NTP REPORT ON THE

TOXICITY STUDIES OF *n*-HEXANE IN B6C3F$_1$ MICE

(INHALATION STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233 Research Triangle
Park, NC 27709

January 1991

NTP TOX 2
NIH Publication No. 91-3121

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Toxicity Study Report were performed under the direction of the NIEHS and were conducted in compliance with NTP chemical health and safety requirements and must meet or exceed all applicable Federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals.

These studies are designed and conducted to characterize and evaluate the toxicologic potential of selected chemicals in laboratory animals. Chemicals selected for NTP toxicology studies are chosen primarily on the bases of human exposure, level of production, and chemical structure.

Anyone who is aware of related ongoing or published studies not mentioned in this report, or of any errors in this report is encouraged to make this information known to the NTP. Comments and questions should be directed to Dr. J.R. Bucher, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709(919-541-4532).

These NTP Toxicity Study Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Toxicity Study Report are available without charge while supplies last from the NTP Public Information Office, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709 (919-541-3991).

TOXICITY STUDIES

OF *n*-HEXANE

(CAS NO. 110-54-3)

IN F344/N RATS AND B6C3F$_1$ MICE

(INHALATION STUDIES)

June K. Dunnick, Ph.D., Study Scientist

NATIONAL TOXICOLOGY PROGRAM
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CONTENTS

$CH_3CH_2CH_2CH_2CH_2CH_3$

***n*-HEXANE**

CAS No. 110-54-3

C_6H_{14} Molecular weight 86.2

Synonym: Hexyl hydride

ABSTRACT

Thirteen-week inhalation toxicity studies of *n*-hexane were conducted with B6C3F$_1$ mice of each sex exposed to 0, 500, 1,000, 4,000, or 10,000 ppm, 6 hours per day, 5 days per week or to 1,000 ppm, 22 hours per day (referred to as 1,000c), 5 days per week. All mice lived to the end of the studies. The final mean body weights of mice exposed to 1,000c ppm or 10,000 ppm were 10% or 17% lower than that of the controls for males and 0% or 6% lower for females.

Hematologic analyses were performed on whole blood samples collected at the end of the 13-week exposure. Segmented neutrophils were significantly increased in male mice exposed to 10,000 ppm.

A battery of behavioral measurements was conducted on mice, and the only parameter affected was locomotor activity, which was decreased in female mice at 1,000c ppm and 10,000 ppm. The test battery performed included forelimb and hind limb grip strength, motor activity and exploratory behavior, acoustic startle response, foot splay, and analgesia response.

Compound-related lesions of the nasal turbinates were seen in all groups of exposed mice except males exposed to 500 or 4,000 ppm. At the 10,000-ppm concentration, nasal lesions included inflammatory, erosive, and regenerative lesions of the olfactory and respiratory epithelium; luminal exudation and metaplastic lesions of the olfactory epithelium; and fibrosis of the submucosa. Lymphoid hyperplasia of the mandibular lymph nodes and neutrophilic hyperplasia of the bone marrow were also seen. At lower concentrations, lesions were not present in all mice and were limited to minimal regeneration or metaplasia of the olfactory epithelium.

A few paranodal swellings in the teased fibers of the tibial nerve were observed in 3/4 males and 3/4 females exposed to 10,000 ppm, 3/4 males and 3/4 females exposed to 1,000c ppm, and 0/4 male and 0/4 female controls; the severity of the lesions was minimal. Neither segmental demyelination nor distal axonal degeneration was seen.

Exposure of mice to *n*-hexane at concentrations up to 10,000 ppm resulted in only minimal toxicity. Paranodal swellings seen in nerves at 1,000c ppm and at 10,000 ppm were considered to be minimal nerve damage that would not result in paralysis. Exposure-related lesions of the nasal cavity occurred after *n*-hexane exposure, but minimal or no effects were seen at 1,000 ppm or below.

CONTRIBUTORS

The NTP Report on the Toxicity Studies of *n*-Hexane is based on studies that began in January 1986 at Brookhaven National Laboratory (Upton, NY).

National Toxicology Program
(Evaluated Experiment, Interpreted Results, and Reported Findings)

June K. Dunnick, Ph.D., Study Scientist

John R. Bucher, Ph.D.
Michael Elwell, D.V.M., Ph.D.
Joel Leininger, D.V.M., Ph.D.
B.A. Schwetz, D.V.M., Ph.D.
James K. Selkirk, Ph.D.
M.B. Thompson, D.V.M., Ph.D.

NTP Pathology Working Group
(Evaluated Slides and Prepared Pathology Report on 9/4/87)

John Seely, D.V.M. (Chair) (PATHCO, Inc.)
Roger Brown, D.V.M. (Experimental Pathology Laboratories, Inc.)
Michael Elwell, D.V.M., Ph.D. (NTP)
Linda Uraih, D.V.M. (NTP)

Principal Contributors at Brookhaven National Laboratory
(Conducted Studies)

Robert T. Drew, Ph.D.
Sonja B. Haber, Ph.D.
Gunnar Senum, Ph.D.

Principal Contributor at Experimental Pathology Laboratories, Inc.
(Evaluated Tissues)

Roger Brown, D.V.M.

Principal Contributor at Duke University
(Evaluated Nerve Specimens)

Doyle Graham, M.D.

Principal Contributor at Pathology Associates, Inc.
(Provided Pathology Quality Assurance)

William Hall, V.M.D., Ph.D.

Principal Contributors at Analytical Sciences, Inc.
(Contractor for Statistical Analysis)

Steven Seilkop, M.S.
Janet Teague, M.S.

Principal Contributors at Carltech Associates, Inc.
(Contractor for Technical Report Preparation)

William D. Theriault, Ph.D.
Abigail C. Jacobs, Ph.D.
John Warner, M.S.
Naomi Levy, B.A.

PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft report on the Toxicity Studies on *n*-hexane on March 13,1989, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, Panel members have four major responsibilities: (a) to ascertain that all relevant literature data have been adequately cited and interpreted, (b) to determine if the design and conditions of the NTP studies were appropriate, (c) to ensure that the Technical Report presents the experimental results and conclusions fully and clearly, and (d) to judge the significance of the experimental results by scientific criteria.

National Toxicology Program Board of Scientific Counselors Technical Reports Review Subcommittee

Robert A. Scala, Ph.D. (Chair)
Senior Scientific Advisor, Medicine and Environmental Health Department
Research and Environmental Health Division, Exxon Corporation
East Millstone, NJ

Michael A. Gallo, Ph.D.
Associate Professor, Director of Toxicology
Department of Environmental and Community Medicine, UMDNJ - Robert Wood Johnson Medical School, Piscataway, NJ

Frederica Perera, Dr. P.H.
Division of Environmental Sciences
School of Public Health
Columbia University
New York, NY

Ad Hoc Subcommittee Panel of Experts

John Ashby, Ph.D.
Imperial Chemical Industries, PLC
Central Toxicology Laboratory
Alderley Park, England

Robert H. Garman, D.V.M. (Principal Reviewer)
Bushy Run Laboratories
Export, PA
Consultants in Veterinary Pathology
Murrysville, PA

Lois Swirsky Gold, Ph.D.
University of California
Lawrence Berkeley Laboratory
Berkeley, CA

Curtis D. Klaassen, Ph.D.
Professor, Department of Pharmacology and Toxicology
University of Kansas Medical Center
Kansas City, KS

William Lijinsky, Ph.D.
Director, Chemical Carcinogenesis
Frederick Cancer Research Facility
Frederick, MD

Barbara McKnight, Ph.D.
Assistant Professor, Department of Biostatistics, University of Washington
Seattle, WA

Franklin E. Mirer, Ph.D.*
Director, Health and Safety Department
International Union, United Auto Workers, Detroit, MI

Paul M. Newberne, D.V.M., Ph.D.
Professor, Mallory Institute of Pathology
Boston, MA

James A. Popp, D.V.M., Ph.D. (Principal Reviewer)
Head, Department of Experimental Pathology and Toxicology
Chemical Industry Institute of Toxicology
Research Triangle Park, NC

*Unable to attend

SUMMARY OF PEER REVIEW COMMENTS ON THE TOXICITY STUDIES OF *n*-HEXANE

On March 13, 1989, the draft Technical Report on the toxicity studies of *n*-hexane received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.K. Dunnick, NIEHS, introduced the short-term toxicity studies of *n*-hexane by reviewing the rationale, experimental design, and results.

Dr. Popp, a principal reviewer, said the report was generally well written. No significant changes in format were needed. He said that the designation of animals for neuropathologic examination and the rationale for groups selected for this examination required clarification. Dr. Dunnick said that this would be done. Dr. Popp thought that the discussion should conclude by stressing the similarity between the findings in the mice studies and those from studies in rats reported by the Chemical Industry Institute of Toxicology.

Dr. Garman, a second principal reviewer, said that there needed to be clarification of the numbers of animals from which nerve fibers were examined and the numbers of teased nerve fibers examined per animal. He asked that statements be made as to which of the nasal turbinate lesions were biologically significant. Dr. Dunnick said that a table would be added with a grading of the lesions in the nasal cavity which would show the lesions in the high dose group (10,000 ppm) to be most prominent.

Dr. Scala commented that it was important to distinguish between the *n*-hexane used in the studies and *n*-hexane as a component of commercial hexane. Most hexane sold and used is the latter, which contains about 40% *n*-hexane. Dr. Scala concluded by saying that the Panel would accept the Report with the modifications noted.

