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TOXICITY AND METABOLISM OF THE NEUROTOXIC HEXACARBONS *n*-HEXANE, 2-HEXANONE, AND 2,5-HEXANEDIONE

D. Couri and M. Milks

Ohio State University, Division of Toxicology, Columbus, Ohio 43210

INTRODUCTION

Until recent years, ketone solvents have generally been considered safe, and thought to pose little hazard from their use in industry. Considering ketones as a class, the toxicity to man largely involves irritation to mucous membranes which by itself will limit further exposure and often preclude systemic toxicity. This is not to say, however, that all ketones are innocuous. Upon repeated or prolonged exposure to ketones, symptoms might include headache, drowsiness and perhaps nausea, all of which are common complaints following exposure to any narcosis-inducing compound. Ketones are not readily metabolized by the body to any great extent. In fact, nearly half the dose of the lower boiling ketones may be eliminated unchanged in expired air.

One very important syndrome of toxicity recently associated with an industrial exposure to 2-hexanone (methyl *n*-butyl ketone) has been described as a central-peripheral distal axonopathy, referring to the "dying-back" process of the nerve axons that accompanies this potentially debilitating neuropathy (1–4). Furthermore, a similar neuropathological condition may be produced by certain other hexacarbon compounds including *n*-hexane and 2,5-hexanedione which share common metabolites with each other and with 2-hexanone (5–7). The interrelationship between the metabolism and the toxicity of these three hexacarbon compounds is the focus of emphasis for this chapter. It is not our intent to provide an exhaus-

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tive account of the area, but rather to discuss, in sufficient detail, those aspects of metabolism and toxicity which should allow for a better understanding of the basic mechanisms of toxic actions of these compounds.

PHYSICAL AND CHEMICAL DATA

n-Hexane, 2-hexanone, and 2,5-hexanedione are all colorless liquids at room temperature. For any given temperature, each compound exerts a characteristic vapor pressure which of course reflects its volatility and therefore its potential for inhalational exposure. Another important chemical property of these solvents is water solubility. *n*-Hexane is insoluble in water, whereas 2-hexanone is slightly soluble and 2,5-hexanedione is soluble. Water solubility of these compounds can be used to explain various pharmacokinetic and pharmacodynamic differences. Also, lipid solubility determines the bioavailability to the central and peripheral neural tissue where the narcotic and neurotoxic effects are exerted. Table 1 below summarizes some physicochemical properties of *n*-hexane, 2-hexanone, and 2,5-hexanedione which are relevant to the metabolism and toxicity of these agents.

SOURCES AND USES

The simple aliphatic hydrocarbon, *n*-hexane, is produced by the cracking of crude oil and the subsequent fractional distillation of the resultant volatile alkanes. The use of *n*-hexane is indeed widespread throughout industry, and this hydrocarbon is contained in countless commercial products. *n*-Hexane is an excellent and inexpensive solvent found in glues, varnishes, paints, and inks, to mention just a few. Commercially, *n*-hexane is used to extract the vegetable oils from various seeds such as soybean and cottonseed. It is also a minor component of gasoline and is sometimes used to denature alcohol.

The hexacarbon ketone, 2-hexanone, commonly referred to as methyl *n*-butyl ketone, is also used in industry as a solvent, although its use has been curtailed due to its recently recognized neurotoxic potential. 2-Hexanone is produced by a catalytic reaction between acetic acid and ethylene under pressure. The primary uses for 2-hexanone involve its application as a solvent in lacquers and in lacquer or varnish removers. It is also a useful solvent for oils, resins, fats, waxes and nitrocellulose.

The hexacarbon diketone, 2,5-hexanedione can be produced from acetoacetic acid with alkylated iodine as a catalyst or by the hydrolysis of 2,5-dimethylfuran. The limited industrial applications of 2,5-hexanedione include its use as a solvent for various compounds and as an intermediate in the synthesis of other organic chemicals.

Table 1 Physicochemical properties of *n*-hexane, 2-hexanone, and 2,5-hexanedione

	n-Hexane	2-Hexanone	2,5-Hexanedione
Common name	Normal hexane Hexane	Methyl *n*-butyl ketone	Acetonyl acetone 1,2-Diacetylethane
Chemical formula	C_6H_{14}	$C_6H_{12}O$	$C_6H_{10}O_2$
Structural formula	$CH_3CH_2CH_2CH_2CH_2CH_3$	$CH_3\overset{\overset{O}{\|}}{C}CH_2CH_2CH_2CH_3$	$CH_3\overset{\overset{O}{\|}}{C}CH_2CH_2\overset{\overset{O}{\|}}{C}CH_3$
Mol wt	86.18	100.16	114.15
bp (°C^{760})	69	128	194
d_4^{20}	0.660	0.811	0.974
Water solubility (g/100 at 20°C)	I[a]	1.4	3.1
Partition coefficient 1-octanol/water, 25°C)[b]	>4100	28	0.22
Vapor pressure (mm Hg, 25°C)	149.0	3.8	1.6

[a] I, insoluble
[b] Unpublished data, this laboratory

EXPOSURE GROUPS

n-Hexane

Because of the excellent solvent capabilities of *n*-hexane, its use in industry is nearly ubiquitous. In fact, an estimated 2.5 million workers are potentially exposed to *n*-hexane vapors (8). The current threshold limit value-time weighted average (TLV-TWA) for *n*-hexane, which represents the airborne concentration of *n*-hexane believed to be without adverse effects upon repeated workplace exposure day after day, is 100 ppm with an intended change to 50 ppm. Occupational exposures to *n*-hexane have been reported to produce peripheral neuropathies in workers (9–12). A far more common cause of hexacarbon peripheral neuropathy, however, is deliberate inhalation of vapors from glues, lacquers, or solvents which contain *n*-hexane (13–17). This abuse of inhalational substances by individuals for the purposes of achieving an intoxicating "high" is an increasing problem in our society.

