

4.4. OTHER STUDIES	54
4.4.1. Acute Toxicity Data	54
4.4.2. Studies with Mixtures Containing n-Hexane	58
4.4.2.1. Oral Exposure	59
4.4.2.2. Inhalation Exposure	59
4.4.2.2.1. Prechronic Studies	59
4.4.2.2.2. Chronic Exposure	59
4.4.2.2.3. Reproduction/Developmental Studies	61
4.4.3. Potentiation and Antagonism Studies	63
4.4.4. Mode of Action Studies	70
4.4.5. Genotoxicity Studies	76
4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION-ORAL AND INHALATION	79
4.5.1. Oral Exposure	79
4.5.2. Inhalation	80
4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION-SYNTHESIS OF HUMAN, ANIMAL, AND OTHER SUPPORTING EVIDENCE, CONCLUSIONS ABOUT HUMAN CARCINOGENICITY, AND LIKELY MODE OF ACTION	90
4.6.1. Summary of Overall Weight-of-Evidence	90
4.6.2. Synthesis of Human, Animal, and Other Supporting Evidence	90
4.7. SUSCEPTIBLE POPULATIONS AND LIFE STAGES	92
4.7.1. Possible Childhood Susceptibility	92
4.7.2. Possible Gender Differences	92
5. DOSE RESPONSE ASSESSMENT	94
5.1. ORAL REFERENCE DOSE (RfD)	94
5.2. INHALATION REFERENCE CONCENTRATION (RfC)	95
5.2.1. Choice of Principal Study and Critical Effect - with Rationale and Justification	95
5.2.2. Methods of Analysis	99
5.2.2.1. Adjustment to a Human Equivalent Exposure Concentration	101
5.2.3. RfC Derivation - Including Application of Uncertainty Factors (UFs) .	102
5.2.4. Previous Inhalation Assessment	104
5.3. CANCER ASSESSMENT	105
6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE	106
6.1. HUMAN HAZARD POTENTIAL	106
6.2. DOSE RESPONSE	107
6.2.1. Noncancer	107
6.2.2. Cancer	108

LIST OF TABLES AND FIGURES

Table 1. Concentration of n-hexane in blood and tissues of pregnant F-344 rats immediately after a 6-hour exposure to 1000 ppm n-hexane	5
Table 2. Tissue distribution of radioactivity in male F-344 rats 72 hours after a 6-hour inhalation exposure to various concentrations of [1,2- ¹⁴ C]-n-hexane	6
Table 3. Steady state concentration of n-hexane concentrations in male F-344 rats exposed via inhalation	7
Table 4. Metabolism of n-hexane following a 6-hour exposure of pregnant F-344 rats on gestation day 20	10
Table 5. Apparent kinetic parameters for n-hexane hydroxylation in rat liver and lung microsomes	11
Table 6. Metabolites excreted in urine during a 72-hour period following inhalation exposure to n-hexane in male F-344 rats	17
Table 7. n-Hexane metabolite levels in urine of Wistar rats co-exposed to n-hexane and toluene	18
Table 8. Persistent and transient neurological symptoms* following occupational exposure to n-hexane in a tungsten carbide alloy factory	24
Table 9. Results of neurological tests in control subjects and those occupationally exposed to n-hexane in a tungsten carbide alloy factory	25
Table 10. Nerve stimulation in control subjects and those occupationally exposed to n-hexane in a tungsten carbide alloy factory	26
Table 11. Motor neurographic findings in patients with n-hexane polyneuropathy	32
Table 12. Nerve conduction study findings in printers with n-hexane induced polyneuropathy	33
Table 13. FM-100 Hue test scores of n-hexane-exposed and non-exposed groups	35
Table 14. Experimental protocol for phase I of a 6-month inhalation study of n-hexane and mixtures containing n-hexane plus hydrocarbon isomers in male Sprague-Dawley rats	45
Table 15. Organ weight changes (relative to body weight) in male Sprague Dawley rats exposed to n-hexane 22 hours/day, 7 days/week for 6 months	47
Table 16. Experimental protocol for phase II of a 6-month inhalation study of n-hexane and mixtures containing n-hexane plus hydrocarbon isomers in male Sprague-Dawley rats	47
Table 17. Incidence of nasal turbinate and neuropathological lesions in B6C3F1 mice exposed to n-hexane for 13 weeks	49
Table 18. Skeletal variations in live fetuses of pregnant Sprague-Dawley rats exposed to n-hexane via inhalation	52
Table 19. Total red blood cells and nucleated cells in bronchial lavage from n-hexane-challenged New Zealand white rabbits	55
Table 20. Enzyme activities in lung homogenates of rabbits exposed to n-hexane	55
Table 21. Concentration of biochemicals and enzyme activities in bronchial lavage extracted from male Sprague-Dawley rats inhaling n-hexane	56
Table 22. Changes in the sciatic and sural nerve action potentials induced by n-hexane and 2,5-hexanedione	58
Table 23. Incidence of liver and pituitary tumors in male and female B6C3F1 mice exposed to commercial hexane for 2 years	60
Table 24. Time-to-onset for the appearance of axonal swellings in 75% of explanted cultures of	

