

Provisional Peer Reviewed Toxicity Values for

Hexanedioic acid
(CASRN 124-04-9)

Superfund Health Risk Technical Support Center
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Acronyms and Abbreviations

bw	body weight
cc	cubic centimeters
CD	Caesarean Delivered
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act of 1980
CNS	central nervous system
cu.m	cubic meter
DWEL	Drinking Water Equivalent Level
FEL	frank-effect level
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	grams
GI	gastrointestinal
HEC	human equivalent concentration
Hgb	hemoglobin
i.m.	intramuscular
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
i.v.	intravenous
kg	kilogram
L	liter
LEL	lowest-effect level
LOAEL	lowest-observed-adverse-effect level
LOAEL(ADJ)	LOAEL adjusted to continuous exposure duration
LOAEL(HEC)	LOAEL adjusted for dosimetric differences across species to a human
m	meter
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
MRL	minimal risk level
MTD	maximum tolerated dose
MTL	median threshold limit
NAAQS	National Ambient Air Quality Standards
NOAEL	no-observed-adverse-effect level
NOAEL(ADJ)	NOAEL adjusted to continuous exposure duration
NOAEL(HEC)	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration

p-RfD	provisional oral reference dose
PBPK	physiologically based pharmacokinetic
ppb	parts per billion
ppm	parts per million
PPRTV	Provisional Peer Reviewed Toxicity Value
RBC	red blood cell(s)
RCRA	Resource Conservation and Recovery Act
RDDR	Regional deposited dose ratio (for the indicated lung region)
REL	relative exposure level
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	Regional gas dose ratio (for the indicated lung region)
s.c.	subcutaneous
SCE	sister chromatid exchange
SDWA	Safe Drinking Water Act
sq.cm.	square centimeters
TSCA	Toxic Substances Control Act
UF	uncertainty factor
µg	microgram
µmol	micromoles
VOC	volatile organic compound

PROVISIONAL PEER REVIEWED TOXICITY VALUES FOR HEXANEDIOIC ACID (CASRN 124-04-9)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA's) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. EPA's Integrated Risk Information System (IRIS).
2. Provisional Peer-Reviewed Toxicity Values (PPRTV) used in EPA's Superfund Program.
3. Other (peer-reviewed) toxicity values, including:
 - ▶ Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
 - ▶ California Environmental Protection Agency (CalEPA) values, and
 - ▶ EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's Integrated Risk Information System (IRIS). PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by two EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multi-program consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because science and available information evolve, PPRTVs are initially derived with a three-year life-cycle. However, EPA Regions or the EPA Headquarters Superfund Program sometimes request that a frequently used PPRTV be reassessed. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV manuscripts conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and RCRA program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

Neither IRIS (U.S. EPA, 2006) nor the HEAST (U.S. EPA, 1997) list an RfD, RfC, or cancer assessment for hexanedioic acid (adipic acid). The Drinking Water Standards and Health Advisories list (U.S. EPA, 2004) does not include an RFD or cancer assessment for hexanedioic acid. The CARA list (U.S. EPA, 1991, 1994) does not include any documents for hexanedioic acid. ACGIH (2001, 2006) established a time-weighted average - threshold limit value (TWA-TLV) of 5 mg/m³ for hexanedioic acid based on a report from the Russian literature of irritant and neurological effects in exposed workers (Krapotkina et al., 1981). No occupational exposure limits have been proposed by NIOSH (2006) or OSHA (2006) for this compound. Hexanedioic acid is considered a GRAS (Generally Recognized as Safe) substance by the U.S. Food and Drug Administration (U.S. FDA, 2003) and is used as a food additive. Reviews for FDA were performed by Informatics (1974) and FASEB (1976). WHO (1967, 1977, 1978, 2000) derived an ADI of from 0 to 5 mg/kg-day for hexanedioic acid and salts based on a NOAEL of 1% in feed in a two-year rat study. Toxicity data for hexanedioic acid were recently reviewed by Kennedy (2002). ATSDR (2006), IARC (2006), and NTP (2006) have not published documents for this compound. Literature searches were conducted for the period from 1965 to July, 2003 in the following databases: TOXLINE (including NTIS and BIOSIS updates), CANCERLIT,

MEDLINE, CCRIS, GENETOX, HSDB, EMIC/EMICBACK, DART/ETICBACK, RTECS, and TSCATS. Additional literature searches from July 2003 through September 2004 were conducted by NCEA-Cincinnati using MEDLINE, TOXLINE, Chemical and Biological Abstracts databases.