2-Hexanone

Used principally in the lacquer industry, 2-hexanone represents a considerably more limited exposure risk than does *n*-hexane. The National Institute for Occupational Safety and Health (NIOSH) estimated that 222,000 workers in the United States are potentially exposed to 2-hexanone, which has

been shown to cause peripheral neuropathy in man (1, 16, 18–20). The current TLV-TWA for workplace exposure to 2-hexanone is 25 ppm, but due to its low vapor pressure (3.8 mm Hg at 25° C) percutaneous absorption of 2-hexanone may represent a more critical route of exposure than does inhalation (21, 22). This is not to imply that inhalation of 2-hexanone vapor is not an important route of exposure, but rather that topical absorption of this solvent may result in an accumulation to toxic levels which would be unexpected according to ambient air concentrations of 2-hexanone. Without a doubt both routes of exposure must receive due consideration in order to protect the workers from potential toxicity. Whereas industrial exposure does pose a certain health hazard, the intentional inhalation of 2-hexanone vapors is not a significant problem, as the use of this ketone in consumer products is very limited.

2,5-Hexanedione (Acetonyl Acetone)

Owing to its low vapor pressure (1.6 mm Hg at 25° C) and to its infrequent use in industry, 2,5-hexanedione has not been reported to produce any cases of human neuropathy. Vapors of 2,5-hexanedione do cause some irritation to mucous membranes, but topical application results in a staining of the skin rather than inflammation. 2,5-Hexanedione is known to be absorbed percutaneously, but even so, no incidents of toxicity from its industrial use have been reported (23). Animal toxicity data indicate, however, that 2,5-hexanedione can indeed produce peripheral neuropathy (7, 24, 25). In fact, 2,5-hexanedione has 3.3 times greater neurotoxin potency than 2-hexanone, and 38 times greater than hexane (7). Although 2,5-hexanedione is not without toxic effects, the likelihood for human exposure, fortunately, is minimal.

An important fact to bear in mind, and one which will be addressed later in this chapter, is that both 2-hexanone and 2,5-hexanedione are metabolites of *n*-hexane. These metabolites, particularly 2,5-hexanedione, appear to mediate the neurotoxic syndrome of the hexacarbon central-peripheral distal axonopathy.

PHARMACODYNAMICS

The pharmacokinetics of *n*-hexane and 2-hexanone have been widely investigated. Much less is known about their common neurotoxic metabolite 2,5-hexanedione, but primary exposure to this agent is very limited as was mentioned earlier.

Absorption and Distribution

n-HEXANE Various studies have shown that *n*-hexane can be absorbed following inhalation of *n*-hexane vapors, ingestion of the solvent, and topi-

cal application to the skin. The rate and extent of absorption, as would be expected, are dependent upon both the route of administration and the dose or concentration of exposure.

Following absorption, *n*-hexane is widely distributed throughout the body and, not unexpectedly, the highest concentrations are achieved in those tissues having the highest lipid contents. Interestingly, Bus et al (26) noted a marked concentration in sciatic nerve of rats exposed to *n*-hexane by inhalation. When Böhlen et al (27) exposed rats to a hexane-air mixture, tissue saturation in blood, brain, adrenals, kidneys, and spleen was reached within 4–5 hours, whereas in liver a saturation value was not achieved even within 10 hours of exposure. In hepatic tissue, *n*-hexane was shown to actually cause an increase in total lipid content which in turn would allow an increasing uptake into that tissue. No changes in the lipid content of the other tissues were observed. Tissue accumulation of *n*-hexane was directly proportional to the total lipid content of the tissue, blood being the only exception. Plasma proteins were postulated to significantly contribute to hexane solubility in blood.

Bus et al (28) examined the absorption and distribution of *n*-hexane following inhalation by pregnant rats. An investigation of the tissue concentrations of *n*-hexane in the dams found the highest levels of the alkane in kidney followed by liver, blood, and brain. The levels of *n*-hexane in the fetuses were approximately equal to those found in maternal blood. In this study, the plasma half-life for *n*-hexane was determined to be about 60 min.

The pharmacokinetics of *n*-hexane administered intraperitoneally to guinea pigs was studied by Couri et al (29). When 132 mg of *n*-hexane was injected intraperitoneally, a peak blood level of 200 μg/ml was reached at 30 min. The solvent was eliminated initially with a half-life of about 36 min followed by a slower β-phase half-life of 4 hours.

The oral absorption of *n*-hexane was documented by a study of Krasavage et al (7), in which the solvent, when given by gavage to rats, was shown to produce the peripheral neurotoxicity. Plasma levels of the metabolite 2,5-hexanedione were measured and correlated with the exposure to *n*-hexane and also to the development of the peripheral neuropathy.

A study of the respiratory uptake, retention, and elimination of *n*-hexane in human volunteers revealed that approximately 28% of the solvent is taken up by the lungs, reaching a constant level after 2 hours exposure. The excretion rate of *n*-hexane in expired air was determined to be roughly 22% and retention, the difference between uptake and excretion, was just slightly less than 6% (30). Upon cessation of exposure large amounts of retained *n*-hexane were rapidly eliminated through the lungs unchanged (31).

Urinary metabolites of *n*-hexane in man were demonstrated by Perbelini et al (32) following workplace exposure to hexane and other solvents. The

neurotoxic metabolite 2,5-hexanedione was found to be the main *n*-hexane metabolite in the urine of these workers, in contrast to 2-hexanol being the major urinary metabolite found in experimental animals (29, 33).

