Acceptable Limits for n-Hexane in Spacecraft Atmospheres

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INTRODUCTION: The Spacecraft Maximum Allowable Concentrations (SMACs) for C2-C9 alkanes set by NASA in 2008 under the guidance and approval of the National Research Council specifically excluded SMACs for n-hexane. Unlike other C2-C9 alkanes, n-hexane can cause polyneuropathy after metabolism in humans or rodents and so requires more stringent SMACs than the other members of this group do. This document reviews the relevant published studies of n-hexane toxicity to develop exposure duration-specific SMACs for n-hexane of 200 ppm for 1 hour, 30 ppm for 24 hours, and 2.4 ppm for 7 days, 30 days, 180 days, and 1000 days.

KEYWORDS: SMACs, polyneuropathy, alkanes, n-hexane.

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Spacecraft Maximum Allowable Concentrations (SMACs) are limits set by NASA for the airborne concentration of selected contaminants in manned spacecraft to which humans may be safely exposed for specific durations. SMACs set for exposures of 1 h or 24 h permit mild, reversible adverse effects in exposed astronauts if exposure becomes necessary to perform emergency operations such as stopping or cleaning up a leak of a potentially toxic chemical. SMACs for continuous exposure durations of 7, 30, 180, and 1000 d are set to protect astronauts from all adverse effects.

Normal hexane (n-hexane), a commonly used liquid organic solvent, is volatile. Exposures to its vapors at concentrations up to 500 ppm for 3 to 5 min are nonirritating to human eyes, nose, or throat,[3] but at higher concentrations and/or longer durations, n-hexane vapors can cause dizziness and a variety of other adverse central nervous system effects similar to many volatile organic solvents. Unlike most other solvents, however, repeated or chronic exposure of humans and animals to n-hexane can cause progressive damage to both sensory and motor neurons, resulting in polyneuropathy. Standards for n-hexane exposure were set decades ago by the U.S. government and by nongovernmental agencies, but some standards have not been revised to incorporate more recent data which show that neurotoxicity can be caused by concentrations of n-hexane considerably lower than the current allowable limits.[28] An attempt by OSHA to lower the Permissible Exposure Limit (for 8 h/d occupational exposures for a working lifetime of 40 yr) for n-hexane from 500 ppm to 50 ppm was vacated by the 11th U.S. Court of Appeals in 1992, so the Permissible Exposure Limit remains at 500 ppm despite scientific evidence that 500 ppm is not protective against polyneuropathy (see **Table I**).[28]

Uses, Chemical and Physical Properties of n-Hexane[1]

Hexane is a minor constituent in gasoline and other petroleum products. Hexane is used as a solvent to extract edible oils from seeds and vegetables and to clean textiles, as well as a degreasing agent.[3] It is also a solvent for many consumer/industrial products, including glues used in shoemaking and roofing, and in varnishes and inks. Hexane could remain in these products as a trace impurity or residue. Hexane has (rarely) been used as an ingredient in experiments conducted on the International Space Station or Shuttle.

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Table I. n-Hexane Exposure Limits Set by Other Organizations.

ORGANIZATION	LIMIT	ppm	mg · m^{-3}	DURATION
OSHA	PEL	500	1800	Occupational
NIOSH	REL	50	180	Occupational
ACGIH	TLV	50	176	Occupational
DFG	MAK	50	180	Occupational
NAC/AEGL	AEGL-1	—	—	—
NAC/AEGL	AEGL-2	2900	10,000	1 h

OSHA: Occupational Safety and Health Administration; NIOSH: National Institute of Occupational Safety and Health; ACGIH: American Congress of Governmental and Industrial Hygienists; DFG: Deutsche Forschungsgemeinschaft/German Research Foundation; NAC: National Advisory Council; AEGL: Acute Exposure Guideline Levels; PEL: Permissible Exposure Limit; REL: Recommended Exposure Limit; TLV: Threshold Limit Value; MAK: Maximale Arbeitsplatz Konzentration/Maximal Admissible Concentration.