fetal mouse spinal cord incubated with mixtures of n-hexane and methyl ethyl ketone .	66
Table 25. Effect of 2,5-hexanedione, acetone, ethanol, and mixtures of 2,5-hexanedione with acetone or ethanol in drinking water on average motor nerve conduction velocity in m/sec	69
Table 26. Pyrrole adduct formation in proteins from γ -diketone-treated rats	75
Table 27. Summary of <i>in vitro</i> studies on the mutagenicity/genotoxicity of n-hexane	76
Table 28. Summary of <i>in vivo</i> studies on the mutagenicity/genotoxicity of n-hexane	77
Table 29. Summary of <i>in vivo</i> and <i>in vitro</i> studies on the mutagenicity/genotoxicity of commercial hexane mixtures	78
Table 30. Inhalation studies for n-hexane	87
Table 31. Benchmark dose modeling results of n-hexane inhalation toxicity studies for selection of the principal study	100
Figure 1. Biotransformation of n-hexane (Adapted from Couri and Milks, 1982 and Soriano et al., 1996)	8
Figure 2. Physiological toxicokinetic model of the distribution of n-hexane in the body and the urinary excretion of 2,5-hexanedione	20

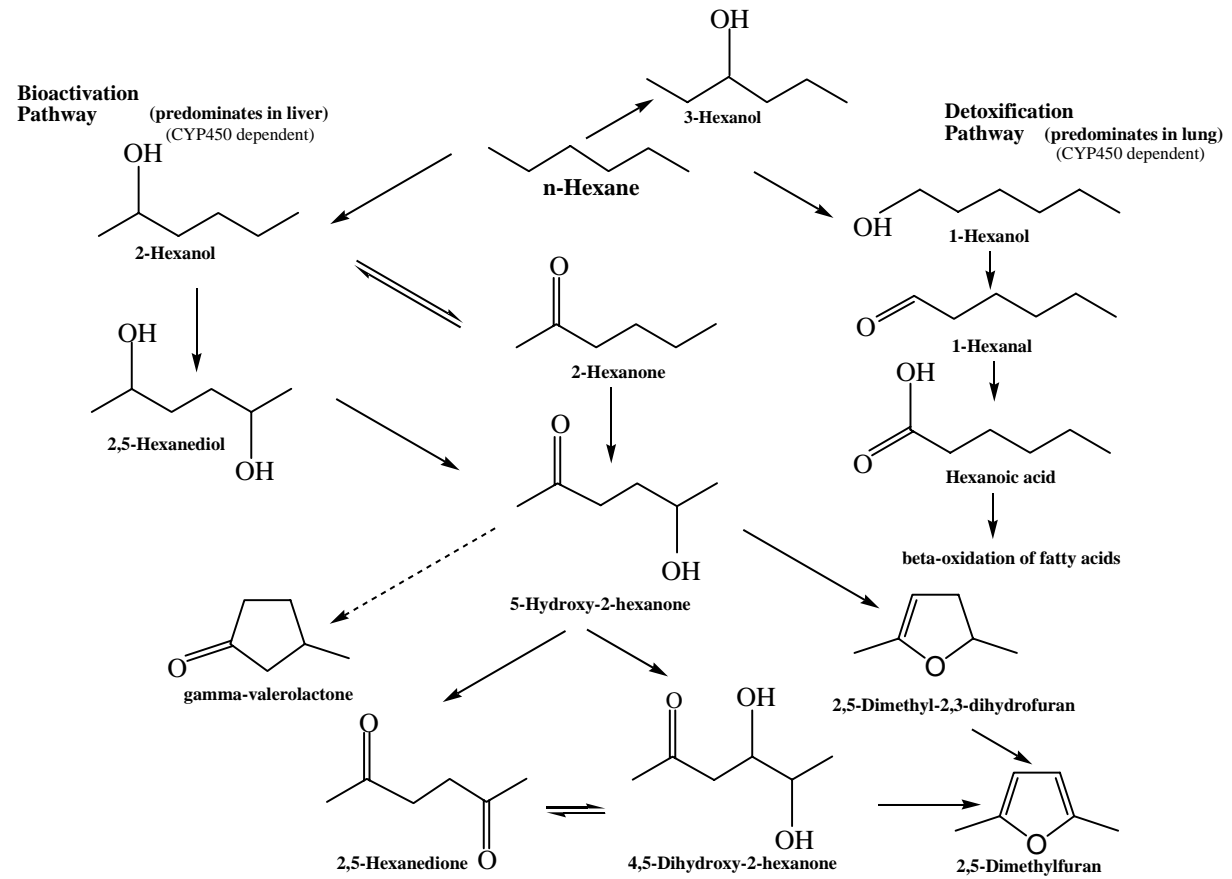


Figure 1. Biotransformation of n-hexane (Adapted from Couri and Milks, 1982 and Soriano et al., 1996)