In reference to human exposure to *n*-hexane, certainly the inhalational route is of prime importance. Percutaneous and oral routes of absorption, although perhaps not as significant as inhalational exposure, represent potential routes of human exposure which have not been thoroughly studied to date.

2-HEXANONE (METHYL *n*-BUTYL KETONE) Prior to 1973, little was known about the absorption or distribution of 2-hexanone, or about its potential neurotoxicity. The harmful effects of 2-hexanone were thought to be limited to irritation and narcosis with no record of any injury from its industrial use (23, 34). However, following an outbreak of peripheral neuropathy in workers at a plastic coating-printing plant, much research was devoted to examining the neurotoxic properties of 2-hexanone (1, 19, 20, 35). Upon continuous exposure of rats to 400 ppm 2-hexanone for up to 60 days, Abdel-Rahman et al (36) found blood levels of 2-hexanone and its metabolite 2,5-hexanedione to be nondetectable by gas chromatographic analysis. When 160 mg of 2-hexanone was injected intraperitoneally into rats, the peak blood level (650 μg/ml) was reached at 30 min and declined biphasically. The half-life for the rapid elimination phase was about 10 min, whereas the half-life of 2-hexanone in the following slower phase was 7 hours (36). In a more recent study, Katz et al (37) exposed rats weekly for 72 hours per week to 700 ppm 2-hexanone. The mean serum concentration of 2-hexanone after 8 weeks of treatment was 0.7 μg/ml. The metabolites 5-hydroxy-2-hexanone and 2,5-hexanedione were reported to be present in serum at concentrations more than 100 times those of the parent ketone.

DiVincenzo et al (38) determined the half-life of 2-hexanone in guinea pigs to be 78 min following intraperitoneal injection of 450 mg/kg of the ketone. The amount of 2-hexanone found in the blood compartment at 1 hour after dosing was 1.4% of the original dose, reflecting the extensive distribution of the ketone.

Using ^{14}C-labeled 2-hexanone, DiVincenzo et al (39) also studied the metabolic fate and disposition of the solvent in rats. Following oral doses of 20 or 200 mg/kg, 2-hexanone was virtually completely absorbed, extensively metabolized, and rapidly eliminated in expired air and urine. Forty-eight hours after dosing, approximately 83% of the radioactivity was recovered in breath and urine, with about 16% remaining in the carcass and 1% excreted in the feces. A more detailed account of the metabolism and elimination of 2-hexanone will be given in a subsequent section of this chapter.

In an extensive study of the respiratory uptake and skin absorption of 2-hexanone in humans and dogs, DiVincenzo et al (21, 40) showed that 2-hexanone was efficiently absorbed by the lungs, by the gastrointestinal tract, and through the skin. About 75–92% of the 2-hexanone inhaled by humans was absorbed by the lungs and respiratory mucosa. Following inhalational absorption, 2-hexanone is not extensively eliminated in expired air, its apparent retention no doubt owing to such factors as water solubility, widespread tissue distribution, and metabolism. Human serum levels of 2-hexanone were detectable (0.3 μg/ml) after 1 hour exposure to 100 ppm of the ketone, but were not detectable at the lower 10 or 50 ppm exposure concentrations. In that same study, DiVincenzo et al reported a 65.8% cumulative recovery in breath and urine after 8 days following ingestion of 0.1 mg/kg of body weight (1-^{14}C)-2-hexanone by human volunteers. Also reported at that time was the unequivocal percutaneous absorption of 2-hexanone through the skin of humans and of dogs.

The amount of information currently available of the absorption and distribution of 2-hexanone allows for a more detailed account of the neurotoxic activity of this solvent.

The water solubility of 2-hexanone enables the compound not only to achieve wide tissue distribution but also contributes to its retention and therefore to its subsequent metabolism to 2,5-hexanedione. 2-Hexanone is 12 times more potent than *n*-hexane as a neurotoxic agent when evaluated according to the exposures required to produce signs of neuropathy. Furthermore, about five times the amount of the metabolite 2,5-hexanedione is produced from 2-hexanone compared to equimolar doses of *n*-hexane (7). Both inhalational and topical absorption of 2-hexanone represent significant routes of human exposure which must be carefully considered in order to protect workers from industrial solvent-induced neuropathy.

2,5-HEXANEDIONE Having been identified as a metabolite of *n*-hexane and 2-hexanone, 2,5-hexanedione was soon shown to produce an indistinguishable neuropathy in experimental animals given subcutaneous injections of the diketone (2, 25). 2,5-Hexanedione was also shown to produce neuropathy when given by oral gavage (2, 7, 41) and in drinking water (5, 24, 42, 43). As a more water-soluble compound 2,5-hexanedione has a greater persistence in serum than does *n*-hexane, 2-hexanone, and the other metabolites thereof, including 2-hexanol, 2,5-hexanediol, and 5-hydroxy-2-hexanone. This persistence is believed to contribute to the pronounced neurotoxicity of 2,5-hexanedione and its progenitor compounds (7, 38). As was mentioned earlier, 2,5-hexanedione does not pose a serious occupational hazard as its vapor pressure is very low (1.6 mm Hg at 25° C) and its use in industry is rather restricted. By far, human exposure to 2,5-hexanedione occurs subsequent to the oxidative metabolism of *n*-hexane,

2-hexanone, or similar hexacarbon compounds. The topic of metabolism and elimination will be discussed in the following section.

