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n-Hexane and its toxicologic effects – a review

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n-Hexane and its toxicologic effects

A review [1]

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JØRGENSEN NK, COHR K-H. n-Hexane and its toxicologic effects: A review. *Scand j work environ health* 7 (1981) 157—168. This review is a critical survey and evaluation of the recent literature relevant as medical background for a discussion of hygienic threshold values for hexane. Polyneuropathy and maculopathy, as well as subclinical effects, eg, functional disturbances (conduction velocity of the motor and sensory nerves), are included.

Key terms: exposure, functional disturbances, maculopathy, motor conduction velocity, occupational exposure limit, polyneuropathy, sensory conduction velocity.

n-Hexane is used widely in industry as a solvent and thinner, eg, in the rubber industry in the production of tires and the impregnation of materials; in glue products, eg, in the gluing of soles in the shoe industry and in the production of tape and bandages; in the food industry for the extraction of vegetable oils; in the pharmaceutical industry in the production of tablets; and in the perfume industry. It is also used as a cleaning agent for, among other things, textiles, furniture, and leather products; in the chemical industry for the production of polyethylene and polypropylene; as a component in the extraction of benzene (common gasoline contains approximately 1.5 % n-hexane); in laboratories; and in low temperature thermometers.

Pure n-hexane is seldom used. It is more often mixed with other aliphatic hydrocarbons and toluene.

This report is only concerned with n-hexane and not with any of the other hexane isomers.

Physical and chemical characteristics

Chemical name:	n-hexane
CAS number:	11—54—4
Molecular formula:	C_6H_{14}
Structural formula:	$CH_3CH_2CH_2CH_2CH_3$
Common properties:	It is a colorless liquid which easely vaporizes and has a characteristic odor. It is nonsoluble in water, but it can be mixed with lipophilic organic solvents, eg, ether or chloroform.
Molecular weight:	86.17
Boiling point (at 101.3 kPa):	68.95°C
Vapor pressure (at 24.8°C):	19.99 kPa (696,000 mg/m³)
Conversion factors:	1 ppm = 3.52 mg/m³ 1 mg/m³ = 0.384 ppm

Metabolic model

Hexane is taken up through the lungs. Workers exposed to an n-hexane concen-

[1] This review is a slightly revised version of a criteria document prepared for the Nordic Expert Group on Hygienic Threshold Values under the Nordic Council of Ministers. Of the available publications only those considered to form a reliable and relevant medical base for the establishment of a hygienic threshold value have been included as references.

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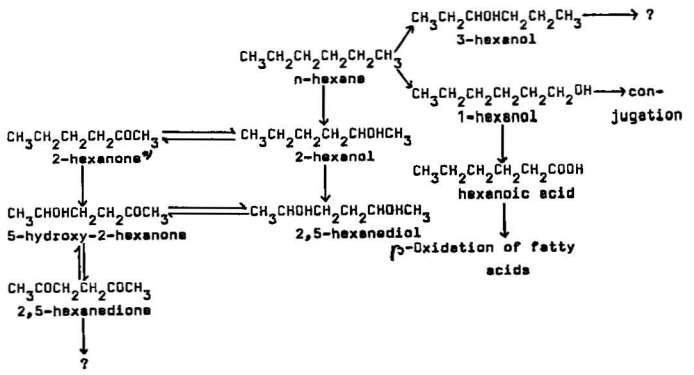

Fig 1. Possible transformation products of n-hexane. The products have been demonstrated in research and in animals which have received larger doses of n-hexane.

*) 2-hexanone = methyl-n-butyl ketone (= MBK)

tration of approximately 160—1,400 mg/m³ (and acetone) for 1—4.5 h have nearly constant alveolar air concentrations of 85 % of the concentration in the inspired air. Retention is thus approximately 15 %. There is a linear relationship between the concentration in venous blood in milligrams per kilogram (y) and the concentration in alveolar air in milligrams per liter (x) after 4.5 h of exposure: $y = 0.77\ x - 107$ (12).

After 4—5 h of exposure to an n-hexane concentration of 170 g/m³ the n-hexane concentrations reach an equilibrium in the venous blood and expired air of rats. Under such circumstances the concentration in venous blood has been found to be 0.15 g/kg (51).