REVIEW OF PERTINENT DATA

Human Studies

Oral Exposure. No relevant data were located regarding the toxicity or carcinogenicity of hexanedioic acid to humans following oral exposure.

Inhalation Exposure. Functional disorders of the autonomic nervous system, gastrointestinal tract, and upper respiratory tract were reported in a study of Russian workers exposed to hexanedioic acid dust during its manufacture (Krapotkina et al., 1981, as cited in ACGIH, 2001). No additional details regarding the observed effects are available.

No relevant data were located regarding the carcinogenicity of hexanedioic acid to humans following inhalation exposure.

Animal Studies

Oral Exposure. Oral toxicity studies for hexanedioic acid in animals include several repeated-dose studies in rats ranging in duration from 5 days to 33 weeks, a series of teratogenicity studies in rats, mice, hamsters, and rabbits, and a chronic 2-year bioassay in rats. These data are summarized below.

Lang and Bartsch (1953) conducted a series of studies designed to characterize the short-term toxicity of hexanedioic acid in rats (unspecified strain). Four repeated-dose experiments were conducted. In the first experiment, female rats (17-20 per group; average weight of 92 grams at study initiation) were fed diets providing 0, 10, 20, or 40 mg/day of hexanedioic acid for 4 weeks. Based on body weight data reported in the article, the average daily doses are estimated to have been 0, 85, 160, and 320 mg/kg-day, respectively. Weight gain and behavior were monitored over the 4-week treatment period. No other toxicological parameters were evaluated. No effects were observed in any treatment group. The high dose of 40 mg/day (320 mg/kg-day) is a NOAEL in this study; A LOAEL was not achieved.

In the second experiment (Lang and Bartsch, 1953), groups of 15-18 male rats (unspecified strain, weighing 40-60 grams at study initiation) were fed diets providing 0, 200, 400, or 800 mg/day of hexanedioic acid neutralized with sodium hydroxide for five weeks. Based on body weight data in the paper, these doses correspond to approximately 0, 1900, 4400, and 11,000 mg/kg-day (rounded to 2 significant digits). Clinical signs of toxicity and body weights were monitored over the treatment period. No other toxicological parameters were evaluated. High-dose animals were observed with dull and ruffled fur and diarrhea for the first 2 to 3 weeks of treatment (incidence rates not reported). These signs of toxicity were not observed

at the end of the study. Body weight gain was reduced in the two high-dose groups. After 5 weeks of treatment, mean body weights were 35% lower than controls in the high dose group and 10% lower than controls in the mid-dose group. Statistical analysis was not conducted by the researchers, but a Student's t-test on the body weight data performed for the current evaluation determined that the differences from controls were statistically significant in both dose groups. The NOAEL in this study was 200 mg/day (1900 mg/kg-day), and the LOAEL was 400 mg/day (4400 mg/kg-day) based on reduced body weight gain.

In the third experiment (Lang and Bartsch, 1953), groups of 13-15 rats (males and females combined, unspecified strain, weighing 60-80 grams at study initiation) were exposed to hexanedioic acid, neutralized with sodium hydroxide in the diet at doses of 0, 400, or 800 mg/day for up to 33 weeks. Based on time weighted average body weights, rats were dosed at approximately 0, 1800, and 3900 mg/kg-day, respectively, over the 33-week treatment period. Body weight and clinical signs of toxicity were recorded throughout the treatment period. After 33 weeks of treatment, an unspecified number of tissues were microscopically examined, and hemoglobin, red and white blood cell count, and differential white blood cell counts were determined (it is not clear whether these parameters were evaluated in animals that died prior to scheduled sacrifice). A histopathology examination was also conducted on a group of animals (unspecified number per dose) designated for interim sacrifice at Weeks 23 or 25. Clinical signs of toxicity were similar to those observed in Experiment 2; diarrhea and dull and ruffled fur were observed at 800 mg/day during the first 3 weeks of the study. In addition, high-dose animals exhibited lethargy, which was not observed in high-dose animals of experiment 2. Mortality incidence was higher in the 800 mg/day group (10 deaths) than in the control and low-dose groups (4 deaths each). All deaths occurred within the first 4 treatment weeks. Body weight gain was reduced in treated rats early in the study (after 8 weeks, average body weight was 26% less than controls in the 800 mg/day group and 12% less than controls in the 400 mg/day group), but reportedly recovered by Week 33 (control data for Week 33 were not shown). Histopathology examinations revealed slight histological changes in the liver (including enlarged cell nuclei, increased cell size and cell volume, and a decrease in Kupffer cells) and kidneys (increased mitosis) and marked chronic inflammation in the intestinal mucosa at 400 and 800 mg/day. In addition to the animals discussed above, an unspecified number of pregnant female rats were treated with 400 mg/day; the researchers reported that hexanedioic acid treatment did not affect their ability to bear litters or nurse their young. The LOAEL in this study was 400 mg/day (1800 mg/kg-day), the lowest dose tested, based on reduced body weight gain and lesions of the gastrointestinal mucosa, liver, and kidney. A NOAEL was not observed.