$CH_3CH_2CH_2CH_2CH_2CH_3$

***n*-HEXANE**

CAS No. 110-54-3

C_6H_{14} Molecular weight 86.2

Synonym: Hexyl hydride

I. INTRODUCTION

Use and Exposure

The consumption of hexane in the United States in 1982 was estimated at 400 million pounds (USITC, 1987). *n*-Hexane is used as a solvent for extraction of oils from seeds, a component of paints, glues, and gasoline, and a solvent for other chemicals (Kirk-Othmer, 1980). Neuropathy has occurred in workers exposed to *n*-hexane in various industries (NIOSH/OSHA, 1981; ACGIH, 1988), including the manufacture of sandals (Yamamura, 1969), shoes, and tungsten carbide, as well as in press-proofing workers and workers in other industrial settings (Battershill et al., 1987; USEPA, 1988a,b). Although there are no data on the carcinogenicity of *n*-hexane in humans, there is an association between leukemia risk in the rubber industry and exposure to a variety of substances including hexane (Checkoway et al., 1984; Wilcosky et al., 1984).

Artists and other workers who use spray adhesives and paints may be exposed to *n*-hexane. The U.S. Consumer Product Safety Commission estimates that 2.5 million workers in the United States may be exposed to *n*-hexane (USCPSC, 1980); the route of exposure is usually by inhalation (NIOSH, 1977).

n-Hexane was found at waste sites and in some water supplies (Kool et al., 1982) and was found to contaminate air in and around underground fuel storage tanks (Rappaport et al., 1987; Shamsky and Samimi, 1987).

The current Occupational Safety and Health Administration standard for *n*-hexane is 500 ppm (1,800 mg/m^3) averaged over an 8-hour workday (NIOSH/OSHA, 1981). The American Conference of Governmental Industrial Hygienists has established a threshold limit value (TLV) of 50 ppm (180 mg/m^3) for *n*-hexane (ACGIH, 1988). The National Institute for Occupational Safety and Health has recommended that the permissible exposure limit be reduced to 100 ppm (350 mg/m^3) averaged over a workday of up to 10 hours per day, 40 hours per week, with a ceiling level of 500 ppm (1,800 mg/m^3) averaged over a 15-minute period (NIOSH, 1981).

Physical Properties

The physical properties of *n*-hexane are given in Table 1.

Toxicity in Humans

The most common and best-characterized toxic response in humans exposed to *n*-hexane is the development of neuropathy (Jorgensen and Cohr, 1981; Oryshkevich et al., 1986; Wang et al., 1986; Chang, 1987), the severity of which is dependent on the length of exposure (Table 2). Long-term exposure to *n*-hexane is characterized by lower extremity weakness and sensory loss (Ruff et al., 1981) and by neuropathy characterized by distal and retrograde axonal degeneration in nerve fiber tracts in the central and

TABLE 1. PHYSICAL PROPERTIES OF *n*-HEXANE (a)

Boiling point	68.7° C
Lower explosion level (by volume in air)	1.2% (12,000 ppm)
Upper explosion level (by volume in air)	7.5% (75,000 ppm)
Freezing point	−95.6° C
Flash point	−30.0° C
Density between 4° and 20° C	0.6603
Autoignition temperature	261.0° C
Vapor pressure (15.8° C)	100.0 mm mercury
Relative vapor density (air = 1)	2.97
Solubility	
Water	Poorly soluble (0.1 mg in 100 ml)
Ethanol (20° C)	50 g in 100 ml
Ether (20° C)	Soluble
Chloroform (20° C)	Soluble
ppm = 1 mg/liter	284
mg/m^3 = 1 ppm	3.52

(a) CIIT (1977); Sax (1979); AIHA (1980); Patty's (1981); CRC (1982-1983); Merck (1983); Battershill et al. (1987).

TABLE 2. EFFECTS ON HUMANS OF INHALATION EXPOSURE TO *n*-HEXANE AT SELECTED CONCENTRATIONS AND FOR VARYING LENGTHS OF TIME

Concentration (ppm)	Length of Exposure	Effects	Reference
500	3-5 min	None	Nelson et al., 1943
2,000	10 min	None	Gerade, 1963
5,000	10 min	Narcotic effects, dizziness	Patty and Yant, 1929
500-1,000	8 h/d for 3-7 mo	Neuropathy	Inoue et al., 1970
Up to 44,000 with methyl ethyl ketone or toluene	10-12 h/d for 5-7 y	Neuropathy, proximal weakness, cranial nerve palsy	Gonzales and Downey, 1972

peripheral nervous systems (Spencer et al., 1980a).

Many instances of *n*-hexane neuropathy have been reported in humans since the first outbreak among workers in the laminating industry in Japan during the 1960s. In many of the clinical episodes, individuals were exposed to a mixture of *n*-hexane and other solvents such as toluene or methyl ethyl ketone (Spencer et al., 1980a). Methyl ethyl ketone exacerbates the effects of *n*-hexane, and repeated exposure to *n*-hexane at concentrations of less than 500 ppm together with methyl ethyl ketone produces neuropathy. Exposure of humans to *n*-hexane at 1,500-5,000 ppm produces acute central nervous system symptoms such as dizziness, headache, and eye and throat irritation. In humans, the characteristic lesion associated with *n*-hexane exposure is peripheral neuropathy, which is seen as axonal swelling in the peripheral nerves. *n*-Hexane can penetrate human skin (Loden, 1986).

Toxicity in Animals

n-Hexane caused deaths of 50% or more of the animals at inhalation exposure concentrations of 48,000 ppm (4-hour exposure) (CIIT, 1977) and 74,000 ppm (Hine and Zuidema, 1970) for rats and 35,000 ppm (Fuhner, 1921) and 43,736 ppm (Patty and Yant, 1929) for mice. The oral LD_{50} is 30 g/kg for adult rats, 16 g/kg for 14-day-old rats, and 0.7 g/kg for newborn rats (Battershill et al., 1987). Table 3 lists some of the

TABLE 3. EFFECTS ON RATS AND MICE OF INHALATION EXPOSURE TO *n*-HEXANE AT SELECTED CONCENTRATIONS AND FOR VARYING LENGTHS OF TIME

Concentration (ppm)	Length of Exposure	Effects	Reference
RATS (a)			
400-600	Continuous for 23 wk	Axonal swellings	Schaumburg and Spencer, 1976
500	24 h/d, 7 d/wk for 9 wk	Hind limb paralysis	Altenkirch et al., 1982a
700	8 h/d, 7 d/wk for 40 wk	No paralysis	Pryor et al., 1982
1,000	24 h/d, 5 d/wk for 11 wk	Neurotoxic signs	Pryor et al., 1982
3,000, 6,500, or 10,000	6 h/d, 5 d/wk for 13 wk	Mild axonal swelling at 10,000 ppm	Cavender et al., 1984
5,000	9 h/d, 5 d/wk for 14 d	Axonal degeneration	Frontali et al., 1979
10,000	8 h/d, 7 d/wk for 19 wk	Hind limb weakness after 8 wk	Altenkirch et al., 1978
MICE (a)			
100-2,000	24 h/d, 6 d/wk for 1 y	Muscle atrophy; no neuropathy at 100 ppm; mild neuropathy at 250 ppm	Miyagaki, 1967

(a) The LC_{50} value is approximately 50,000 ppm.

toxic effects in animals exposed by inhalation to *n*-hexane.

When groups of five F344 rats were exposed to *n*-hexane by inhalation at 3,000, 6,500, or 10,000 ppm, 6 hours per day, 5 days per week for 90 days, males, but not females, showed decreased body weight gain at 10,000 ppm (Cavender et al., 1984). In 4/5 male rats exposed to 10,000 ppm and 1/5 to 6,500 ppm, axonal swelling, a lesion characteristic of *n*-hexane neuropathy, was seen in the peripheral nerves. One of five male rats also had axonal swelling in the spinal cord. No axonal swellings were seen in any of the female rats or in the male 3,000-ppm group. No other exposure-related lesions were observed.

Rats exposed to *n*-hexane and methyl ethyl ketone had lesions of the lung characterized as fatty degeneration and changes of lamellar bodies in type II pneumocytes (Schnoy et al., 1982). Male New Zealand rabbits exposed to *n*-hexane at 3,000 ppm, 8 hours per day, 5 days per week for 24 weeks, had exposure-related lesions in the lung, including airway enlargement of respiratory bronchioles and alveolar ducts, scattered foci of pulmonary fibrosis, and papillary tumors of bronchiolar epithelial cells (Lungarella et al., 1984). In rats exposed to *n*-hexane at 1,000-8,000 ppm for 8 hours, there was a decrease in brain acetylcholine (Honma, 1983).

Studies indicate that 2,5-hexanedione (the major metabolite of *n*-hexane) reacts with lysine residues, forming a pyrrole ring that is oxidized and then forms covalent cross-links in protein. It is hypothesized that the effects of 2,5-hexanedione are localized in the large myelinated axons of the peripheral nervous system because these axons contain a greater number of neurofilaments and the nodes of Ranvier have a comparatively smaller diameter. 2,5-Hexanedione produces cross-links in the neurofilaments, and these filaments, which are formed in the cell body, move slowly down the axon. As the number of cross-links increases, this progressive movement through the nodes is restricted and the characteristic swelling occurs (Graham and Gottfried, 1984; Rosenberg et al., 1987a,b; Genter et al., 1987; Lynch et al., 1989).

The neurotoxicity of *n*-hexane in rodents is dependent on the length of exposure (see Table 3) (Altenkirch et al., 1982a,b; Takeuchi et al.,

1983). More severe neuropathologic effects are seen after continuous *n*-hexane exposure than after intermittent exposure. Rats exposed to *n*-hexane at 700 ppm, 8 hours per day for 40 weeks, did not develop clinical or morphologic signs of neurotoxicity (Altenkirch et al., 1982a), whereas rats continuously exposed to 500 ppm had hind limb paralysis after 9 weeks (Altenkirch et al., 1982b). Rats exposed to 3,000, 6,500, or 10,000 ppm *n*-hexane 6 hours per day, 5 days per week for 13 weeks, developed neuropathologic effects only at the highest concentration (Cavender et al., 1984).