Nakajima et al. (1991) used phenobarbital, n-hexane, 2-hexanone, and 2,5-hexanedione to induce different CYP450s to which they also raised monoclonal antibodies. The enzyme activities, i.e., benzene aromatic hydroxylase, toluene side chain oxidation, ethoxyresorufin O-deethylase (EROD), and pentoxyresorufin O-depentylase (PROD) were measured as indirect indicators of the activity of the CYP450 species, CYP2E1, CYP2C2/6, CYP1A1/2, and CYP2B1/2, respectively. There was increased activity of benzene aromatic hydroxylase in liver microsomes from n-hexane-treated rats indicating the induction of CYP2E1. Conversely, there was no increase in PROD or EROD activities in microsomal preparations from n-hexane-treated rats compared to control preparations, indicating that n-hexane probably did not specifically induce CYP2A1/2 or CYP2B1/2. 2,5-Hexanedione induced CYP2E1 and, to some extent, CYP2B1/2, suggesting that more than one CYP450 species may be involved in the overall conversion of n-hexane to its metabolic products. n-Hexane and 2-hexanone increased CYP2E1 to a similar extent in immunoinhibition analysis of toluene side-chain oxidation. In addition, 2-hexanone induced CYP2B1/2 to a lesser extent than phenobarbital treatment.

Iba et al. (2000) demonstrated in *in vivo* studies that CYP2E1 may be involved in the metabolism of n-hexane to 2,5-hexanedione. CYP2E1 knockout mice and control mice were administered daily intraperitoneal injections of n-hexane (200 mg/kg) for up to 21 days. CYP2E1 knockout and control mice had similar levels of 2,5-hexanedione on day 10 of administration (6.1 and 4.3 µg/mL in the CYP2E1 knockout and control mice, respectively). Levels of 2,5-hexanedione continued to increase in control mice on days 14 and 21 (22.9 and 16.1 µg/mL), but not in CYP2E1 knockout mice. These data indicate that CYP2E1 may be involved in the metabolism of n-hexane to 2,5-hexanedione following prolonged daily exposures.

In vitro studies also indicate the involvement of CYP450 enzymes in n-hexane metabolism (specifically the CYP2B1 enzyme), primarily leading to the formation of 2- and 3-hexanol (bioactivation pathway). Toftgard et al. (1986) evaluated the role of the phenobarbital-inducible CYP450 isozymes (CYP2B1 and CYP2B2) and beta-naphthoflavone-inducible CYP450 isozyme (CYP1A1) in n-hexane hydroxylation. Specific isozyme preparations were isolated from rat livers after exposure to the appropriate inducer and the isozymes involved in the production of hexanols were identified. 1-, 2-, and 3-Hexanol production was measured relative to time and expressed as nmoles metabolite/nmole enzyme protein/minute (turnover number or K_{cat}). The enriched CYP2B1 preparation produced all 3 alcohols with a turnover ratio of approximately 30:10:1 for 2-, 3-, and 1-hexanol, respectively. The CYP2B2 turnover ratio was similar to CYP2B1 (20:8:1 for the 2-, 3-, and 1-hexanol, respectively). Turnover numbers were highest for the CYP2B1 and lowest for the CYP2A1 isozyme. The CYP2B1/2 isozymes primarily resulted in the production of 2-hexanol where the CYP2A1 isozyme primarily resulted in the production of 3-hexanol. All three isozymes had low turnover numbers for production of 1-hexanol.

Although Toftgard et al. (1986) did not unequivocally demonstrate the identities and number of the CYP450 isozymes involved in the hydroxylation of n-hexane in the liver, the isozyme turnover data in combination with the kinetic data presented above support the hypothesis that 2-hexanol is the favored product in the liver.

Sanagi et al. (1980) also carried out a number of neurological tests to identify exposure-related neurological signs of n-hexane toxicity (Table 9). No objective neurological symptoms related to muscle strength by manual testing, muscle wasting, or muscle tone were reported. A statistically significant exposure-related deficit in muscle strength (as determined by jump test on one foot) and reduced vibration sensation of the radial processes (determined by the tuning fork test for vibration sensation) in the exposed group compared to controls (average group values) was observed.