In the fourth experiment, male rats (unspecified number and strain, weighing 40-60 grams at study initiation) were maintained on protein restricted diets (11% protein, composed of wheat and cod liver oil) supplemented with 0, 50, 100, 200, or 400 mg of hexanedioic acid daily for 19 weeks (Lang and Bartsch, 1953). Based on body weight data reported in the paper, these doses corresponded to approximately 0, 410, 880, 1600, and 4100 mg/kg-day (rounded to two significant digits). Body weights and clinical signs of toxicity were monitored throughout the treatment period. After 19 weeks of treatment, the animals were sacrificed, unspecified tissues were microscopically examined, and hemoglobin, red and white blood cell count, and differential white blood cell counts were determined (it is not clear whether these parameters were determined for animals that died prior to the scheduled sacrifice). Also, three rats per group

were designated for interim sacrifice after 8 weeks of treatment. These animals were subjected to histological examinations (unspecified tissues). Mortality was similar in all groups. Clinical signs of dull and ruffled fur and diarrhea (which were observed in previous experiments) were not observed at any dose level in this experiment. Mean body weights of rats exposed at 400 mg/day were 22% less than controls after 6 weeks and 28% less after 19 weeks. Rats exposed at 400 mg/day were also observed to have slight histological changes in the liver and kidneys and marked chronic inflammation in the intestinal mucosa. No treatment-related hematological effects were noted. The NOAEL in this study was 200 mg/day (1600 mg/kg-day), and the LOAEL was 400 mg/day (4100 mg/kg-day), based on reduced body weight gain and lesions of the gastrointestinal mucosa, liver, and kidney.