Biotransformation and Elimination

The metabolism of *n*-hexane and 2- hexanone to 2,5-hexanedione appears to be causally related to the development of the peripheral neuropathy associated with exposure to these agents. There are several distinctly different metabolic pathways that *n*-hexane and 2-hexanone can follow, some leading to the production of the neurotoxic metabolite, 2,5-hexanedione, and others which ultimately degrade the hexacarbon to CO_2. Of course, the body can also inactivate the potentially neurotoxic compounds by conjugating available hydroxyl groups to form glucuronides and sulfates. These conjugates are then rapidly eliminated in urine and bile and are unavailable for further biotransformation and/or toxicological interactions.

n-HEXANE When *n*-hexane is incubated with rat liver microsomes, hydroxylation is seen to occur at all positions, though carbon 2 (ω-1) is preferentially oxidized (44). By examining the effects on *n*-hexane hydroxylation of various inducers and inhibitors of cytochrome P_{450} mediated drug metabolism, Frommer et al (44) concluded that more than one monooxygenase is involved in the hydroxylation of aliphatic hydrocarbons, and that the cytochrome P_{450} dependent (ω-1) hydroxylase is induced by phenobarbital pretreatment. Indeed, Couri et al (29) showed an increased microsomal biotransformation of *n*-hexane to 2-hexanol in phenobarbital pretreated guinea pigs. The same study reported an increase in the metabolism of 2-hexanone to 2,5-hexanedione as well as the identification of 2,5-hexanedione as a metabolite common to the biotransformation of *n*-hexane and 2-hexanone (29).

Other *n*-hexane metabolites which have been identified include 1-hexanol (45), 3-hexanol (33), 5-hydroxy-2-hexanone, 2,5-hexanediol, γ-valerolactone, and 2,5-dimethylfuran (7, 32, 39). The cyclic metabolite 2,5-dimethylfuran is postulated as arising from either metabolic cyclization or, more likely, arising artifactually upon gas chromatography of some hydroxylated urinary metabolite (32, 38, 39). Whereas oxidation of the (ω-1)-carbon atom can lead to metabolic production of the neurotoxic metabolite, 2,5-hexanedione, ω-hydroxylation of *n*-hexane results in the formation of 1-hexanol (45) which can follow the pathway of fatty acid β-oxidation following its successive oxidation to hexanal and to hexanoic acid (46–49). The ω-hydroxylation product, 1-hexanol, has not been shown to produce peripheral neuropathy or to cause a reduction in motor nerve conduction.

One very interesting feature of *n*-hexane metabolism is that the metabolite 2-hexanol produced from the hydroxylation of *n*-hexane can in turn be

further oxidized to 2-hexanone (7, 38). Likewise, 2-hexanone has been shown to be metabolically reduced to the corresponding alcohol, 2-hexanol (7, 29, 38, 39). This metabolic interconversion intimately links the disposition of *n*-hexane and 2-hexanone; therefore, what shall now be discussed concerning 2-hexanone metabolism may be applied to the biotransformation of *n*-hexane as well.

2-HEXANONE The biotransformation and elimination of 2-hexanone has been fairly well characterized. Couri et al (29) have demonstrated that 2-hexanone can be oxidized and reduced by the microsomal and cytosolic fractions of liver, respectively, leading to the production of 2,5-hexanedione or 2-hexanol. Metabolites of 2-hexanone which have been identified in serum include 5-hydroxy-2-hexanone and 2,5-hexanedione (7, 39, 50). Urinary metabolites of 2-hexanone have been reported to include 2-hexanol, 2,5-hexanediol, 5-hydroxy-2-hexanone, and 2,5-hexanedione (29, 36, 39, 41, 50).

By administering ^{14}C-labeled 2-hexanone orally to rats, DiVincenzo et al (39) were able to extensively characterize the metabolic fate and disposition of 2-hexanone. Radioactive products identified in the breath were unchanged 2-hexanone and carbon dioxide, representing approximately 5 and 40% of the administered dose, respectively. The expired $^{14}CO_2$ most likely originates by α-oxidation to α-ketohexanoic acid and its subsequent decarboxylation, leaving 1-pentanal which in turn can enter intermediary metabolism after oxidation to pentanoate. No radiolabeled acetate was detected in urine, suggesting minimal breakdown of 2-hexanone via intermediary metabolism. Likewise, there was little incorporation of ^{14}C into neutral lipids or phospholipids. The identification of ^{14}C-norleucine (2-amino-hexanoic acid) as a urinary metabolite of 2-hexanone supports an α-oxidation mechanism since the postulated metabolite α-ketohexanoate could undergo transamination as well as decarboxylation. The detection of ^{14}C-urea in urine was not unexpected, based upon the identification of large amounts of $^{14}CO_2$ formed.

In the same study, DiVincenzo et al (39) investigated the effects on 2-hexanone disposition of pretreatment with the mixed-function oxidase inhibitor SKF 525A. SKF 525A produced an increase in respiratory $^{14}CO_2$ and a decrease in radiolabeled urinary metabolite production. These results further support the (ω-1) oxidation pathway as a microsomal mixed function oxidase system.

In an earlier study of DiVincenzo et al (38), when 5-hydroxy-2-hexanone was administered intraperitoneally to guinea pigs, both the oxidation and reduction products (2,5-hexanedione and 2,5-hexanediol, respectively) were identified in serum, although the diketone was the predominate metabolite.

Similarly, when 2,5-hexanediol was administered to guinea pigs, (38, 50), or to rats (41), 5-hydroxy-2-hexanone and 2,5-hexanedione were identified as the major metabolites.

2,5-HEXANEDIONE When 2,5-hexanedione was administered to guinea pigs, the only metabolite detected was the reduction product 5-hydroxy-2-hexanone (38, 50). The half-lives of the two compounds were determined to be 100 min for the diketone and 156 min for 5-hydroxy-2-hexanone (38). These two compounds have been shown to be the most neurotoxic of all the hexacarbon compounds and metabolites studied (7). The biotransformation and the common metabolic pathways of *n*-hexane, 2-hexanone, and 2,5-hexanedione are shown in Figure 1.