Distribution

The distribution coefficient of n-hexane is approximately 0.8—1.0 between blood and air at 27°C (12).

The solubility of n-hexane in water is limited. Therefore it must be bound to other components in the blood, one such component being lipids. The distribution coefficient between olive oil and air is approximately 204 at 37°C (K-H Cohr, unpublished results). In addition n-pentane and n-heptane data indicate that approximately 40 g of n-hexane can be bound to 10^4 g of protein in a 5 % water-based solution of protein (41, 42).

In inhalation experiments with rats (170 g/m³) equilibrium was found to occur in the brain, liver, kidneys, and adrenal glands after 4—5 h of exposure. The equilibrium concentrations were 0.39, 0.14, 0.20, and 0.49 g/kg of tissue, respectively. These values correspond to the distribution coefficients for tissue to blood of 2.60, 0.93, 1.33, and 3.27 (13). Accumulation in these tissues depends on the lipid content. Four milligrams of n-hexane accumulates per gram of lipid. The blood is able to bind approximately 25 mg of n-hexane per gram of lipid (13). Equilibrium does not develop in connection with the accumulation of n-hexane in rat liver. The n-hexane content continues to rise in the liver with the simultaneous accumulation of lipid (12).

Biotransformation

The biotransformation of n-hexane has mainly been investigated with microsomal fractions of animal liver (34). Other tissues, eg, lung and liver, can very likely also transform hexane (33). n-Hexane is biotransformed in an oxidation process that involves microsomes of the monoxygenic system (24, 33, 34). Rats exposed to an n-hexane concentration of 90,000 mg/m³ (2.5 %) in 4 d showed an increase in liver microsome protein and an increased activity of the following: cytochrome P-450, NADPH-dependant[4] cytochrome P-450-reductase, and cytochrome b_5 (34).

The possible transformation products are shown in fig 1. These products have been demonstrated in research in which experimental animals have received larger doses of n-hexane (19, 24, 34, 46, 47). The primary transformation product is 2-hexa-

[4] NADPH = reduced nicotinamide dinucleotide phosphate.

nol (24). This transformation is further increased by exposure to n-hexane (34).

The oxidation products of n-hexane have not been found in occupationally exposed workers (46, 47).

Elimination

Only the elimination of n-hexane through the lungs and kidneys is described.

Lungs

The n-hexane concentration in expired air 10 min after the cessation of exposure is 2—5 % of the equilibrium concentration during exposure (52). Within the first 4 h after the end of exposure 50—60 % of the retained quantity is eliminated through the lungs (44).

Kidneys

The transformation products 1,2,3-hexanol, methyl-n-butyl ketone and 2,5-hexanedione (46, 47) that have been found in animal experiments have not been found in 24-h urine specimens from workers exposed to n-hexane concentrations of 350—750 mg/m³ (100—200 ppm) of n-hexane for 4.5 h.

In 24-h urine specimens 5 % of the n-hexane injected intraperitoneally into guinea pigs was recovered as 2-hexanol (2); 1.7 % of the n-hexane (330 mg/kg) injected intraperitoneally into rats was recovered as 2-hexanol in 24-h urine specimens (however, no 1-hexanol was recovered) (46, 47). In another experiment 0.7 % the n-hexane (73—363 mg/kg) intraperitoneally injected into rats was recovered in the urine as 1-hexanol; 70 % of the 1-hexanol was in the glucuronide form and 30 % in the free form. In addition 27 mg of 2-n-hexanone was found irrespective of the dose (21).

Biological half-time

Ten humans were experimentally exposed to n-hexane (306—429 mg/m³; 87—122 ppm) for 4 h. A two-compartment kinetic model described the elimination through the lungs for the first 4 h after the cessation of exposure. The half-times were 13 min and 2.5 h for the smallest and largest exposure, respectively (44).

The maximal blood concentration occurs 30 min after an intraperitoneal injection of 2-hexanone into rats. Thereafter, the elimination follows the two-compartment kinetic model with half-times of 10 min and 7 h (2).