The neurotoxicity of *n*-hexane is enhanced in Wistar rats when administered by inhalation in combination with methyl ethyl ketone (Takeuchi et al., 1983). *n*-Hexane is metabolized by the cytochrome P450 system (Morohashi et al., 1983), and pretreatment with xylene or phenobarbital increases the metabolism of *n*-hexane to 2,5-hexanedione (Toftgard et al., 1983). Mixture of *n*-hexane with other solvents brings about an earlier onset of neurotoxicity (Hewitt et al., 1980; Altenkirch et al., 1982a,b; Abou-Donia et al., 1985).

Metabolism

n-Hexane is oxidized by liver microsomal enzymes; the transformation products of *n*-hexane are summarized in Figure 1 (Kramer, 1974; Couri et al., 1978; Perbellini et al., 1978; Jorgensen and Cohr 1981; DeCaprio et al., 1983; Toftgard et al., 1986).

The metabolite responsible for neurotoxicity is reported to be 2,5-hexanedione (Governa et al., 1987). The elimination half-life of *n*-hexane is 1-2 hours (Nomiyama and Nomiyama, 1974; Bus et al., 1979a). Studies by Toftgard et al. (1986) suggest that after inhalation exposure, *n*-hexane is transported to the liver, where it is first metabolized to hexanol; the cytochrome P450-PB (phenobarbital-inducible)-B_2 fraction is primarily responsible for this change. The major urinary metabolite of *n*-hexane in humans (Mutti et al., 1984; Governa et al., 1987), as well as in rats (Iwata et al., 1983), is 2,5-hexanedione. Baker and Rickert (1981) reported on the tissue distribution of *n*-hexane in male F344 rats after inhalation exposure to 500, 1,000, 3,000, or 10,000 ppm. The half-life of *n*-hexane in all tissues was 1-2 hours except in the kidney, where the half-life was 5-6 hours. *n*-Hexane was distributed to all major organ systems; the highest concentrations of 2,5-hexanedione were found in the blood, kidney, and sciatic nerve.

Teratogenicity and Reproductive Toxicity Studies

Exposure of pregnant F344 rats to *n*-hexane (94% pure) at 1,000 ppm on days 8-16 of gestation resulted in depression of postnatal growth of pups for up to 3 weeks after birth; by 7 weeks post partum, the body weights of exposed and control groups were similar (Bus et al., 1979b). An increase in the incidence of pyelectasia (enlarged renal pelvis) was seen in fetuses from the exposed groups. The number of implants, number of fetuses, or percentage resorptions was not affected. Pregnant CD®-1 albino mice administered *n*-hexane (99% pure) in cottonseed oil by gavage at doses up to 9.9 g/kg per day on days 6-15 of gestation gave birth to litters that had no signs of an increased teratogenic effect (Marks et al., 1980). Average fetal weights in the exposed groups were lower than that in the control group. Some studies indicate that weanling male F344 rats are more resistant than young adult rats to neuropathy induced by *n*-hexane (95% pure) (Howd et al., 1983).

Gillies et al. (1981) and Chapin et al. (1982) showed that exposure to 2,5-hexanedione at 10,000 ppm in drinking water for up to 6 weeks resulted in testicular atrophy, alterations in lipid metabolism, decreases in enzyme levels (β-glucuronidase), and inhibition of spermatogenesis in dosed male F344 rats. Based on changes in enzymes localized in Sertoli cells, which occurred before the onset of azoospermia, researchers hypothesized that 2,5-hexanedione may have a direct effect on the testis (Chapin et al., 1983).

In studies performed for the National Toxicology Program (NTP), timed-pregnant Sprague Dawley rats (Mast, 1987) and Swiss CD®-1 mice (Mast, 1988) were exposed to *n*-hexane by inhalation at 0, 200, 1,000, or 5,000 ppm (20 hours per day) for 14 days (rats) or 12 days (mice) (consecutive on gestation days 6-19 for rats and days 6-17 for mice). No major abnormalities were

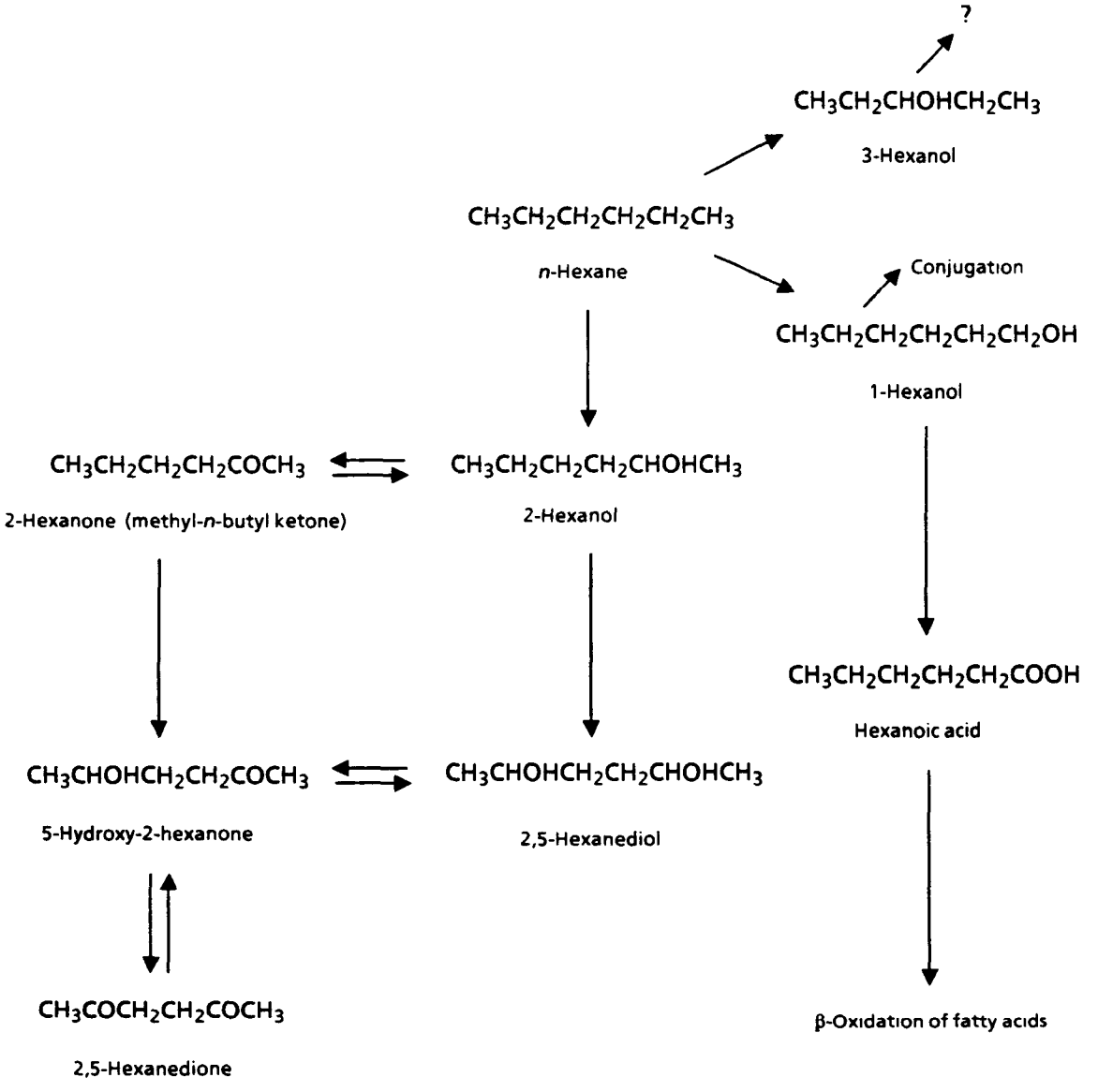

FIGURE 1. SOME TRANSFORMATION PRODUCTS OF *n*-HEXANE

seen in any of the fetuses. A reduction in rat fetal body weights was seen at 1,000 and 5,000 ppm; an increased incidence of reduced ossification of sternebrae in rat fetuses was observed at 5,000 ppm. Extragestational maternal weight gain for rat dams on gestational days 0-20 was 20%, 23%, or 45% lower than that of controls for the 200-, 1,000-, or 5,000-ppm groups, respectively. Thus, developmental toxicity was observed in rats in the presence of maternal toxicity at 1,000 and 5,000 ppm but not at 200 ppm. In mice, the number of resorptions per litter at all exposure concentrations increased. This effect was seen in the absence of any reduction of extragestational maternal weight gain.

Genetic Toxicology

Unpublished NTP data are as follows. *n*-Hexane was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested with a preincubation protocol at doses up to 1,000 µg/plate with or without Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver S9. Treatment with *n*-hexane, at doses up to 5,000 µg/ml in the presence or absence of Aroclor 1254-induced male Sprague Dawley rat liver S9, did not induce chromosomal aberrations in cultured Chinese hamster ovary (CHO) cells. Sister chromatid exhanges (SCEs) were induced in CHO cells by *n*-hexane but only in the presence of S9; this effect did not correlate with the dose. In an in vivo mouse bone marrow cytogenetics assay, doses of 500, 1,000, or 2,000 mg/kg *n*-hexane dissolved in corn oil and administered by intraperitoneal injection did not increase the incidence of SCEs; chromosomal aberrations were slightly increased in the dosed groups, but this increase was not significant. There was no increase in the incidence of micronucleated normochromatic erythrocytes (NCEs) or polychromatic erthyrocytes (PCEs) in the peripheral blood of male and female mice exposed to 1,000, 4,000, or 10,000 ppm *n*-hexane, 6 hours per day, 5 days per week for 13 weeks; mice exposed to 1,000 ppm for 22 hours per day for 13 weeks also had no increase in the incidence of micronucleated PCEs or NCEs.