"n-Hexane" refers specifically to the straight-chain isomer, whereas "hexane" usually refers to a mixture of hexane isomers and closely related compounds present in technical grade or commercial grade hexane; nevertheless, the word "hexane" is occasionally used to refer to n-hexane. Commercial hexane may contain from 20 to 85% n-hexane together with various amounts of other hexane isomers: 2-methylpentane, 3-methylpentane, 2-3-dimethylbutene, and cyclohexane; the mixture may also contain small quantities of cyclopentane, pentane and heptane isomers, acetone, methyl ethyl ketone, dichloromethane, and trichloroethylene. Trace amounts of benzene may be present.[47] The solubility of n-hexane in water is low, but it is miscible with alcohol, chloroform, and ether.[47] The properties of n-hexane include:

- Molecular weight: 86.10
- Specific gravity: 0.660 @ 20°C
- Freezing point: −95°C
- Boiling point: 68.95°C
- Vapor pressure: 124 torr @ 20°C
- 145 torr @ 25°C
- Flash point: −22°C, closed cup
- Explosive limits: lower = 1.18% (11,000 ppm); upper = 7.8% by volume in air
- Conversion factors: 1 ppm = 3.52 mg · m^{-3}; 1 mg · m^{-3} = 0.284 ppm

n-Hexane in Spacecraft

Only rarely has hexane been detected (method detection limit is 0.035–0.05 mg · m^{-3}, depending on which air quality monitor was used) in the atmospheres of U.S. and international spacecraft, and then only at concentrations < 3.5 mg · m^{-3} (< 1 ppm) [Gazda D. Emails on 12/16/2019 and 12/17/2019 providing measurement data from the Air Quality Monitor on the International Space Station; and unpublished laboratory measurements of grab samples of spacecraft atmospheres: levels of n-hexane in spacecraft atmospheres 2015–2019; 2019.].

Absorption, Distribution, Metabolism, and Excretion of n-Hexane

n-Hexane is absorbed after inhalation, ingestion, or topical application to the skin. It easily crosses the alveolar-capillary membrane and enters the bloodstream but is poorly absorbed from the gastrointestinal tract and dermal absorption is slow. Most pharmacokinetic studies have been done in rats, but in human volunteers, the lungs took up about 28% of inhaled n-hexane, reaching a constant level after 2 h of exposure.[15] Alveolar retention is about 25% of the inhaled dose. Final absorption is 15–17% of the total respiratory uptake.[32] In numerous workers, percutaneous absorption of n-hexane represented as much as 50% of the total absorbed dose.[9] Peak blood levels occur within 1 h after inhalation or percutaneous exposure.[22]

After single or repeated inhalation exposures of rats to n-hexane vapors at 1000 ppm for 6 h/d, n-hexane was preferentially distributed to tissues with a high lipid content, such as nerves and brain, as well as to the kidneys.[8] Target tissue concentrations of n-hexane and several of its metabolites, including 2,5-hexanedione (2,5HD), the metabolite of greatest toxicological interest, have been measured in rats at various times after 6-h inhalation exposures to 500, 1000, 3000, and 10,000 ppm of n-hexane.[4] The authors concluded that "… *n*-hexane exposure concentration cannot be directly correlated with tissue 2,5HD concentrations, and severity of neuropathy may not be directly related to *n*-hexane exposure concentration, particularly at concentrations above 1000 ppm."[4]

In rats, n-hexane is metabolized in the liver into 2-hexanol, 2-hexanone, 2,5HD, 2,5-hexanediol, and 5-hydroxy-2-hexanone.[24] In humans, the major urinary metabolite is 2,5HD.[39] Many studies of n-hexane metabolism have implicated 2,5HD as the toxic metabolite in rats and humans. Some studies, however, have proposed that the toxic chemical species is not 2,5HD itself, but a pyrrole formed from 2,5HD reacting with free amino groups in proteins.[17,19,20,41] The pyrroles, after undergoing further oxidation, can covalently cross-link with proteins.