Experimentally, rats were exposed to an n-hexane concentration of 3,520 mg/m³ (1,000 ppm) for 6 h a day for 5 d. The elimination of hexane, 2-hexanone, and 2,5-hexanedione from the blood, liver, kidneys, brain, and sciatic nerve was studied after the cessation of exposure. Hexane and 2-hexanone was not detected in any of the tissues after 4—8 h. Less than 0.05 μg of 2,5-hexanedione/g of tissue was discovered in the blood, liver, kidneys, and brain after 24 h. Approximately 0.6 μg/g and approximately 0.5 μg/g was recovered from the sciatic nerve after 12 and 24 h, respectively (15).

Factors influencing the metabolic model

Rats that had been exposed 8 h a day for 15 d to an n-hexane concentration of 31,700 mg/m³ (9,000 ppm) and a butanone concentration of 2,933 mg/m³ (1,000 ppm) developed polyneuropathy more rapidly than rats that had been exposed to 35,200 mg/m³ (10,000 ppm) of the former. Rats exposed to a butanone concentration of 17,600 mg/m³ (6,000 ppm) developed no neurological abnormalities (4). This experiment was set up because of findings among sniffers (38). The concentrations of hexane, the hexane transformation products, and the butanone content of the blood were examined.

Rats exposed to 2-hexanone (900 mg/m³; 225 ppm) and butanone (2,200 mg/m³; 750 ppm) had the following blood concentrations: 240 mg/kg (24 mg%) and 2 mg/kg (0.2 mg%), respectively. 2,5-Hexanedione was not detected. 2-Hexanone and its transformation products were not found in the blood after exposure to 2-hexanone alone (2). Rats exposed only to 2-hexanone developed no polyneuropathy, whereas rats exposed to both compounds developed serious polyneuropathy (2).

Liver microsomes from rats that had received phenobarbital (80 mg/kg) intraperitoneally once a day for 2 d had an increased monooxygenase activity in vitro. The biotransformation of n-hexane to 2-

and 3-hexanol was increased six to seven times (24).

n-Hexane and its oxidation products 2,5-hexanedione and 2-hexanone increase the hepatotoxic and nephrotoxic effects of chloroform in rats (30). n-Hexane causes the greatest increase in the toxicity of chloroform, whereas 2-hexanone causes the least (30).

Toxicologic mechanisms

The exact mechanism of the hexacarbon neuropathy symptom-complex is not known.

Savolainen (54) is of the opinion that man must separate the short- and long-term effects. Narcosis, coma, and, eventually, respiratory arrest are the short-term effects. They may be caused by the incorporation of the solvent molecule into the nerve cell membranes in the central nervous system (CNS), a phenomenon which influences the transportation of ions.

The long-term effects after n-hexane exposure are probably caused by the oxidation of n-hexane to 2,5-hexanedione. Both n-hexane and methyl-n-butyl ketone are metabolized to 2-hexanol and further metabolized to 2,5-hexanediol and 2,5-hexanedione (46). Methyl-n-butyl ketone, 2,5-hexanedione, and 2-hexanol have been shown to cause the same functional and morphological changes as n-hexane after occupational exposure and in animal experiments (47, 60).

The relative toxicity of n-hexane, methyl-n-butyl ketone, and their metabolites has been measured by noting the time it took for rats to develop clincal paralysis of the hind legs. The following compounds, mentioned in decreasing order of potency, have all caused identical clinical and morphological changes: 2,5-hexanediol, methyl-n-butyl ketone, 2-hexanol, and n-hexane (35).

The 10-nm neurofilaments are important in the rapid protein transport of axons. One can imagine the nerve-toxin interference with the tertiary structure of the filaments. Such interference would bring about an accumulation of neurofilaments in the axons and thus produce a transport block (53).

The rapid axon transport system is energy demanding and needs glutaraldehyde-3-phosphate dehydrogenase. In vitro experiments with larger doses have demonstrated that n-hexane metabolites and methyl-n-butyl ketone inhibit the activity of both crystalline and endogenous glutaraldehyde-3-phosphate dehydrogenase in the central and peripheral nervous systems. However, nonneurotoxins do not inhibit the enzyme (53).