TOXICOLOGY

Neither *n*-hexane, 2-hexanone, nor 2,5- hexanedione can be considered to be highly toxic agents based upon their acute oral exposures to rats (see Table 2). Aside from irritation to ocular and nasal mucous membranes and narcosis upon exposure to very high concentrations, these three hexacarbon compounds were generally thought to be without significant toxic effects. In the late 1960s there were several reports from Japanese investigators discussing the neuropathy that *n*-hexane could produce in humans and animals, but the findings were largely unappreciated (9, 51). The 1973 outbreak of peripheral neuropathy at a fabrics-coating plant in Columbus, Ohio (1, 19, 20, 52–54) with the eventual identification of 2-hexanone as the toxic agent responsible for the neuropathies, and the subsequent proliferation of hexacarbon research, quickly changed the status of these solvents from innocuous to potentially dangerous. Consequently, the threshold limit value (TLV-TWA) for 2-hexanone had been reduced from 100 ppm to 25 ppm, and was further reduced to 5 ppm in 1981 (55). The TLV-TWA for *n*-hexane was likewise reduced from 500 ppm to 100 ppm and eventually lowered to 50 ppm in 1980. Due to the very low vapor pressure of 2,5-hexanedione and its limited industrial use, a TLV-TWA has not been established for this compound. Toxicity data (oral LD_{50} and inhalational toxicity data) and the adopted and intended TLV-TWA for the three solvents are listed below in Table 2.

General Toxicity

n-HEXANE The toxicity of *n*-hexane, aside from its potential neurotoxic effects, is largely unremarkable. The oral median lethal dose (LD_{50}) in young rats was determined to be roughly 32 g/kg (56), far from being a

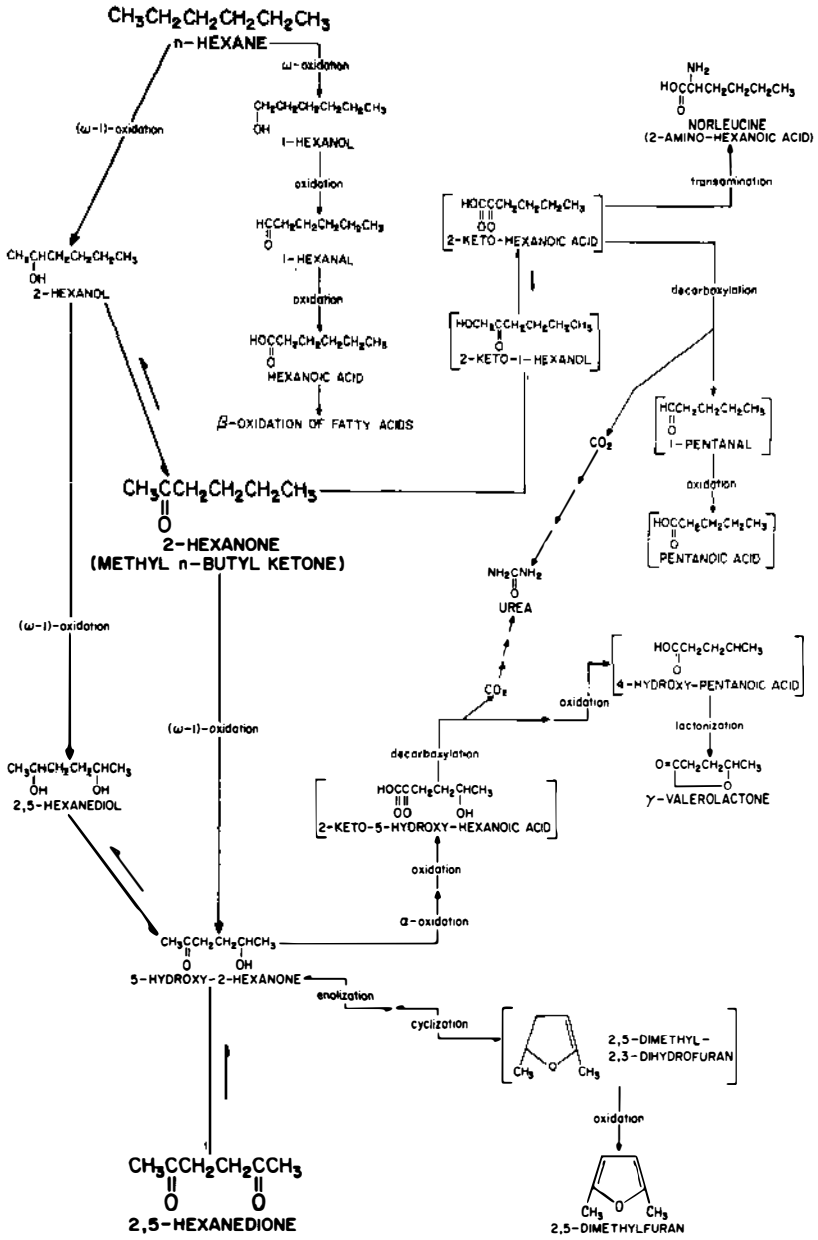

Figure 1 Biotransformation of *n*-hexane, 2-hexanone, and 2,5-hexanedione (tentative intermediates are enclosed in brackets).