Co-exposure to other solvents can modify the rate of metabolism of n-hexane in rats. In addition to hexane, solvent mixtures often contain toluene, xylene, acetone, and methyl ethyl ketone. Several studies have shown that acetone and methyl ethyl ketone can potentiate n-hexane-induced neurotoxicity in rats.[10,26,27,29] In one of the more recent studies, Cardona et al.[10] reported that co-exposure of workers to acetone and n-hexane increased the proportion of free 2,5HD in urine.

Lung uptake and excretion of n-hexane were studied in 10 workers in a shoe factory.[32] Alveolar excretion was monitored during a 6-h postexposure period. Uptake was calculated from lung ventilation, the retention coefficient, and environmental concentrations. The amount of exhaled n-hexane was calculated from the decay curve. The postexposure alveolar excretion was about 10% of the total uptake. The main metabolites of n-hexane were identified and measured by capillary gas chromatograph/mass spectrometer in spot urine samples collected before, at the end, and 15 h after the same working shift. Urinary concentrations were low, though related to n-hexane in the air. The concentration of 2,5HD in the end-of-shift urine samples gave the best estimate of overall exposure. About 3 mg of 2,5HD/g creatinine in urine corresponded to about 50 ppm of n-hexane in the air (mean daily exposure).

In a study of blood concentrations of n-hexane in volunteers exposed to 102 or 204 ppm n-hexane for 4 h, steady-state concentrations of n-hexane were achieved within 100 min, with a rapid decline to half those levels in the first 10 min after ceasing

exposure, followed by a slower decline with a half-life of 1.5 to 2 h.[46]

Adverse Effects of Acute Inhalation Exposures of Humans to n-Hexane

There are inconsistent values reported in the scientific literature for the concentrations at which n-hexane can cause irritation. n-Hexane vapor is reported to be irritating to the eyes and nose at 511 ppm (1800 mg · m^{-3}).[40] In human volunteers, exposures to 5000 ppm (17,600 mg · m^{-3}) for 10 min caused marked vertigo and nausea.[5] The American Conference of Governmental Industrial Hygienists reports that, "In humans, inhaling 2,000 ppm for 10 min resulted in no effects, but 5,000 ppm caused dizziness and… giddiness. …Slight nausea, headache, eye and throat irritation occurred at 1,400–1,500 ppm. …No ocular or mucous membrane irritation was found at 5,000 ppm in un-acclimated subjects."[1] Lewis[30] reports that inhalation of n-hexane at 2500–1000 (sic) ppm for 12 h produces drowsiness, fatigue, loss of appetite, and paresthesia in distal extremities. Conflicting data reported by Lewis state that 2000 ppm for 10 min produces no symptoms but that 2500–500 (sic) ppm for 10 min produces muscle weakness, cold pulsation in the extremities, blurred vision, headache, anorexia, and onset of polyneuropathy.[30] No reports were found to confirm that exposure to any concentration of n-hexane for 10 min would lead to the onset of polyneuropathy.

Adverse Effects of Sub-Chronic Inhalation Exposures of Rodents to n-Hexane

For subchronic exposures of rats to n-hexane, the dose rate and duration of exposure determine the incidence, rapidity of onset, and severity of neuropathy as illustrated in the following examples. Rats continuously exposed to 400 to 600 ppm n-hexane developed neuropathy after 7 wk, whereas no changes were found in rats exposed intermittently (9 to 10 h/d, 5 to 6 d/wk) to 500 ppm for 30 wk.[2,18,42] No morphological changes to nerves were seen in rats exposed 9 h/d, 5 d/wk for 30 wk to 500 ppm n-hexane or to 1500 ppm for 14 wk.[18] In rats exposed to 5000 ppm n-hexane intermittently (9–10 h/d, 5–6 d/wk), however, giant axonal swellings appear at 14 wk.[18]