Table 9. Results of neurological tests in control subjects and those occupationally exposed to n-hexane in a tungsten carbide alloy factory

Test	Exposed Group	Controls
Muscle Strength		
Grip power (kg)	45.3 ± 2.9	44.9 ± 5.2
Jumping on one foot (cm)	21.3 ± 3.6*	26.0 ± 6.2
Vibration Sensation		
Radial Processes (s/16s)	13.8 ± 2.4*	15.4 ± 1.6
Medial malleoli (s/16s)	12.2 ± 2.1	13.4 ± 2.0
Position Sense		
Barrany's test (cm)	0.8 ± 0.4	0.7 ± 0.5
Mann's test (%)	21	0
Co-ordination skills		
Knee slapping (times/15s)	24.8 ± 4.8	24.5 ± 2.8
Floor tapping (times/15s)	39.9 ± 7.7	42.6 ± 6.0

Source: Adapted from Sanagi et al. (1980)

Values are means ± SD

*Statistically significant versus controls (p<0.05)

Neurophysiological findings indicated a slowing of MCV in the posterior tibial nerve, with delayed recovery in exposed groups compared to control (Table 10). These findings are consistent with the neurological signs and subject-reported symptoms generally associated with n-hexane exposure. Consistent with the neurological signs and subject-reported symptoms, these responses may be related to n-hexane exposure. The findings of Sanagi et al. (1980) include minor neurological changes among workers exposed to n-hexane at a level close to the ACGIH recommended TLV of 50 ppm (ACGIH, 2003). However, the degree to which these changes represent impairment of neurological function is uncertain, because most neurophysiological findings and neurological signs in the n-hexane exposed group were indistinguishable from those of controls. The data suggest that exposure in the workplace to n-hexane at 58 ppm may be at or near the threshold for the onset of neurological effects.

Air levels of n-hexane taken over a 2-month period were found to be as high as 4060 mg/m³ (1152 ppm) in the plant.

Wang et al. (1986) evaluated a group of 59 press proofing workers from 16 factories who were employed for at least 2 months. All but four of these workers had regular contact with solvents in the process of cleaning the rollers. Two exposure measures using personal air samplers were taken in 14 of the 16 factories. Samples of the bulk cleaning solvents were found to contain n-hexane at concentrations ranging from 10–65%. Referent neurological data were collected from 150 healthy individuals (50 persons from three age groups, 10–35, 36–50, and 51–80 years, sex unstated). MCVs were consistently lower among the workers exposed to n-hexane than among the controls. The results of the neurological examination identified 15 workers with polyneuropathy and two asymptomatic workers with abnormal MCVs. All but one of these workers were employed in factories that used solvents with n-hexane concentrations in excess of 50%. While no association was found with length of employment, statistically significant associations were found to exist between (1) frequency of polyneuropathy and n-hexane concentration in the cleaning solvents and air samples, and (2) abnormal MCV and n-hexane concentration in the cleaning solvents. Among the workers with polyneuropathy, a high percentage worked in factories with n-hexane air concentrations greater than 100 ppm. However, a significant reduction in the MCV was found among workers exposed to air concentrations less than 25 ppm, a result that the authors considered to be related to the prolonged exposure due to overtime work.

The 15 cases of polyneuropathy from the study by Wang et al. (1986) were included in a group of 28 color printers with polyneuropathy studied by Chang and Yip (1987). This study of EMG changes also included 5 subclinical cases, 45 workers with no apparent symptoms, and 72 normal subjects who served as the control group. Among the clinical and subclinical cases, a significant decrease in MCVs and in amplitude of MAPs and SNAPs, and a significant prolongation of latencies was seen compared to controls. Among the exposed workers with no apparent symptoms, MCVs were slower, motor DL were prolonged, and SNAP amplitudes were attenuated compared to controls. The percentage difference in these myographic changes from the control values increased with increasing severity of symptoms.

Chang (1990) followed 11 of the 28 polyneuropathy cases for 4 years. The authors observed the patients monthly for the first two years, bimonthly for the third year, and once every 3 months for the final fourth year. All 11 cases had moderate to severe polyneuropathy. There was some worsening of motor function and electrographic findings in nine of the cases even after exposure to n-hexane ceased. Delayed worsening of sensory function was not observed. Sensory disturbances usually disappeared within 4 months. All patients, including the most severely affected, who was a quadriplegic, regained full motor nerve capacity within 1–4 years. Tightness in the legs, which appeared early in the course of recovery for six of the more severe cases, was replaced by muscle cramps which persisted up until the last clinical visit 4 years after the onset of neuropathy. Two of the six also had hyperreflexia and residual muscle atrophy in the lower extremities, and one had only residual atrophy. The inability of two of the subjects to perceive colors correctly (dyschromatopsia) persisted until the end of the study. These patients also had macular retinopathy.