Table 2 Toxicology of *n*-hexane, 2-hexanone, and 2,5-hexanedione

Compound	*n*-Hexane	2-Hexanone		2,5-Hexanedione	
LD_{50}, rat (p.o., g/kg)	32.0	2.6		2.7	
Inhalational toxicity, rat					
Concentration (ppm)	48,000	4000	8000	200	1700
Duration (hr)	4	4	4	4	>1
Effect	LC_{50}[a]	No deaths	LC_{100}	LCLo[b]	LC_{100}
TWA ppm					
Adopted	100	25		NE[c]	
Intended	50	5		NE	

[a] LC_{50}, concentration in air lethal to 50% of animals tested
[b] LCLo, lowest concentration in air reported to cause death in rats
[c] NE, not established

highly toxic agent. A 5 min inhalational exposure of mice to various concentrations of *n*-hexane revealed: at 8000 ppm, no anesthesia; at 16,000 ppm, light anesthesia; at 32,000 ppm, deep anesthesia; and at 64,000 ppm, respiratory arrest (57, 58). In humans, giddiness and dizziness are experienced after 10 min at 5000 ppm; at 1500 ppm for 10 min, eye and throat discomfort, headache, and less commonly, nausea, are reported by some subjects (8). Human exposure for several minutes to 500 ppm *n*-hexane was reported to be without adverse effect (59). The neurotoxic effects of *n*-hexane, which will be discussed in a subsequent section, are the major toxic effects of this solvent. Little information has been reported on other organ or tissue pathology resulting from *n*-hexane exposure. The perinatal toxicity of *n*-hexane was investigated in pregnant rats by Bus et al (28). No significant alterations in fetal resorptions, body weights, visible anomalies, or soft tissue and skeletal anomalies were noted in the *n*-hexane treated group as compared to controls. The postnatal growth of pups born to dams exposed to *n*-hexane was temporarily depressed (up to 3 weeks after birth) but litter weights of the treated pups had returned to control values by 7 weeks. The possibility that the hexacarbon alkane may cause liver damage is suggested by the work of Böhlen et al (27) which related *n*-hexane inhalation to hepatic lipid accumulation. Also, DiVincenzo & Krasavage (60) have found *n*-hexane to produce an increase in serum ornithine-carbamyl transferase activity when administered to guinea pigs. Hepatotoxicity is certainly not the most prominent toxic effect of *n*-hexane, but perhaps it should be considered in the clinical evaluation of occupationally exposed workers or intoxicated inhalant abusers.

2-HEXANONE The salient features of 2-hexanone toxicity include irritation, narcosis, and of course, peripheral neuropathy. Based on a comparison of the oral LD_{50} in rats, 2-hexanone ($LD_{50} = 2.6$ g/kg) is considerably more toxic than *n*-hexane ($LD_{50} = 32$ g/kg), but the toxicity of 2-hexanone itself is not great (61). The major symptoms in animals which occur almost immediately upon exposure to higher concentrations of 2-hexanone include irritation of the eyes and nose. Ataxia ensues within 3–5 min and narcosis in 20–120 min, but only with concentrations of 6500 ppm or greater. Death due to 2-hexanone inhalation, which may require hours of exposure to these concentrations, is apparently due to narcosis rather than irritation to lungs. Congestion and hemorrhage of the lungs, moderate congestion of liver and kidneys, and slight congestion of the brain have been noted upon necropsy (23).

In humans, exposure for 4 hours to 50 or 100 ppm of 2-hexanone resulted in no ill effects (21). Irritation to the eyes and nose is noticeable at 1000 ppm. The neurotoxic effects of 2-hexanone will be discussed in a subsequent section.

2,5-HEXANEDIONE Although recognized as the neurotoxic metabolite of *n*-hexane and 2-hexanone, and known to produce the neuropathy following administration to rats, 2,5-hexanedione is not especially toxic when given acutely. The oral LD_{50} in rats for 2,5-hexanedione is 2.7 g/kg which is about the same as that for 2-hexanone. The acute toxicity of 2,5-hexanedione to animals consists of narcosis and irritation to mucous membranes. The main effect of this diketone on the skin is that of staining rather than inflammation, and no toxic effects from human exposure have ever been reported (23). It would appear that the hazard of 2,5-hexanedione from primary exposure is minimal indeed.

Neurotoxicity

Humans and experimental animals chronically exposed to relatively high concentrations of *n*-hexane or 2-hexanone, and animals administered 2,5-hexanedione subacutely, develop a neurotoxic syndrome which has come to be known as hexacarbon peripheral neuropathy. Pathologically the condition is characterized by giant axonal swellings consisting of focal accumulations of 10 nm neurofilaments. These swellings occur along the nerve fibers, originating on the proximal side of the nodes of Ranvier in the more distal sections of the axons, but eventually developing more proximally and in the internodal regions. Retrograde fiber demyelination and degeneration ensue and the neurotoxic process resembles the dying-back neuropathies associated with acrylamide, carbon disulfide, and organophosphate (1, 10, 11, 13–16, 25, 47, 62–66).

In the hexacarbon peripheral neuropathy, the clinical symptoms which occur include a symmetrical sensory dysfunction in the hands and feet progressing to a distal muscle weakness and loss of deep tendon reflexes (1, 16). Neurophysiological studies indicate that nerve conduction velocities are significantly lowered prior to the overt symptomatology and the slowing becomes more pronounced with the progression and intensity of the neuropathy (1, 15, 16, 46, 47, 67, 68). Changes in visual color discrimination secondary to macular damage have also been reported (69). Clinical laboratory tests have not revealed any consistent pattern of hepatic, renal, hematologic, or glucose metabolic dysfunction (16). Following discontinuance of exposure to the insulting hexacarbon compound, the prognosis for recovery is generally quite good, although the disorder may intensify for several weeks or months. However, permanent neural damage, both centrally and peripherally, have been reported following recovery from severe hexacarbon neuropathy (10, 11, 13–16).