Negative results in Salmonella tests have been reported by several other investigators (Kawachi et al., 1980; McCarroll et al., 1980; Ishidate et al., 1984). *n*-Hexane was also reported to be negative for growth inhibition due to DNA damage in *Bacillus subtilis* (McCarroll et al., 1981a) and *Escherichia coli* (McCarroll et al., 1981b). *n*-Hexane was reported to induce dose-related increases in chromosomal abnormalities and mitotic disruption in *Vicia faba* root tip anaphase cells (Gomez-Arroyo and Villalobos-Pietrini, 1981). Kawachi et al. (1980), in their summary of the results from mutagenicity studies conducted over several years in Japan, list *n*-hexane as negative both for induction of gene mutation in *Bombyx mori* and induction of chromosomal aberrations in cultured Chinese hamster fibroblasts. No induction of gene mutations was observed in Chinese hamster V79 cells treated with 10.5 µg/ml *n*-hexane (Lankas et al., 1978). Ishidate et al. (1984) reported induction of polyploidy in Chinese hamster lung fibroblasts by *n*-hexane without S9, and Perocco et al. (1983) observed an inhibition of DNA synthesis in human lymphocytes treated with 0-862 µg/ml *n*-hexane in the absence of S9.

The structural analog *n*-pentane was also reported to be negative in Salmonella gene mutation assays (Simmon et al., 1977; Kirwin et al., 1980). No information on the genotoxicity of 2,5-hexanedione was found in the literature (Environmental Mutagen Information Center; Hazardous Substance Data Bank; Chemical Abstracts).

Study Rationale

n-Hexane was nominated for toxicity and carcinogenicity studies in rats and mice because of widespread exposure by the inhalation route in industrial settings and because there were no adequate 2-year rodent studies. The U.S. Environmental Protection Agency, under section 4(a) of the Toxic Substance Control Act, has requested toxicity and carcinogenicity studies of commercial hexane (a mixture of different hexanes) (USEPA, 1988a,b,c). The NTP performed the 13-week studies in mice; previously published results of 13-week studies in rats were available (Cavender et al., 1984). The highest exposure concentration for the 13-week studies in mice was set at 10,000 ppm because it is just below

the explosive limit of 12,000 ppm and because the previous 13-week studies in rats showed no severe toxic effects at exposures up to 10,000 ppm. Toxicity at 1,000 ppm for 6 hours per day was compared with that at 1,000 ppm for 22 hours per day because the shorter duration of exposure each day would allow for some repair of nerve damage (Altenkirch et al., 1982a; Graham and Gottfried, 1984).

II. MATERIALS AND METHODS

Procurement and Characterization of *n*-Hexane

n-Hexane, specified by the manufacturer as 99% pure, was obtained in two lots (lot no. D-941 and lot no. H-088) from Phillips Chemical Company (Bolger, TX). Purity and identity analyses were conducted at Midwest Research Institute (MRI) (Kansas City, MO). MRI reports on the analyses performed in support of the *n*-hexane studies are on file at the National Institute of Environmental Health Sciences.

In an initial analysis of lot nos. D-941 and H-088, benzene concentrations were found to be 914 and 1,350 ppm, respectively. Because of concern over the level of benzene in the two lots, a purification step was developed. Fuming sulfuric acid was added to *n*-hexane to react with the benzene impurity. After sulfonation, samples were distilled and filtered in the presence of additional sulfuric acid.

After purification, both lots were identified as *n*-hexane by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. The infrared and nuclear magnetic resonance spectra were consistent with those expected for the structure and with spectra in the literature (Sadtler Standard Spectra). The ultraviolet/visible spectra were consistent with that expected for the structure of *n*-hexane.

The purity of both lots was determined by elemental analysis, Karl Fischer water analysis, and gas chromatographic analysis. Gas chromatography was performed with flame ionization detection and either an 80/100 mesh Carbopack C/0.1% SP1000, 1.8 mm × 4 mm i.d., glass column (system 1) or an 80/100 mesh Porapak QS, 1.8 mm × 4 mm i.d., glass column (system 1). Benzene was quantitated by using a 10% 1,2,3-tris(cyanoethoxy)propane column (system 3) and either a mass spectrometric detector (lot no. D-941) or a flame ionization detector (lot no. H-088).

Cumulative data indicated a purity of greater than 99% for both lots. The results of elemental analysis of both lots were in agreement with the theoretical values for hydrogen and carbon. Water content by Karl Fischer analysis of each lot was 0.003%. Gas chromatography with system 1 indicated one impurity with an area 0.19% that of the major peak for lot no. D-941 and a relative area of 0.17% for lot no. H-088; no impurities were detected by system 2. Benzene was determined with gas chromatographic system 3 to be present at a concentration of 720 and 162 ppb in lot nos. D-941 and H-088, respectively.

The heat stability of the bulk chemical was determined over a 2-week period. Analysis by gas chromatography (system 1) indicated that *n*-hexane was stable as a bulk chemical when stored protected from light for 2 weeks at temperatures up to 60° C. Further confirmation of the stability of the bulk chemical was obtained by periodic gas chromatographic analysis with system 1. No degradation was seen over the course of the studies. The identity of the chemical at the study laboratory was confirmed by infrared spectroscopy.

Some emptied bottles, which had been allowed to evaporate to dryness at the study laboratory, contained a brownish-black residue and were therefore returned, together with other bottles containing approximately 1 liter of *n*-hexane, to

the analytical chemistry laboratory for analysis. The additional bottles contained no visible residue except for a fine film coating the inside of the bottles. The residues were characterized by elemental analysis, Karl Fischer water analysis, and titration with sodium hydroxide to determine sulfuric acid content.

The residues contained approximately 50% water, 33% sulfuric acid, and 17% oxidized organic material. Based on the color of the residue and the low hydrogen to carbon molar ratio, the organic portion was believed to be primarily hexane oxidation/sulfation/sulfonation products. The estimated weight percent of the residue was less than 0.1%. Since the residue was not volatile and the generator was never allowed to run dry, the probability that any of the residue was introduced into the inhalation chamber was concluded to be negligible.

Generation and Measurement of Chamber Concentrations

Vapor Generation System: *n*-Hexane, inside a 2-liter anodized aluminum reservoir, was vaporized at 50° C by passing air through the liquid with a fritted bubbler. The *n*-hexane/air stream was then metered into 0.5 m^3 Hinners-type chambers. Five generators were used: one for the 1,000c-ppm (continuous exposure) chamber, one for the 500- and 1,000-ppm chambers, one for the 4,000-ppm chamber, and two for the 10,000-ppm chamber.

Vapor Concentration Monitoring: A Miran 1A infrared spectrophotometer was used to monitor the 10,000-ppm exposure chamber, and a Miran 980 infrared spectrophotometer was used to monitor all other exposure chambers. A cell-path length of 11.25 mm and a wavelength of 11.196 μ were used. Monitors were calibrated by injecting *n*-hexane into the Miran spectrophotometers, each equipped with a closed loop. Relative humidity was shown to have a minimal effect on the response, i.e., less than 0.1 ppm hexane with a 25% change in the relative humidity. Chambers were sampled at least once per hour. Daily mean exposure concentrations over the course of the studies are given in Table 4. As a confirmatory measure, the chamber concentrations were also monitored biweekly with two gas chromatographs equipped with a flame ionization detector and a 6 ft × 2 mm glass column packed with 0.075% SP1000 on 60/80 Carbopack F. One gas chromatograph was dedicated to the 10,000-ppm chamber, and the second gas chromatograph was used for all other chambers. Calibration of the gas-chromatographs was accomplished by injection of gas phase *n*-hexane standards.

Chamber Characterization: The uniformity of the vapor concentration in each exposure chamber was measured two times during the studies. Distribution studies were first conducted without animals in the chambers and then once during the first week of the studies with animals present. The vapor concentrations were measured without animals present at all eight positions sampled within the chamber and ranged from 93% to 111% of the target concentrations. The vapor concentrations measured for the eight

TABLE 4. MEAN CHAMBER CONCENTRATIONS OF *n*-HEXANE IN THE THIRTEEN-WEEK INHALATION STUDIES

Target Concentration (ppm)	Range	Determined Mean Concentration (a) (ppm)
500	551-605	580
1,000	1,048-1,138	1,109
(b) 1,000c	1,053-1,203	1,099
4,000	4,260-4,750	4,421

(a) Data represent the mean and range of the daily mean concentrations.
(b) Animals in this group were exposed to *n*-hexane for 22 h/d.

positions in the chambers with animals present ranged from 91% to 123% of the target concentrations.

The buildup (T_{90}) and bleedoff (T_{10}) times for the chamber concentrations were similar for all concentrations except for the T_{90} value for the 10,000-ppm chamber. The time to build up to 90% of the target chamber concentration (T_{90}) as well as the time to bleed off to 10% of the target concentration after generation was discontinued (T_{10}) were both approximately 7 minutes. However, for the 10,000-ppm chamber, the T_{90} value was approximately 13 minutes. The difference in T_{90} values is not considered significant.

The stability of the chemical in the chamber was evaluated by using the Miran 980. Infrared spectra of the bulk chemical and of the chemical in the chamber revealed no significant differences, confirming that the generated *n*-hexane had not been degraded.

Thirteen-Week Study Design

Groups of 18 mice (10 animals for core studies and 8 animals for neurobehavioral studies) of each sex were exposed to air containing *n*-hexane at concentrations of 0 (chamber controls), 500, 1,000, 4,000, or 10,000 ppm for 6 hours per day, 5 days per week for 13 weeks, and to 1,000 ppm for 22 hours per day (1,000c-ppm groups), 5 days per week for 13 weeks. Mean concentrations are summarized in Table 4.