Organ effects

Skin, mucous membranes and conjunctivas

The inhalation of vapor is the most frequent type of contact with hexane. Both spills and spray can lead to eye and skin contact.

The toxicologic reference books report that hexane is irritating for human mucous membranes in the respiratory system and the eye in concentrations of about 5,000 mg/m^3 (1,500 ppm) (9, 10, 11, 56). Yamamura (66) examined 93 shoe and leather workers, 59 % of whom had areas of rough, cold, and erythematous skin on the distal parts of their legs.

It has been shown that hexane is a mild skin irritant of rabbits after primary contact (32).

One milliliter of n-hexane dropped on guinea pigs causes pycnotic nuclei in all layers of the epidermis. Longer exposure leads to progressive degeneration with karyolysis and perinuclear edema. The epidermis can be separated from the corium between the basal membrane and the basal cell layer. Pseudoeosinophil cells infiltrate the outer layer of the dermis after 4 h (36).

Prolonged and repeated skin contact causes erythema and scar formation in rabbits (32).

According to the Darize system, dropping 0.1 ml of n-hexane into the conjunctiva of rabbits results in minimal irritation, grade one (32).

Respiratory organs

In spite of the frequent contact of n-hexane with the respiratory organs, no references show human damage to that area.

The aspiration of 0.2 ml of hexane by rats results in a few seconds in death due

to cardiac arrest, respiratory paralysis and asphyxia. The weight of the lungs increases, on the average, by approximately 2 g, which indicates transudation from the alveolar capillaries into the alveolar spaces. Hemorrhaging has been not demonstrated (27).

Liver

Workers and sniffers who have breathed hexane vapors have significantly lower serum cholinesterase levels than control groups (3, 45). The authors who studied this subject concluded that this phenomenon is caused by a primary toxic action on the liver. The workers in this experiment were exposed to 360—2,160 mg/m³ (100—600 ppm) for two to six months. The exact exposure of the sniffers was unknown. The content of n-hexane in the handled products was high, between 50—95 %. However, in addition to hexane, toluene, other alcanes, and other solvents were present.

Liver examinations have for the most part been normal in the existing reports and examinations. However, in a few individual cases high values of serum creatine phosphokinase, lactate dehydrogenase, and ornithine carbamoyl transferase, have been demonstrated, as well as positive urobilinogen reactions in the urine (28, 66).

It has also been demonstrated that n-hexane can lead to a decrease in the serum triglyceride level (13).

The distribution of n-hexane was measured in the blood, liver, brain, lungs, kidneys, spleen, and adrenals of rats that had been exposed to n-hexane vapor in the concentration of 170,000 mg/m³ (47,200 ppm) for 2—10 h. After 10 h the liver was still not fully saturated. A linear accumulation of fat (triglycerides) was noted. In this manner the affinity of the liver for hexane was increased. Thus, the complete saturation of the liver did not take place as long as the content in fat was increasing (13).

Other studies have not shown liver damage in guinea pigs after skin contact (36) or in rats after oral intake (30). Hexane potentiated the liver toxicity of trichloromethanes when the two were given simultaneously to rats (30).

Blood and blood-forming organs

In humans exposed to n-hexane vapor several investigators have found mild hypochronic anemia that becomes normal under hospitalization (51, 62, 66). In addition pleocytosis has been found in a young sniffer (38).

Rabbits were given intravenous and subcutaneous daily injections of n-hexane for 6 d. After the first day, a drop in the leucocyte count from 2,800/mm³ to 600/mm³ was noted. Thereafter, the count gradually normalized over the five remaining days. After the sixth day the bone marrow was microscopically examined and found to be normal (8).

Mild changes in the reticuloendothelial system of the spleen in the form of hemosiderin pigmentation and the occurrence of giant cells has been described for rats exposed to an n-hexane concentration of 3,060 mg/m³ (850 ppm) for 143 d (37).

Kidneys and the gastrointestinal and cardiovascular systems

No pathological changes in the kidneys, stomach, gastrointestinal system, heart, or blood vessels have been described after exposure to n-hexane.