Polyneuropathy in Workers Chronically Exposed to n-Hexane

Neurotoxic effects to both the peripheral and the central nervous systems and both sensory and motor neurons have been reported after occupational exposure or recreational abuse of n-hexane.[3] After exposure ceases, gradual recovery is generally quite good, although adverse effects may temporarily intensify for several weeks or months. In cases of severe neuropathy, however, permanent neural damage, both centrally and peripherally, has been reported.[15]

A 1993 report[12] documented peripheral sensorimotor neuropathy in 56 workers (all men) in an offset printing factory in Hong Kong. Symptoms were found in 20/56 workers and subclinical electrophysiological effects were found in 26 of the 36 asymptomatic workers. These offset machine workers worked 12 h/d, 6 d/wk for durations ranging from 1 mo to 12 yr (mean = 2.6 yr). Time-weighted average n-hexane concentrations from personal air samples were 80 ppm to 210 ppm (mean 132 ppm). A direct relationship between the development of neuropathy and the duration of employment was not observed in this study. Because of this lack of correlation, one should conservatively assume that exposures to the lowest reported concentration (80 ppm) for the shortest duration (12 h/d, 6 d/wk, 1 mo) could cause neuropathy. Since 10 of the workers were asymptomatic, however, it is possible that 80 ppm exposures for 1 mo are below a threshold for inducing adverse effects. Alternatively, the lack of adverse effects in some workers could be due to individual differences in adsorption, metabolism, or excretion.

Similar results were reported in a study of workers in printing factories in Taipei, Taiwan.[13] Of the workers examined (number not reported), 44 had polyneuropathy, 10 had subclinical decrements in nerve conduction velocities, and 14 showed no detectable adverse effects. Unfortunately, however, this study did not report the n-hexane concentrations to which the workers had been exposed nor the durations of the exposures. Nevertheless, the fact that 14 of the exposed workers exhibited no adverse effects suggests the existence of a threshold concentration below which n-hexane does not induce adverse effects, even for chronic exposures.

Occupational exposure to n-hexane at 23.6 ppm (83.2 mg · m^{-3}) time-weighted average in 27 Iranian male shoemakers produced no symptoms, but electrophysiological studies showed that the amplitudes of sensory nerve action potential (SNAP) for median and sural nerves were significantly lower in exposed subjects compared to 20 unexposed normal controls.[34] Similarly, a study of 20 workers exposed for prolonged periods to n-hexane and with urinary 2,5HD concentrations exceeding the 5 mg · L^{-1} Biological Exposure Index were found to have significantly reduced amplitudes of SNAPs of the sural and ulnar nerves, but no other neurological anomalies.[35] The amplitude of the SNAP in the sural and median nerves correlated significantly with the number of years worked. These electrophysiological effects are likely indicators of some n-hexane-induced nerve damage (loss of function of axons). Although it is not known whether these electrophysiological effects would have progressed to symptomatic neuropathy upon continued exposure to low concentrations of n-hexane, it is prudent to assume that this could be the case. Thus, 23.6 ppm (83.2 mg · m^{-3}) will be considered a Lowest Observed Adverse Effect Level (LOAEL) rather than a No Observed Adverse Effect Level (NOAEL) when it is used as a point-of-departure for calculating an acceptable concentration (AC) for long-term exposures.

In addition to numerous reports of sensorimotor neuropathies involving peripheral nerves, case reports of neuropathy in workers exposed to n-hexane occasionally include reports of cerebral and brain stem dysfunctions (changes in behavior, nystagmus, maculopathy, visual evoked potentials, and Parkinson's disease[38,40,44]).

Continuous vs. Intermittent Exposures. In rats inhaling 500 ppm n-hexane, clinical or morphological signs of neuropathy were seen only in animals continuously exposed (24 h/d, 7 d/wk),

but not in animals exposed intermittently (8 h/d) for 40 wk.[2] This is consistent with partial recoveries after removal from exposure that have been reported in many cases of polyneuropathies in workers,[14,21,23,25,31,36] despite the total exposure duration in rats being higher (320 h) in the intermittently exposed group than in the continuously exposed group (168 h).

