon disulfide, is the formation of axonal swellings containing an abnormally large number of 10 nm neurofilaments. Savolainen[189] has suggested that hexacarbons, like carbon disulfide and acrylamide, might bind to neurofilaments and affect their normal (unknown) function(s). Graham[87] has proposed that 2,5-HD reacts with ε-amino groups of neurofilaments to form a conjugated imine.

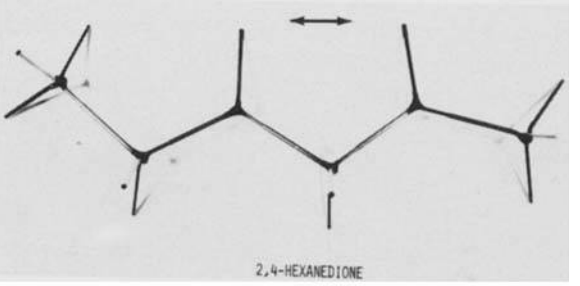
Any theory that postulates a direct effect on neurofilaments must explain their initial accumulation in the distal part of the nerve fiber. One possibility is that neurofilaments are denatured slowly and progressively during their course along the axon, so that eventually they are unable to be transported further. The neurofilaments exposed for the longest time, i.e., those at the distal end of the longest nerve fibers would be the first to be affected. With time, neurofilaments in more proximal regions would become similarly affected.

C. Metal Chelation

Goldstein[74] has suggested M*n*BK and 2,5-HD produce cell toxicity (unspecified) by chelating mitochondrial calcium.

VIII. CONCLUDING REMARKS

The subject of hexacarbon neuropathy comprised less than 1% of J.B. Cavanagh's



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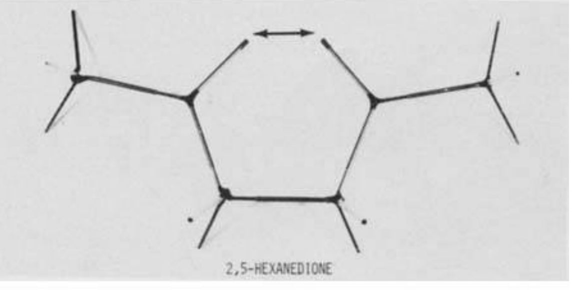

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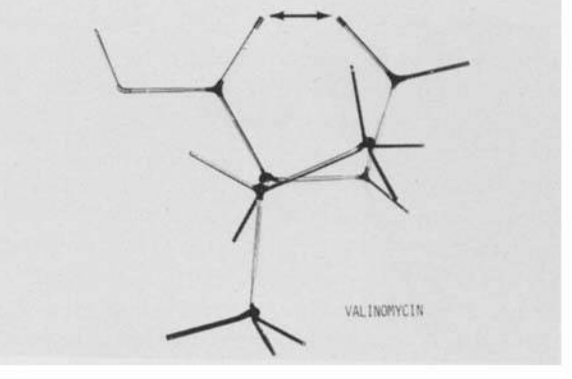

C

FIGURE 30. Possible conformations for the sterochemical structures of (A) 2,4-hexanedione, (B) 2,5-HD, and (C) the ionophore valinomycin.[49] Double-headed arrows show spacing between adjacent carbonyl groups is identical for 2,5-HD and valinomycin; this is required in the latter to form coordination bonds with ions, thereby allowing the molecule to act as an ionophore.

1973 review article[41] on peripheral neuropathies. The foregoing summarizes the intensive investigation of hexacarbon neurotoxicity, most of which has occurred during the past 6 years. This period has been marked by a close collaboration of scientists and physicians from the toxicological and neurological communities. As a result of their combined efforts, we may stand at the brink of understanding for the first time the precise molecular mechanisms by which a compound can elicit axonal degeneration. Such information appears likely to lay the groundwork for determining the causation, prevention, and treatment of many other polyneuropathies.

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