Source and Specifications of Animals: Male and female B6C3F$_1$ (C57BL/6N, female × C3H/HeN MTV$^-$, male) mice used in these studies were produced under barrier conditions at Frederick Cancer Research Facility. Animals were progeny of defined microflora-associated parents that were transferred from isolators to barrier-maintained rooms.

Animal Maintenance: Mice were housed individually. Feed was available ad libitum during nonexposure periods; water was available at all times. For the 1,000c-ppm groups, feed was available overnight during exposure. Further details of animal maintenance are summarized in Table 5.

Clinical Examinations and Pathology: Details of clinical examinations and outlines of pathology procedures are given in Table 5. A necropsy was performed on animals surviving to the end of the studies. The brain, heart, right kidney, liver, lung, spleen, right testis, and thymus were weighed. Hematologic analyses were performed on blood obtained from the retro-orbital sinus; data were gathered for leukocytes, lymphocytes, monocytes, eosinophils, segmented neutrophils, hematocrit, hemoglobin, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean cell volume, erythrocytes, reticulocytes, and platelets. All data except those for reticulocytes and differential counts were obtained by using a Coulter Electronic Counter, Model S-Plus IV.

A necropsy was performed on the 10 core animals. Organs and tissues were examined for gross lesions. Tissues were preserved in 10% neutral buffered formalin and routinely processed for preparation of histologic sections for microscopic examination. Tissues and groups examined are listed in Table 5.

Upon completion of the histologic evaluation by the laboratory pathologist, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory where quality assessment was performed, and the results were reviewed and evaluated by the NTP Pathology Working Group (PWG). The final diagnoses represent a consensus of the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman et al. (1985).

Neurobehavioral and Neuropathologic Examinations: The eight animals in the neurobehavioral assessment groups were evaluated for forelimb and hind limb grip strength, motor activity and exploratory behavior, acoustic startle response, foot splay, and analgesia response (Appendix A).

Four of eight animals were randomly picked from animals used in the neurobehavioral studies for perfusion, fixation, and collection of nerve specimens. Perfusion, fixation, and collection of

TABLE 5. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE THIRTEEN-WEEK INHALATION STUDIES OF *n*-HEXANE

Strain and Species	$B6C3F_1$ mice
Animal Source	Frederick Cancer Research Facility (Frederick, MD)
Study Laboratory	Brookhaven National Laboratory
Size of Study Groups	18 males and 18 females, individually caged (10 mice for core studies, 8 mice for neurobehavioral assessment group)
Exposure Concentrations and Durations	0, 500, 1,000, 4,000, or 10,000 ppm *n*-hexane by inhalation, 6 h/d, 5 d/wk, 1,000 ppm, 22 h/d (1,000c ppm), 5 d/wk
Method of Animal Distribution	Distributed to weight classes and then assigned to cages according to a table of random numbers The cages were assigned to groups according to another table of random numbers
Feed	NIH 07 Rat and Mouse Ration (Zeigler Bros., Inc., Gardners, PA), available ad libitum during nonexposure periods; feed available ad libitum during overnight exposure for 1,000c-ppm group
Chamber Environment	Temp--21.0°-24.8° C (mean); hum--40%-84%; fluorescent light--12 h/d
Time Held Before Study	14 d
Age When Placed on Study	7 wk
Duration of Dosing	13 wk
Age When Killed	21 wk
Type and Frequency of Observation	Observed 2 × d; weighed 1 × wk
Necropsy, Histologic Examinations, and Supplemental Studies	Core animals--necropsy performed on 10 males and 10 females per group; the following tissues examined histologically for control and 10,000-ppm groups: adrenal glands, brain, bronchial lymph nodes, cecum, colon, duodenum, esophagus, gallbladder, gross lesions and tissue masses, heart, ileum, jejunum, kidneys, larynx, liver, lungs and mainstem bronchi, mammary gland (including surface skin), mandibular and mesenteric lymph nodes, mediastinal lymph nodes, nasal cavity and turbinates, pancreas, parathyroid glands, pituitary gland, rectum, salivary glands, sciatic nerve, spinal cord, spleen, sternum including marrow, stomach (including forestomach and glandular stomach), testes with epididymis/prostate/seminal vesicles or ovaries/uterus, thymus, thyroid gland, trachea, and urinary bladder. Mandibular lymph nodes, nasal cavity, and sternum with marrow examined for all other groups. The liver was examined for all males. Weights of brain, heart, right kidney, liver, lung, spleen, right testis, and thymus recorded at necropsy. Hematologic analyses performed for all groups Neurobehavioral groups--an additional 8 males and 8 females were included for neurobehavioral assessment. Behavioral assessment included grip strength, locomotor activity and exploratory behavior, startle response, foot splay, and analgesia response; neuropathologic evaluations performed on 4/8 males and 4/8 females at 0, 1,000c, and 10,000 ppm (performed at Duke University as a research project)

nerve specimens were done at Brookhaven National Laboratory by Experimental Pathology Laboratories, Inc., according to Spencer et al. (1980b); the collected nerve tissue specimens were sent to Dr. D. Graham, Duke University (Durham, North Carolina), for processing and examination (Graham and Gottfried, 1984).

The fixed brain, spinal cord, and sciatic nerve system, which were shipped in buffer, were stored under refrigeration until they were processed. The nerve specimens from 4/8 animals from the control, 1,000c-ppm, and 10,000-ppm groups were processed for neuropathologic evaluation. Two cross sections of cervical spinal cord were taken 6-8 mm below the junction with the medulla oblongata, unless this area had been traumatized during the removal of the cord. In these mice, sections were taken above or below this level. In mice nos. 50047 and 50048, the cervical cord was so disrupted that one section was taken from the medulla and one from the upper thoracic cord. The sections of cord were postfixed in cacodylate-buffered 1% osmium tetroxide for 2 hours, embedded in epon, and two toluidine blue sections were prepared on each block. Microscopic examination of the sections disclosed that the degree of spinal cord disruption was greater than had been apparent by gross examination, and additional sections were taken under visualization with a dissecting microscope and embedded for 16 mice. No axonal swellings were seen in any of the sections of spinal cord.

Cross sections of posterior tibial nerve were taken for osmication, epon embedding, and toluidine blue sections. Two 3-mm sections were taken, one at bifurcation of this nerve and one 12-15 mm above this point. The piece of nerve between these two sections was osmicated and dehydrated in cedar wood oil, and approximately 50 teased fibers (range 46-88 fibers) were placed on a clean glass slide for examination by light microscopy (see Table 10).

Statistical Methods: Organ weight to body weight ratios and hematologic and neurobehavioral data were analyzed by the multiple comparison methods of Dunnett (1980).

III. RESULTS

Survival, Body Weights, and Clinical Signs

All mice lived to the end of the studies (Table 6). Mean body weights of male mice exposed to 1,000 ppm or more and of female mice exposed to 10,000 ppm were lower than those of controls throughout the studies (Figure 2). The final mean body weights of mice exposed to 1,000c ppm or 10,000 ppm were 10% or 17% lower than that of controls for males and 0% or 6% lower for females. Compound-related clinical signs were limited to sneezing in groups exposed to 10,000 ppm.

TABLE 6. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE THIRTEEN-WEEK INHALATION STUDIES OF *n*-HEXANE

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial (b)	Mean Body Weights (grams) Final	Final Weight Relative to Controls (percent)
MALE				
0	10/10	23.5 ± 0.3	33.4 ± 0.5	
500	10/10	23.5 ± 0.3	32.3 ± 1.0	96.7
1,000	10/10	23.3 ± 0.3	31.2 ± 0.6	93.4
(c) 1,000c	10/10	23.6 ± 0.3	30.0 ± 0.6	89.8
4,000	10/10	23.8 ± 0.2	31.2 ± 0.7	93.4
10,000	10/10	23.6 ± 0.5	27.8 ± 0.5	83.2
FEMALE				
0	10/10	18.3 ± 0.3	25.3 ± 0.5	
500	10/10	18.4 ± 0.3	25.1 ± 0.6	99.2
1,000	10/10	19.3 ± 0.3	26.4 ± 0.7	104.3
(c) 1,000c	10/10	19.0 ± 0.3	25.4 ± 0.9	100.4
4,000	10/10	19.3 ± 0.2	25.6 ± 0.8	101.2
10,000	10/10	20.0 ± 0.4	23.8 ± 0.6	94.1

(a) Number surviving/number initially in the group
(b) Initial group mean body weight ± standard error of the mean
(c) Animals were exposed for 22 h/d; all other dosed groups were exposed for 6 h/d.