Central nervous system

Short exposure to high doses of n-hexane causes narcosis in man. Headache and nausea are caused by 5,400 mg/m³ (1,500 ppm). Confusion and dizziness are caused by exposure to 18,000 mg/m³ (5,000 ppm) for 10 min (43).

Headache, nausea, and anorexia are the most commen CNS symptoms after the inhalation of n-hexane.

Altenkirch & Mager (3) found horizontal nystagmus with a rotary component in four young individuals who had sniffed glue containing n-hexane and toluene.

The same morphological changes have been found in both the peripheral and central nervous system. These histopathological changes had been found in the spinal cord, the medulla oblongata, the cerebellum, and the cerebrum (4, 56).

Shaumburg & Spencer (56) postulated that dysfunction at the peripheral nerve level masks signs of disturbance in the CNS. Complete healing of CNS lesions

seldom occurs. This situation can explain the reflex abnormalities and spasiticity of the legs that have been observed 2 a after exposure.

Peripheral nervous system

The outstanding toxic effects of n-hexane involve the peripheral nervous system (table 1). The symptom complex, which is known as hexacarbon neuropathy, is comprised of sensory and motor abnormalities, the motor abnormalities playing the larger role.

Polyneuropathy has been described following occupational exposure to n-hexane, after the sniffing of hexane vapor in order to be "high," and for experimental animals, especially rats and mice, that have been exposed only to n-hexane.

Peripheral nerve biopsies from both man and experimental animals under a light microscope show significant swelling of the nerve with thinning of the myelin sheath and retraction of the paranodal area (3, 28, 37, 38, 51, 56, 58, 59, 61, 63).

Under the electron microscope, the swollen axons have been found to contain an increased number of closely packed neurofilaments interspaced with a few glycogen granules. "Dense bodies" and mitochondria were found spread throughout the myelinized fibers. Within the Schwann cells proliferation processes and cytoplasmic increases of both glycogen and Reichert granules have been described (3, 28, 29, 38, 51, 56, 58, 59, 63).

Herskowitz et al examined the intramuscular sensory and motor end plates in man under the electronic microscope (29). The nerves contained an increased amount of neurofilaments. The mitochondria that contained "dense bodies" had a significant "onion-bulb" formation and abnormal membrane structures. The motor end plates contained a swelling of the terminal axons, an increased amount of degenerated mitochondria, and glycogen granules. In addition "dense bodies," large osmiophil bodies, and an increased number of both synaptic folds and vesicles were described.

The histograms of myelinized nerve fibers from the sural nerve in sniffers

Table 1. Symptoms and clinical findings and electrophysiological and morphological changes in peripheral nerves after exposure to n-hexane.

	Human data based on occupational exposure	Animal data
Exposure dose	360—2,160 mg/m³ (100—600 ppm) with maximum exposure at 9,000 mg/m³ (2,500 ppm)	1,400—36,000 mg/m³ (400—10,000 ppm)
Concentration	70—95 % n-hexane	n-Hexane alone
Latency period	2—6 months	4—5 months
Symptoms	Sensation disturbances; muscle weakness; and distal symmetric pain in the legs	Walking disturbances
Clinical changes	Muscle atrophy; hypotonic, decreased muscle strength; deficient reflexes with foot drop and in some cases hand drop; paresthesia and hypoesthesia distally in the arms and legs	Muscle atrophy and foot drop
Electrophysiological changes	Decreased motor nerve conduction and decreased sensory nerve conduction with an increased distal latency period; electromyographic indications of neurogenic damage	Decreased motor nerve conduction and sensory nerve conduction; increased refractory period; decreased excitability
Light microscope	Swelling of nerves with thinning of the nerve sheath and paranodal retractions; muscle atrophy of fibers	Local dilatation of axons with a thinning of the myelin sheath; ovoid change of the myelin
Electron microscope	Swelling of axons with closely packed masses of neurofilaments; other signs of degeneration	Closely packed masses of long neurofilaments
Maximum	In certain cases between 2—3 months after the cession of exposure	
Recovery time	Very long (0.5—1 a); in some cases symptoms and clinical polyneuropathy after 2 a	
References	1, 14, 18, 29, 45, 48, 51, 66	37, 56, 58, 64