MECHANISM OF NEUROTOXICITY

Hexacarbon neuropathy has been shown to occur in humans exposed to *n*-hexane and 2-hexanone (1, 10, 11, 13–20). In experimental animals the syndrome has been produced by exposures to *n*-hexane, 2-hexanone, and various metabolites thereof including 2-hexanol, 2,5-hexanediol, 5-hydroxy-2-hexanone, and 2,5-hexanedione (7, 24, 25, 35, 36, 62). The term "hexacarbon" neuropathy has been applied to this syndrome of chemically induced central-peripheral distal axonopathy because those agents which are known to produce the neuropathy all have a six carbon molecular skeleton. *n*-Pentane and *n*-heptane were shown not to induce the neuropathy in rats, nor to disturb the nerve conduction velocities (70). Neither was 3-hepatanone (ethyl *n*-butylketone) found to produce clinical or microscopic signs of systemic toxicity or neurotoxicity in rats (71). Although the term "hexacarbon neuropathy" is conveniently descriptive as to the probable chemical structure of these neurotoxic agents, it offers no insight into the possible mechanism of neurotoxicity. Furthermore, there appears to be a definite requirement for the γ-diketone arrangement of the oxygen and carbon atoms for a hexacarbon compound (or its metabolites) to exert its neuropathologic effects. When 1-hexanol was administered to rats for up to 8 months, neither motor conduction velocity was reduced nor were any clinical signs of peripheral neuropathy apparent (46). Similarly, 2,4-hexanedione, 2,3-hexanedione, and 2,6-heptanedione, which lack the 1,4 spacing of the carbonyl groups, as well as 1,6-hexanediol, were all determined to be nonneurotoxic (16, 66, 72–74). On the other hand, the γ-diketones 2,5-heptanedione and 3,6-octanedione have been shown to produce peripheral neuropathologic lesions identical to those produced by 2,5-hexanedione (66, 74).

3-Heptanone (ethyl *n*-butyl ketone) is metabolized, in part, to the neurotoxic γ-diketone 2,5-heptanedione (37). However, rats exposed to 700 ppm 3-heptanone for 24 weeks failed to develop clinical or microscopic signs of neurotoxicity. Apparently, 2-heptanone is not as readily metabolized to its corresponding diketone since the serum concentrations of the 2,5-heptanedione were found to be far less than those of 2,5-hexanedione following equivalent exposure to 2-hexanone (37).

Having presented evidence suggesting a prominent structure-activity relationship for the neurotoxic γ-diketones, any generalized statement of mechanism must take this information into account. At present, however, no single mechanism of neurotoxicity has been postulated which can adequately explain the structure-activity relationship, the slowing of nerve conduction velocities, and the eventual demyelination and degeneration of the affected axons. It is quite well established that the peripheral neuropathy is preceded by a focal accumulation of 10 nm neurofilaments producing axonal swelling above the nodes of Ranvier (2, 10, 11, 13, 15, 17, 25, 35, 42, 62–65, 72). These multifocal accumulations of neurofilaments have been associated with a blockade of fast axonal transport (75). An impairment in energy metabolism has been proposed to explain the mechanism of neurotoxic action of the hexacarbon compounds (47, 76). According to this hypothesis, the neurotoxic hexacarbons are believed to inhibit the sulfhydryl enzymes glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphofructokinase (PFK) in a dose-dependent manner. The energy insufficiency would reduce the axoplasmic supply of glycolytic enzymes from the perikaryon. This in turn results in inadequate energy production in the axons with their subsequent deterioration. Inhibition of GAPDH has been shown to block fast axoplasmic transport (77, 78) and Sabri et al (79) have reported GAPDH inhibition by 2-hexanone and 2,5-hexanedione. Howland et al (80) also found 2,5-hexanedione to inhibit PFK and GAPDH, and to a lesser degree the glycolytic enzyme enolase. The reduction in oxygen uptake by sciatic nerve from 2,5-hexanedione treated rats as reported by Couri & Nachtman (43) suggests that oxidative metabolism is also impaired, or associated with the altered glycolytic activities. Graham & Abou-Donia (81) have also reported that 2,5-hexanedione irreversibly inhibits GAPDH, but they propose that the inhibition is due to a reaction with amino groups rather than sulfhydryl groups on the enzymes. These authors, however, do not believe that a glycolytic impairment is the key biochemical event in hexacarbon neuropathy, and in support of their contention they cite the lack of hemolysis in clinical cases of *n*-hexane- and 2-hexanone-induced peripheral neuropathy. Instead, Graham & Abou-Donia (81) postulate that intermolecular and intramolecular cross-linking of neurofilament proteins results in neurofilament aggregation which, in turn, obstructs axoplasmic transport and leads to axonal degeneration (81, 82). Accordingly, they

suggest that the structure-activity relationship of the neurotoxic γ-diketones may be partially explained by the differing degrees of water solubility of the hexacarbons and also by the propensity of the diketones to form stable conjugated Schiff bases with amino groups of proteins (81). The interaction of the γ-diketones with neurofilaments remains to be elucidated, but an interference with the delicately balanced tertiary structure of the filaments has been proposed (12, 47, 83). No alterations in microtubular binding activity were demonstrated by examining the colchicine binding activities of sciatic nerve in 2,5-hexanedione treated rats (43). Neither was the ability of tubulin subunits to polymerize into microtubules adversely affected by the addition of 2-hexanone or 2,5-hexanedione in brain microtubular reconstitution experiments (84). Reduced microviscosity of sciatic nerve homogenate and loss of phase transition in viscosity-temperature plots were reported in rats receiving 2,5-hexanedione in drinking water (43). The loss of phase transition precedes clinical paralysis and represents a quantitative and qualitative alteration of the myelin preparation which may be related to the neuropathology of hexacarbon-induced peripheral neuropathy (43, 85).