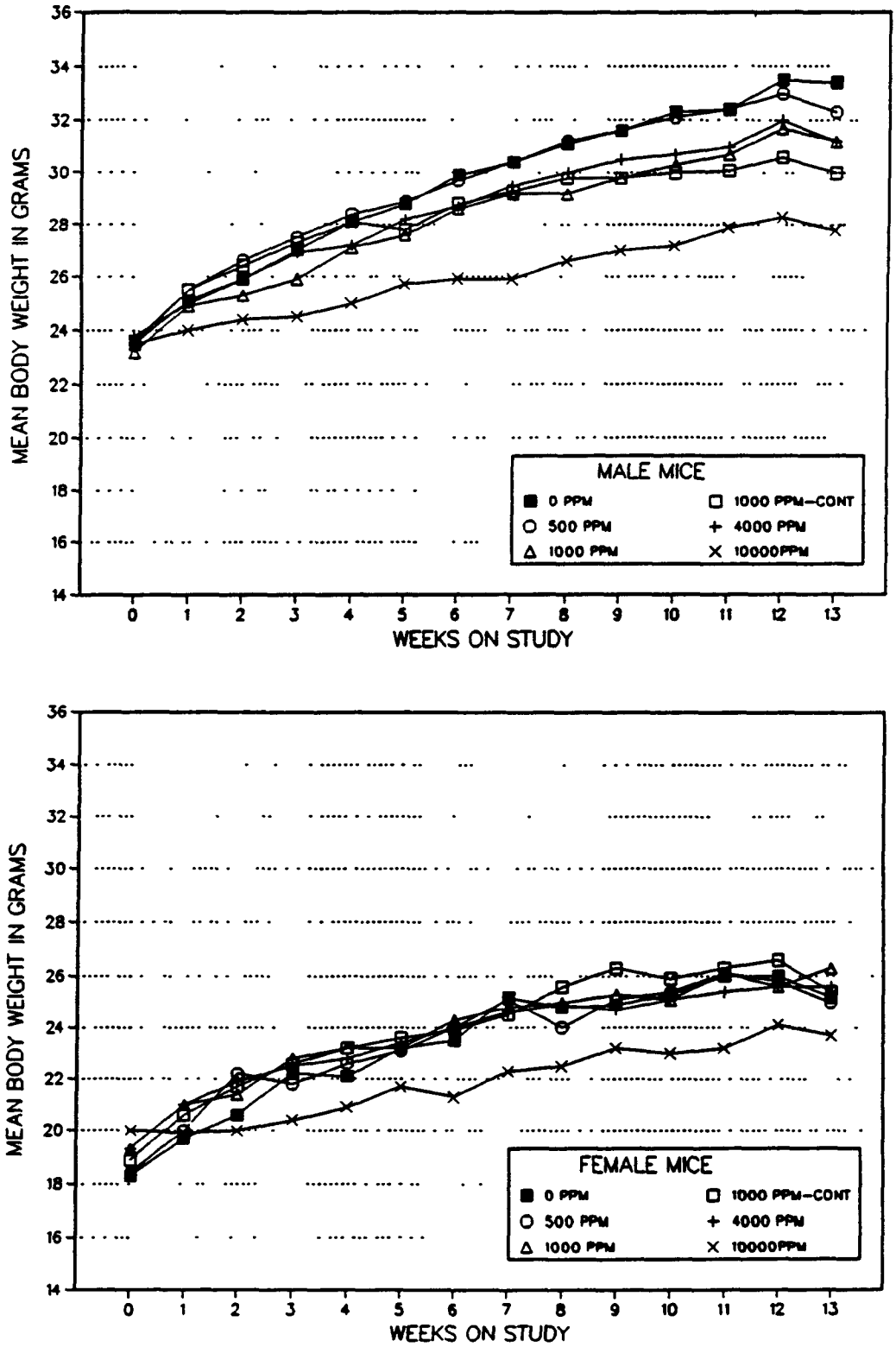

FIGURE 2. GROWTH CURVES FOR MICE EXPOSED TO *n*-HEXANE BY INHALATION FOR THIRTEEN WEEKS

Organ Weights and Hematologic Effects

No changes in organ weight to body weight ratios were observed for male or female mice (Table 7) which could be clearly related to n-hexane exposure; however, liver, kidney, and heart weights appeared to be increased in exposed female mice.

The number of segmented neutrophils was significantly increased for males exposed to 10,000 ppm (Table 8) and may have been a consequence of the more severe chronic active inflammation of the nasal mucosa in a few of these mice. No other changes in the other hematologic parameters measured were considered biologically relevant.

TABLE 7. ORGAN WEIGHTS OF MICE SHOWING SIGNIFICANT CHANGES IN THE THIRTEEN-WEEK INHALATION STUDIES OF *n*-HEXANE (a)

Organ	Control	500 ppm	1,000 ppm	1,000c ppm (b)	4,000 ppm	10,000 ppm
MALE						
Body weight (grams)	33.4 ± 0.55	32.3 ± 0.97	31.2 ± 0.57	**30.0 ± 0.60	31.2 ± 0.75	**27.8 ± 0.50
Brain						
Absolute	492 ± 7.3	485 ± 7.0	492 ± 10.9	491 ± 9.0	486 ± 5.0	475 ± 6.0
Relative	14.8 ± 0.32	15.1 ± 0.45	15.8 ± 0.35	*16.4 ± 0.50	15.7 ± 0.39	**17.1 ± 0.37
Heart						
Absolute	187 ± 7.3	179 ± 5.3	185 ± 8.6	194 ± 8.3	167 ± 3.0	*159 ± 4.8
Relative	5.6 ± 0.25	5.6 ± 0.18	5.9 ± 0.24	*6.5 ± 0.32	5.4 ± 0.09	5.7 ± 0.17
Right kidney						
Absolute	310 ± 7.8	301 ± 10.2	302 ± 4.9	321 ± 11.7	(c) 286 ± 11.4	**255 ± 4.3
Relative	9.3 ± 0.29	9.4 ± 0.31	9.7 ± 0.25	**10.7 ± 0.39	(c) 9.1 ± 0.31	9.2 ± 0.15
Liver						
Absolute	1,759 ± 45.2	1,628 ± 37.9	1,679 ± 51.2	1,623 ± 42.2	1,663 ± 63.7	**1,501 ± 40.5
Relative	52.8 ± 1.23	50.7 ± 1.28	53.8 ± 1.12	54.1 ± 1.33	53.2 ± 1.01	54.0 ± 1.30
Lungs						
Absolute	229 ± 6.7	227 ± 7.2	227 ± 11.2	232 ± 8.5	214 ± 6.0	219 ± 10.4
Relative	6.9 ± 0.20	7.1 ± 0.20	7.3 ± 0.33	7.7 ± 0.26	6.9 ± 0.13	*7.9 ± 0.36
Right testis						
Absolute	129 ± 5.9	131 ± 3.8	141 ± 3.8	*152 ± 9.4	127 ± 3.7	137 ± 6.5
Relative	3.9 ± 0.19	4.1 ± 0.18	4.5 ± 0.12	**5.1 ± 0.29	4.1 ± 0.12	**4.9 ± 0.21
FEMALE						
Body weight (grams)	25.3 ± 0.49	25.1 ± 0.62	26.4 ± 0.66	25.4 ± 0.88	25.6 ± 0.75	23.8 ± 0.57
Heart						
Absolute	142 ± 4.7	144 ± 5.8	151 ± 6.7	**184 ± 9.9	158 ± 8.1	148 ± 5.1
Relative	5.6 ± 0.18	5.8 ± 0.16	5.8 ± 0.31	**7.3 ± 0.36	6.2 ± 0.27	6.3 ± 0.20
Right kidney						
Absolute	183 ± 6.2	194 ± 7.3	206 ± 5.4	**220 ± 8.3	206 ± 7.8	202 ± 11.1
Relative	7.3 ± 0.21	7.8 ± 0.23	7.9 ± 0.25	**8.7 ± 0.32	8.0 ± 0.20	*8.5 ± 0.43
Liver						
Absolute	1,334 ± 30.0	1,321 ± 52.5	1,485 ± 40.7	1,495 ± 52.7	1,485 ± 78.1	1,500 ± 66.4
Relative	52.9 ± 0.80	52.6 ± 1.25	56.4 ± 0.86	**59.0 ± 1.29	*57.7 ± 1.44	**62.9 ± 1.56

(a) Mean ± standard error (absolute organ weights in milligrams; relative organ weights in milligrams per gram) for groups of 10 animals unless otherwise specified; P values vs. the controls by Dunnett's test (Dunnett, 1980).
(b) Animals were exposed for 22 h/d; all other dosed groups were exposed for 6 h/d.
(c) Kidneys of nine animals were weighed.
*$P<0.05$
**$P<0.01$

TABLE 8. *LISTING OF HEMATOLOGIC DATA SHOWING STATISTICALLY SIGNIFICANT CHANGES FOR MICE IN THE THIRTEEN-WEEK INHALATION STUDIES OF n-HEXANE (a)*

	Control	500 ppm	1,000 ppm	1,000c ppm (b)	4,000 ppm	10,000 ppm
MALE						
Number examined	9	10	9	10	10	10
Segmented neutrophils ($10^3/\mu l$)	0.31 ± 0.054	0.40 ± 0.068	0.46 ± 0.108	0.55 ± 0.079	0.25 ± 0.036	**1.16 ± 0.363
Mean red cell volume (μ^3)	48.2 ± 0.45	47.5 ± 0.19	47.4 ± 0.18	48.5 ± 0.30	47.5 ± 0.11	**46.9 ± 0.27
FEMALE						
Number examined	10	10	9	10	10	10
Segmented neutrophils ($10^3/\mu l$)	0.36 ± 0.041	0.36 ± 0.059	0.33 ± 0.094	0.31 ± 0.074	0.43 ± 0.055	0.33 ± 0.072
Lymphocytes ($10^3/\mu l$)	2.7 ± 0.25	2.2 ± 0.14	2.1 ± 0.30	2.0 ± 0.17	2.1 ± 0.18	*1.8 ± 0.18
Mean red cell volume (μ^3)	47.1 ± 0.14	47.2 ± 0.10	47.3 ± 0.17	* 47.8 ± 0.19	47.5 ± 0.10	*47.7 ± 0.20

(a) Mean ± standard error; P values vs. the controls by Dunnett's test (Dunnett, 1980).
(b) Animals were exposed for 22 h/d; all other dosed groups were exposed for 6 h/d.
*P<0.05
**P<0.01

Behavioral Tests

The only exposure-related behavior change affected was a decrease in locomotor activity in female mice at 1,000c ppm and 10,000 ppm (Table A1).