Testicular Toxicity

In rats, 2,5HD testicular toxicity results from alterations in Sertoli cell microtubules caused by pyrrole-dependent cross-linking of cytoskeletal elements. This results in a loss of Sertoli cell paracrine support of the germ cells.[45] Unlike toxicity to the nervous system, 2,5HD-induced toxicity to rat testicles is dependent on the rate of intoxication, independent of the total dose.[7] Low, subneurotoxic doses of 2,5HD, the presumed toxic metabolite of n-hexane, have been reported to cause progressive testicular toxicity in rats,[6,24] but there have been no published reports of sterility or reproductive toxicity in humans after exposures to n-hexane (see **Table II**).

Calculation of ACs

The methodology for setting SMACS is documented in a book published by the National Academy of Sciences.[33] SMACs for 1-h and 24-h exposures are airborne concentrations that permit some minor, temporary adverse effects, but permit astronauts to respond to an unexpected release. Limited data are available in the scientific literature to permit confident calculation of the maximum dose or dose-rate of n-hexane vapor to which humans could be exposed for exposure durations of 1 h or 24 h without risk of unacceptable adverse health effects. Human exposure data summarized below from studies reported by Shibata et al.[44] and by Veuelmans et al.[46] support the conclusion that exposure to concentrations of 54 ppm pure (sic) n-hexane for 2 h exercising at 50 W[44] and 100 or 200 ppm for 4 h at rest or 100 ppm for 3 h exercising at 100 W[46] will not produce any adverse effects. Although the number of subjects in each study was small (four or six volunteers, respectively), the reported lack of adverse effects supports the use of these data for setting the 1-h and 24-h SMACS, since they permit temporary, minor adverse effects.

Based on the data of Veulemans et al., exposures to 200 ppm n-hexane caused no adverse effects in 4 h; therefore, 200 ppm can be set as an AC for a 1-h exposure that would be expected to produce at most, mild, transient adverse effects. Although the small number of subjects (six) would statistically require the application of a factor of 10/sqrt(6) = 0.24 to the observed NOAEL to estimate the true NOAEL, the 1-h SMAC is not meant to be a NOAEL, so this "small n" factor will not be applied. No exposure-duration factor is applied to the 1-h AC because doing so would unacceptably increase the AC beyond what the published data support. Note that if the safety factors were to be applied, the small n factor (0.24) and an exposure duration factor (4 h/1 h) would essentially cancel each other out.

$$1\text{-h AC} = 200\,\text{ppm}\ (720\,\text{mg}\cdot\text{m}^{-3})$$

Although ACs for short-term exposures of 24 h are set to permit mild, temporary effects, no data were found regarding short term exposures to concentrations that caused mild adverse effects. Using the 200 ppm 4-h NOAEL from Veulemans as a point of departure and applying a factor of 4 h/24 h to adjust for the duration of exposure, the 24-h AC can be calculated as 200 ppm × 4 h/24 h = 33 ppm, rounded down to 30 ppm.

$$24\text{-h AC} = 30\,\text{ppm}\ (106\,\text{mg}\cdot\text{m}^{-3})$$

The study of Neghab et al.[34] in Iranian shoemakers is used to calculate ACs for ≥ 7 d. A safety factor of 3 is used to extrapolate from the occupational exposures (12 h/d, 6 d/wk for durations of 1 mo to 12 yr) to continuous exposures. This safety factor of 3 is reduced from the more common factor of 5 because the workers in this study worked 12 h/d, 6 d/wk rather than the more common 8 h/d, 5 d/wk. An additional safety factor of 3 is applied to estimate a subthreshold concentration from data showing subclinical effects. The two factors of 3 are combined and rounded up to a safety factor of 10. The resulting AC of 23.6