Another biochemical alteration resulting from 2,5-hexanedione administration is an inhibition of sterol biosynthesis in rat sciatic nerve. Gillies et al (86, 87) have demonstrated that the inhibition of sterologenesis occurs between acetate and mevalonate (87), and they suggest that the neurotoxicity of 2,5-hexanedione may be related to the reduced biosynthesis of ubiquinone, an essential link in the mitochondrial electron transport chain (86). Again, although in a different light, altered energy metabolism is suggested as an etiologic factor in hexacarbon neuropathy.

Other biochemical effects of the hexacarbon compounds or untested hypotheses of the mechanism of neurotoxicity include thiamine inactivation, ionphore activity of γ-diketones, calcium chelating effects, and acetylcholinesterase inhibition (12, 16, 88). Despite the great strides in research recently taken in elucidating the mechanism of hexacarbon neurotoxicity, a single, comprehensive, and tested hypothesis still eludes us.

MIXED SOLVENT EFFECTS

The identification of *n*-hexane (10, 11, 13–17) and 2-hexanone (1, 19, 20, 52) as neurotoxic agents in various human exposures was the necessary first step toward understanding the mechanism of hexacarbon neurotoxicity. Yet even as the precise mechanism of *single* agent exposure remains to be elucidated, the additional complication of mixed solvent effects must receive due consideration for at least two important reasons. Firstly, human exposure (excepting laboratory studies) is rarely to a single solvent, whether in an occupational setting or by deliberate inhalant abuse. And secondly, most

laboratory testing has been performed using solitary reagent grade solvents. Very little is known about the pharmacodynamics of solvents inhaled or administered as mixtures. Furthermore, there is virtually an infinite number of possible combinations of solvents, and an even greater number if concentration variables are considered. However, by approximating the human exposure conditions known to cause neuropathies, laboratory studies can begin to investigate the mixed solvent effects in a meaningful fashion (53, 54, 89).

Abel-Rahman et al (36) exposed rats to concentrations of either 225 ppm 2-hexanone, 400 ppm 2-hexanone, or 2-hexanone/2-butanone (methyl ethyl ketone) combined (225:750 ppm). The animals exposed only to 400 ppm 2-hexanone for up to 2 months had nondetectable blood levels of the ketone. However, in rats exposed for only 6 days to the combined 225 ppm 2-hexanone/750 ppm 2-butanone, blood levels were 9.5 mg/dl for 2-hexanone with its metabolite 2,5-hexanedione present at 2.5 mg/dl and 2-butanone at 1.3 mg/dl. A longer exposure (23 days) to the solvent mixture resulted in even more dramatic alterations in blood ketone levels, the 2-hexanone blood levels increasing to 24.0 mg/dl and the 2-butanone levels falling to 0.2 mg/dl. Furthermore, 2-butanone was reported as having enhanced the neurotoxicity of 2-hexanone upon concomitant exposure. Animals exposed to mixed solvent vapors showed an earlier onset and greater severity of neuropathy than those exposed to 2-butanone or 2-hexanone alone. These results make evident the potential for significant pharmacodynamic interaction between two solvents.

Couri et al (90) demonstrated a significant stimulation of liver microsomal biotransformation activities in rats exposed to the mixed solvent combination (225 ppm 2-hexanone/750 ppm 2-butanone). Also, a mixed solvent effect (150 ppm 2-hexanone/1000 ppm 2-butanone) on the biotransformation of hexobarbital determined as a diminution in sleep time was demonstrated in mice and guinea pigs in a later study (29). Duckett et al (91) exposed rats via inhalation to 200 ppm 2-hexanone or the solvent mixture 200 ppm 2-hexanone/2000 ppm 2-butanone and found that after 8 hours exposure each day the rats presented with a muscular weakness of all limbs. The 2-hexanone group was reported to have recovered within several hours, while recovery for the mixed solvent exposure group took at least 24 hours. Again, a solvent mixture is seen to have pronounced pharmacodynamic effects. Altenkirch et al have reported epidemiological (92) and experimental (93) evidence that 2-butanone potentiates the neurotoxicity of *n*-hexane. Also, 2-butanone was shown to potentiate the peripheral neurotoxicity of 2-hexanone (65).

The mixed solvent interactions reported above represent only a small fraction of the potential interactions. A better understanding of the basic mechanisms of solvent potentiation would aid in identifying and preventing

other such interactions. Much further research is vitally needed to elucidate the pharmacology and toxicology of solvent mixtures.

SUMMARY

Human exposure to hexacarbon compounds is quite pervasive, including occupational exposures to industrial solvents as well as unintentional and sometimes deliberate exposures to hexacarbon solvents contained in innumerable commercial products. The exact mechanism of hexacarbon neurotoxicity has not yet been identified, but an interference with neuronal axoplasmic flow seems most likely. Metabolism of *n*-hexane and 2-hexanone to 2,5-hexanedione is a prominent feature which appears to be causally related to the neuropathologic syndrome, and mixed solvent effects have been noted in regard to potentiation of hexacarbon neurotoxicity. Continued effort in investigating the chemically induced peripheral neuropathy is essential not only to define the precise molecular mechanism, but to advance our basic understanding of other polyneuropathies as well. Ultimately, progress in these areas should yield such benefits as early diagnosis of potential neuropathology, better measures for the prevention of neurotoxicities, and more effective modalities of treatment. Indeed, sustained research efforts are imperative in maintaining human health and safety throughout our current era of advancing global technology.

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