Gross and Microscopic Pathologic Effects

Compound-related lesions of the nasal turbinates included inflammatory, erosive, and regenerative lesions of the olfactory and respiratory epithelium. These changes were seen in most male and female mice from the 10,000-ppm exposure group (Table 9). At this concentration, inflammatory cells were present within the olfactory and respiratory epithelium as well as the submucosa, where they were often accompanied by edema and fibrosis around the Bowman's glands. In the high exposure mice, an exudate in the nasal lumen consisted of proteinaceous material with a few inflammatory cells. Metaplasia of the olfactory epithelium was characterized by replacement of olfactory cells with a ciliated respiratory epithelium. Regeneration consisted of multifocal areas in the olfactory epithelium with disorganization and thinning of the normal stratified nuclear layer. Olfactory lesions were generally limited to the olfactory epithelium in the dorsal meatus (anterior olfactory region); lesions of the olfactory turbinates in the posterior portion of the nasal cavity were less numerous and less severe. Regeneration of respiratory epithelium consisted of focal areas with a shortened, more cuboidal epithelium. At lower exposure concentrations, the nasal lesions were almost always limited to the olfactory epithelium and rarely involved the respiratory epithelium.

Nasal lesions were present in most female mice at the two highest concentrations, but the severity of lesions and inflammation was reduced in the 4,000-ppm group. The incidence and severity of lesions in the 4,000-ppm group were similar to those in the 1,000c-ppm group and consisted of minimal regeneration and metaplasia of the olfactory epithelium. At 1,000 and 500 ppm, minimal olfactory epithelial changes were present in only a few mice.

Nasal lesions in male mice at 10,000 ppm were similar in incidence and severity to those observed in females, but at concentrations below 10,000 ppm, fewer males than females were affected. Lesions were present in a few males in the 1,000c-ppm exposure group but not in the

TABLE 9. NUMBERS OF MICE WITH NASAL TURBINATE LESIONS IN THE THIRTEEN-WEEK INHALATION STUDIES OF *n*-HEXANE (a)

Site/Lesion	Control	500 ppm	1,000 ppm	1,000c ppm (b)	4,000 ppm	10,000 ppm
MALE						
Lumen						
Exudate, suppurative	0	0	0	0	0	10 (2.3)
Olfactory epithelium						
Chronic active inflammation	0	0	0	0	0	10 (2.1)
Multifocal erosion ulceration	0	0	0	0	0	3 (3.0)
Multifocal regeneration	0	0	2 (2)	3 (1.5)	0	10 (2.2)
Metaplasia	0	0	2 (1)	4 (1)	0	10 (2.8)
Respiratory epithelium						
Chronic active inflammation	0	0	0	0	0	9 (1.9)
Multifocal erosion	0	0	0	0	0	2 (1.5)
Multifocal regeneration	0	0	0	0	0	10 (1.4)
Submucosa						
Focal fibrosis	0	0	0	0	0	5 (1.4)
FEMALE						
Lumen						
Exudate, suppurative	0	0	0	0	0	10 (2.3)
Olfactory epithelium						
Chronic active inflammation	0	0	0	0	0	7 (1.9)
Multifocal erosion	0	0	0	0	0	3 (1.7)
Multifocal regeneration	0	2 (1)	1 (2)	9 (1.7)	9 (2.0)	10 (2.6)
Metaplasia	0	0	1 (1)	8 (1.9)	8 (2.0)	10 (2.6)
Respiratory epithelium						
Chronic active inflammation	0	0	1 (2)	0	0	5 (1.4)
Multifocal erosion	0	0	0	0	0	0
Multifocal regeneration	0	0	0	0	1 (2)	6 (1.2)
Submucosa						
Focal fibrosis	0	0	0	0	1 (1)	9 (1.6)

(a) Ten mice were examined in each group. The numbers in parentheses represent the average grade of the most severe lesions observed in the three sites of the nasal cavity examined and are based on the following scale: 1 = minimal; 2 = slight; 3 = moderate; 4 = moderately severe; 5 = severe.
(b) Animals were exposed for 22 h/d; all other dosed groups were exposed for 6 h/d.

equivalent 4,000-ppm exposure group. This apparent difference in incidence may be attributed to variation in sectioning through this area (dorsal meatus) of olfactory epithelium and to the focal distribution of these lesions of minimal severity. No nasal lesions were seen in males in the 500-ppm group.

Other histologic changes in exposed mice included neutrophilic hyperplasia of the bone marrow in 4/10 males and 4/10 females at 10,000 ppm and in 1/10 females at 4,000 ppm. Lymphoid hyperplasia of the mandibular lymph nodes was increased in mice in the 10,000-ppm group (7/7 males and 5/9 females) compared with controls (0/5 males and 2/10 females).

Neuropathologic Effects

A few paranodal swellings in the teased fibers of the tibial nerve were observed in 3/4 males and 3/4 females exposed to 10,000 ppm, 3/4 males and 3/4 females exposed to 1,000c ppm, and 0/4 male and 0/4 female controls; the severity of the lesions was minimal (Table 10). Neither segmental demyelination nor distal axonal degeneration was seen.

TABLE 10. RESULTS OF NEUROPATHOLOGIC EVALUATIONS OF NERVE SPECIMENS FOR INDIVIDUAL MICE IN THE THIRTEEN-WEEK INHALATION STUDIES OF *n*-HEXANE

Exposure Concentration (ppm)	Epon Sections (a)		Teased Fiber (b)
	Spinal Cord	Tibial Nerve	
MALE			
0	–	–	0/46
0	–	–	0/60
0	–	–	0/72
0	–	–	0/64
(c) 1,000c	–	–	6/60
(c) 1,000c	–	–	4/60
(c) 1,000c	–	–	1/64
(c) 1,000c	–	+	0/49
10,000	–	–	1/59
10,000	–	–	1/59
10,000	–	–	2/59
10,000	–	–	0/55
FEMALE			
0	–	–	0/51
0	–	–	0/61
0	–	–	0/48
0	–	–	0/51
(c) 1,000c	–	–	0/62
(c) 1,000c	–	–	2/69
(c) 1,000c	–	–	2/64
(c) 1,000c	–	–	1/59
10,000	–	–	3/60
10,000	–	–	3/62
10,000	–	–	10/88
10,000	–	–	0/74

(a) – = swellings not present; + = swellings present.
(b) Number of paranodal swellings observed/number of teased tibial nerve axons examined
(c) Animals were exposed for 22 h/d; all other groups were exposed for 6 h/d.

IV. DISCUSSION AND CONCLUSIONS

n-Hexane is a widely used solvent, and exposure in the workplace is primarily via inhalation. No long-term rodent studies have been performed to characterize the toxicity and carcinogenicity of *n*-hexane; these 13-week toxicity studies were performed in B6C3F$_1$ mice to provide information on the toxicity of the chemical. Thirteen-week inhalation toxicity studies of *n*-hexane had previously been performed in F344 rats (Cavender et al., 1984).

In the 13-week studies, n-hexane was administered at concentrations of 0, 1,000, 4,000, or 10,000 ppm (6 hours per day, 5 days per week) or at 1,000c ppm (22 hours per day, 5 days per week). All mice lived to the end of the studies. The final mean body weights of male mice exposed at 1,000c ppm or 10,000 ppm were 10% or 17% lower than that of controls, and the final body weight of females at 10,000 ppm was 6% lower. Exposure-related clinical signs were

seen at 10,000 ppm and included sneezing, an effect that was attributed to irritation of the nasal cavity.

The primary histopathologic lesions observed in male and female mice after the 13-week exposure to *n*-hexane were inflammation and regeneration of the respiratory epithelium and olfactory epithelium and/or metaplasia of the olfactory epithelium to respiratory epithelium at 10,000 ppm. The severity of these changes was exposure related, and minimal or no effects were seen at 1,000 ppm or less. The changes observed are considered to be nonspecific and indicative of inflammatory and regenerative changes in the mucosa secondary to an inhaled irritant. Similar lesions were not reported in rats after exposure for 13 weeks at concentrations up to 10,000 ppm (Cavender et al., 1984). Lymphoid hyperplasia of mandibular lymph nodes and neutrophilic hyperplasia of bone marrow observed at 10,000 ppm are considered to be physiologic responses to the slightly more extensive inflammatory changes in the nasal cavity.

n-Hexane is known to cause nerve damage in humans, and studies were conducted to determine if it caused any nerve damage in mice. Changes were seen in the tibia nerve of males and females exposed to 1,000c ppm or 10,000 ppm (see Table 10). No axonal swellings were seen in other sections of the spinal cord. Teased nerve preparations were more sensitive than were epon sections for the detection of axonal swelling. In the Chemical Industry Institute of Toxicology study in rats, similar nerve lesions were seen in male rats (but not in female rats) after 13 weeks of exposure to 10,000 ppm, 6 hours per day, 5 days per week (Cavender et al., 1984). These nerve lesions in both rats and mice were mild in nature, did not result in any paralysis, and were not considered life-threatening. Humans (with longer nerves) are generally more susceptible than rodents (with shorter nerves) to the development of neurofilament-filled axonal swellings after *n*-hexane exposure, apparently because of the greater probability for cross-linking in the longer axon (Graham and Gottfried, 1984; Friede et al., 1984).

A series of behavioral tests, including grip strength, motor activity, startle response, foot splay, and analgesia response, were conducted at weeks 0, 6, and 13 (Table A1). The only exposure-related effect was a decrease in locomotor activity in female mice at 1,000c ppm and 10,000 ppm.

There was no indication of paralysis, which correlated with the finding of only very minimal nerve damage at 1000c ppm or 10,000 ppm.

Exposure of mice to *n*-hexane at concentrations up to 10,000 ppm resulted in only minimal toxicity. Paranodal swellings seen in nerves at 1,000c ppm and at 10,000 ppm were considered to be minimal nerve damage that would not result in paralysis. Exposure-related lesions of the nasal cavity occurred in all mice exposed to 10,000 ppm *n*-hexane, but minimal or no effects were seen at 1,000 ppm or below. The effects of exposure of mice and rats to *n*-hexane at concentrations up to 10,000 ppm are similar, with no clinical signs of hemotoxicity observed in either species; nasal lesions were seen in mice, but no other target organ toxicity was observed in mice in the current studies or in rats in previous studies.