Table II. Inhalation Toxicity Summary.

n-HEXANE (ppm)	DURATION	SPECIES	EFFECTS	REFERENCE
HUMANS				
Not Reported	Occup, various, not reported	Man, *N* = 28	Polyneuropathy	Chang YC[13]
80–210 ppm mean = 132 ppm	Occup, 12 h/d, 6 d/wk, 1 mo to 12 yr	Man, *N* = 56	36%: peripheral neuropathy 46%: subclinical neuropathy (decr. amplitude of SNAP & MNAP)	Chang CM et al.[12]
23.6 ppm	Occup	Man	Subclinical neuropathy (decr amplitude of SNAP)	Neghab et al.[34]
RATS				
500 ppm	>20 h/d, 7 d/wk, cont.	Rat	Hind-limb paralysis, histological signs of nerve damage	Altenkirch et al.[2]
400–600 ppm	45 d, continuous		Giant axonal swellings and fiber degeneration in CNS & PNS	Schaumburg & Spencer[42]
5000 ppm	16 h/d, 6 d	Rat	Decr. motor nerve conduction velocity	DeMartino et al.[16]
5000 ppm	16 h/d, 6 d/wk, 4 wk	Rat	Paralysis	DeMartino et al.[16]
6500 ppm	6 h/d, 5 d/2k, 13 wk	Rat	Axonopathy in tibial nerve in 1 of 5 male rats	Cavender et al.[11]
10,000 ppm	6 h/d, 5 d/2k, 13 wk	Rat	Axonopathy in tibial nerve in 4 of 5 male rats	Cavender et al.[11]
10,000 ppm	6 h/d, 5 d/2k, 13 wk	Rat	Axonopathy in medulla in 1 of 5 male rats	Cavender et al.[11]

occup., occupational; decr., decreased; SNAP, sensory nerve action potential; MNAP, motor nerve action potential; CNS, central nervous system; PNS, peripheral nervous system.

Table III. Acceptable Concentrations.

END POINT, EXPOSURE DATA[REF.]	UNCERTAINTY FACTORS					ACCEPTABLE ppm @ EXPOSURE DURATIONS					
	SPECIES	NOAEL	TIME	SPECIES	SPACEFLIGHT	1 h	24 h	7 d	30 d	180 d	1000 d
4-h NOAEL = 200 ppm[46]	Human	1	N.A.	1	1	200	—	—	—	—	—
4-h NOAEL = 200 ppm[46]	Human	1	N.A.	1	1	—	30	—	—	—	—
Subclinical electrophysiological decrements, occupational, 23.6 ppm[34]	Human	10	N.A.	1	1	—	—	2.4	2.4	2.4	2.4
Explosion [10% of 110,000 ppm LEL]	N.A.	N.A.	N.A.	1	1	11,000	11,000	11,000	11,000	11,000	11,000
SMACs						200	30	2.4	2.4	2.4	2.4

NOAEL: no adverse effect level; N.A.: not applicable; LEL: lower explosive limit; SMACS: Spacecraft Maximum Allowable Concentrations.

ppm/10 = 2.4 ppm (8.4 mg · m^{-3}) is used for all exposure durations greater than 24 h, with the assumption that 2.4 ppm is effectively below a threshold concentration for production of clinical or subclinical neurological adverse effects.

$$7\text{-d}, 30\text{-d}, 180\text{-d}, 1000\text{-d}, \text{ACs} = 2.4\,\text{ppm}\,(8.4\,\text{mg}\cdot\text{m}^{-3})$$

None of the adverse effects reported for inhalation of vapors of n-hexane are expected to be affected by spaceflight or microgravity, so no spaceflight-specific adjustment factor will be applied.

ACs were calculated for human NOAEL values, subclinical electrophysiological decrements, and explosion. For each exposure duration, the lowest AC among these adverse effects was selected as the SMAC, as shown in **Table III**.

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