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APPENDIX: Behavioral Tests in the Thirteen-Week Inhalation Studies of *n*-Hexane

Methods

Behavioral tests were performed on the same eight males and eight females per group before the beginning of the 13-week studies, during week 6, and at the end of the studies. The test battery performed included forelimb and hind limb grip strength, motor activity and exploratory behavior, acoustic startle response, foot splay, and analgesia response.

Grip Strength: Grip strength was measured by allowing the animal to grip a triangular ring with its forepaws and gently pulling it back along a platform until its grip was broken. As the backward motion continued, its hind paws reached a T-shaped rear-limb grip bar that it was allowed to grasp and then was forced to release by continued pulling. Chatillon push-pull strain gauges were used to record the maximum strain required to break the animal's grip in each case. The average of three valid measurements was taken as the animal's score for either forelimb or hind limb grip strength in adult animals. One measurement was taken for 35-day-old or younger mice.

Locomotor Activity and Exploratory Behavior: A black plexiglass test arena was bisected by a removable partition with an opening in the bottom center. The novel side had black and white alternating stripe and checkerboard murals on each of the three walls; the other half had only black walls. The arena was placed on a Selective Activity Meter (Columbus Instruments, Model S, Columbus, OH), which senses motor activity by electromagnetic field stimulation so that identical movements on either side of the arena will give identical results. The animal was placed on the nonnovel side of the arena, a stopwatch was activated, and time to first cross to the novel side and time spent on the novel side were recorded. The trial lasted for 150 seconds. The number of crossings and general motor activity were recorded.

Startle Response: Acoustic startle response was measured with a Responder IV-I Startle Response Monitor (Columbus Instruments, Columbus, OH). The animal was placed on a startle platform installed within a sound-attenuating chamber (Colbourn Instruments, Lehigh Valley, PA) that contained an acoustic stimulus subsystem with a variable frequency generator, amplifier, loudspeaker, and a white noise generator. After 10 seconds, a 7,000-Hz, 110-db stimulus was presented for 0.1 seconds.

Foot Splay: After the hind paws of each mouse had been lightly inked, the mouse was suspended and dropped a distance of 32 cm onto a white blotter that provided a permanent record of hind foot splay. The distance between the points where the fourth digit of each hind paw first made contact was measured and recorded. The average of three valid measurements was used as the test score of the landing foot spread.

Analgesia Response: The analgesia response was measured by placing the animal on a heated (55° C) plate (Bel-Art Products Model H-46455 Analgesia Meter) for up to 60 seconds. Paw-lick latency was recorded.

Results

Results are presented in Table A1.

TABLE A1. LISTING OF BEHAVIORAL DATA SHOWING STATISTICALLY SIGNIFICANT CHANGES FOR MICE IN THE THIRTEEN-WEEK INHALATION STUDIES OF *n*-HEXANE (a)

	Control	500 ppm	1,000 ppm	1,000c ppm (b)	4,000 ppm	10,000 ppm
MALE						
Body weight (grams)						
Week 0	24.4 ± 1.11	23.4 ± 0.69	23.4 ± 0.67	24.2 ± 0.53	23.7 ± 0.68	23.2 ± 0.57
Week 6	30.1 ± 0.65	29.8 ± 0.65	29.0 ± 0.40	28.7 ± 0.37	28.8 ± 0.28	**27.3 ± 0.50
Week 13	34.3 ± 0.81	34.0 ± 0.99	**31.8 ± 0.74	**31.0 ± 0.39	32.6 ± 0.54	**29.9 ± 0.81
Locomotor activity (instrument units)						
Week 0	(c) 162 ± 6.1	174 ± 9.9	170 ± 6.4	164 ± 6.6	172 ± 7.9	186 ± 5.0
Week 6	175 ± 8.8	195 ± 7.2	174 ± 5.1	166 ± 8.2	184 ± 6.8	187 ± 5.5
Week 13	162 ± 6.5	174 ± 15.1	*(c) 189 ± 8.8	161 ± 11.3	174 ± 8.80	161 ± 10.4
FEMALE						
Body weight (grams)						
Week 0	17.8 ± 0.21	18.4 ± 0.29	17.7 ± 0.52	18.5 ± 0.22	18.3 ± 0.25	17.8 ± 0.52
Week 6	23.8 ± 0.36	24.0 ± 0.47	24.4 ± 0.43	23.0 ± 0.30	24.1 ± 0.34	**22.1 ± 0.42
Week 13	26.4 ± 0.38	26.2 ± 0.53	26.9 ± 0.82	25.6 ± 0.36	26.2 ± 0.33	**24.8 ± 0.38
Hot-plate latency (seconds)						
Week 0	7.8 ± 0.60	9.2 ± 1.08	8.4 ± 1.18	8.4 ± 0.94	8.7 ± 1.28	7.2 ± 1.09
Week 6	5.8 ± 0.62	*9.3 ± 0.61	7.7 ± 0.43	*9.3 ± 1.44	(c) 8.4 ± 1.01	8.3 ± 0.93
Week 13	6.2 ± 0.90	8.4 ± 0.84	6.8 ± 1.02	8.7 ± 1.14	6.4 ± 0.63	7.4 ± 0.58
Locomotor activity (instrument units)						
Week 0	168 ± 4.7	182 ± 7.0	174 ± 7.6	*182 ± 7.1	178 ± 6.5	175 ± 6.0
Week 6	181 ± 7.1	187 ± 5.4	176 ± 4.9	164 ± 9.8	169 ± 6.4	158 ± 6.4
Week 13	170 ± 9.1	168 ± 8.7	166 ± 9.2	**126 ± 10.5	152 ± 6.6	*141 ± 10.6
Startle-response amplitude (instrument units)						
Week 0	179 ± 21.0	168 ± 16.4	132 ± 21.8	180 ± 21.6	201 ± 27.5	184 ± 13.9
Week 6	172 ± 19.8	186 ± 23.0	156 ± 12.4	**90 ± 15.2	161 ± 6.8	112 ± 9.1
Week 13	141 ± 25.8	172 ± 25.4	149 ± 18.5	119 ± 14.9	174 ± 26.0	136 ± 11.9
Startle-response latency (milliseconds)						
Week 0	397 ± 23.3	403 ± 24.4	439 ± 26.7	361 ± 26.7	351 ± 36.4	369 ± 21.1
Week 6	414 ± 16.8	417 ± 25.4	441 ± 12.1	475 ± 5.9	422 ± 7.3	481 ± 2.3
Week 13	409 ± 29.0	387 ± 23.6	434 ± 16.8	*484 ± 0.1	392 ± 28.2	450 ± 12.7

(a) Mean ± standard error for groups of 8 animals unless otherwise specified; P values are vs. the controls by Dunnett's test (Dunnett, 1980).
(b) Animals were exposed for 22 h/d; all other dosed groups were exposed for 6 h/d.
(c) Seven animals were examined.
*$P<0.05$
**$P<0.01$

TABLE A1. LISTING OF BEHAVIORAL DATA SHOWING STATISTICALLY SIGNIFICANT CHANGES FOR MICE IN THE THIRTEEN-WEEK INHALATION STUDIES OF n-HEXANE (a)

	Control	500 ppm	1,000 ppm	1,000c ppm (b)	4,000 ppm	10,000 ppm
MALE						
Body weight (grams)						
Week 0	24.4 ± 1.11	23.4 ± 0.69	23.4 ± 0.67	24.2 ± 0.53	23.7 ± 0.68	23.2 ± 0.57
Week 6	30.1 ± 0.65	29.8 ± 0.65	29.0 ± 0.40	28.7 ± 0.37	28.8 ± 0.28	**27.3 ± 0.50
Week 13	34.3 ± 0.81	34.0 ± 0.99	**31.8 ± 0.74	**31.0 ± 0.39	32.6 ± 0.54	**29.9 ± 0.81
Locomotor activity (instrument units)						
Week 0	(c) 162 ± 6.1	174 ± 9.9	170 ± 6.4	164 ± 6.6	172 ± 7.9	186 ± 5.0
Week 6	175 ± 8.8	195 ± 7.2	174 ± 5.1	166 ± 8.2	184 ± 6.8	187 ± 5.5
Week 13	162 ± 6.5	174 ± 15.1	*(c) 189 ± 8.8	161 ± 11.3	174 ± 8.80	161 ± 10.4
FEMALE						
Body weight (grams)						
Week 0	17.8 ± 0.21	18.4 ± 0.29	17.7 ± 0.52	18.5 ± 0.22	18.3 ± 0.25	17.8 ± 0.52
Week 6	23.8 ± 0.36	24.0 ± 0.47	24.4 ± 0.43	23.0 ± 0.30	24.1 ± 0.34	**22.1 ± 0.42
Week 13	26.4 ± 0.38	26.2 ± 0.53	26.9 ± 0.82	25.6 ± 0.36	26.2 ± 0.33	**24.8 ± 0.38
Hot-plate latency (seconds)						
Week 0	7.8 ± 0.60	9.2 ± 1.08	8.4 ± 1.18	8.4 ± 0.94	8.7 ± 1.28	7.2 ± 1.09
Week 6	5.8 ± 0.62	*9.3 ± 0.61	7.7 ± 0.43	*9.3 ± 1.44	(c) 8.4 ± 1.01	8.3 ± 0.93
Week 13	6.2 ± 0.90	8.4 ± 0.84	6.8 ± 1.02	8.7 ± 1.14	6.4 ± 0.63	7.4 ± 0.58
Locomotor activity (instrument units)						
Week 0	168 ± 4.7	182 ± 7.0	174 ± 7.6	*182 ± 7.1	178 ± 6.5	175 ± 6.0
Week 6	181 ± 7.1	187 ± 5.4	176 ± 4.9	164 ± 9.8	169 ± 6.4	158 ± 6.4
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