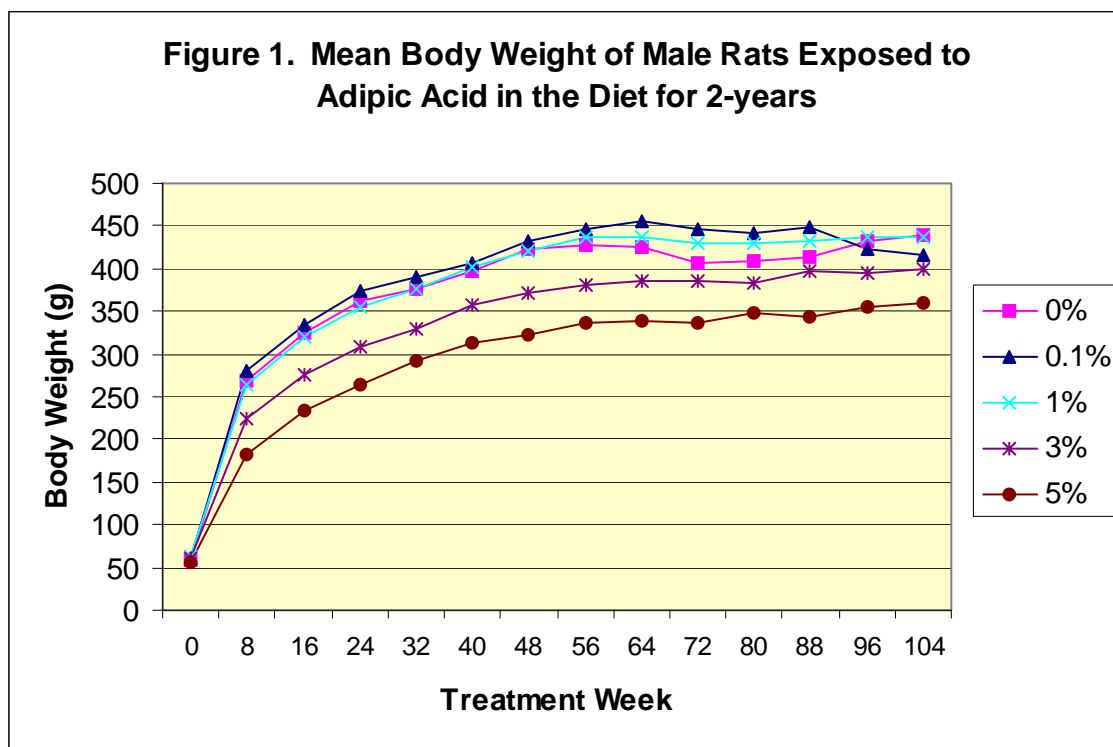
Other subchronic studies also reported reduced body weight gain in rats fed hexanedioic acid in the diet, but apparently did not examine other endpoints. In a 90-day feeding study (Hazleton, 1950, as cited in FASEB, 1976), male albino rats (10 per dose, unspecified strain) were maintained on a diet supplemented with hexanedioic acid at 0, 0.1%, 1.0%, or 5.0% (0, 100, 1000, or 5000 mg/kg-day, assuming a food factor of 10% for growing rats in a subchronic study). Ten females were exposed at 0% or 1% (0 or 1000 mg/kg-day). Body weight and survival were evaluated. Histopathology examinations were not conducted. Mean body weights of rats exposed to 5% hexanedioic acid (5000 mg/kg-day) were substantially decreased compared with controls throughout the exposure period. This effect was attributed to impaired food utilization associated with high acid consumption by the study authors. No effects were observed at the 1% hexanedioic acid concentration (1000 mg/kg-day). The original study report was not available, and additional details were not reported in FASEB (1976). Similar results were observed when male Carworth Farms albino rats (10 per dose) were maintained on a diet supplemented with 5% (5000 mg/kg-day) sodium adipate (Informatics, 1974). Controls (N=5) were fed untreated diet. Five treated rats were sacrificed after 14 weeks of treatment, and the remaining five rats were fed untreated diet for an additional 8 weeks. Body weights were recorded throughout the study, and all rats were subjected to a gross pathology examination at terminal sacrifice. "Retardation of growth" occurred in sodium adipate treated rats. Rapid growth occurred during the 8-week period after sodium adipate treatment stopped. The original study report was not available, and additional details were not reported.

One chronic toxicity study in laboratory animals was located (Horn et al., 1957). Young male Carworth Farms albino rats (20 per dose) were exposed to hexanedioic acid (unspecified purity) in the diet at 0.1%, 1%, 3%, and 5% for two years (Horn et al., 1957). Based on time weighted average body weights, these concentrations correspond to approximately 44, 470, 1500, and 2800 mg/kg-day (rounded to two significant digits). Nineteen female rats were also exposed at 1% hexanedioic acid (\approx 630 mg/kg-day). Controls (twenty males and 10 females) were fed basal diet. The following parameters were recorded during the 2-year exposure period: clinical signs of toxicity (weekly), body weights (recorded weekly, but reported for 8-week intervals), food consumption, and survival. After two years of treatment, surviving animals were sacrificed and the following parameters were evaluated: gross pathology (unspecified tissues), organ weights (heart, liver, spleen, and kidneys [all surviving females and approximately half of each male exposure group], brain, thyroid, lungs, adrenals, stomach, and testes [from approximately half of each male exposure group]), and microscopic examination of 14 tissues (thyroid, lungs, heart, liver, spleen, kidneys, adrenals, stomach, small intestine, large intestine,

pancreas, bone marrow, testes or ovaries, and uterus). Animals that died prior to study termination were subjected to gross pathology evaluations when possible. It is not clear if a complete microscopic evaluation was conducted on animals that died prior to study termination; however, the lungs of animals that died prior to terminal sacrifice were microscopically examined. It does not appear that any of the data were subjected to statistical analyses.

Results are summarized in Table 1 and Figure 1 (Horn et al., 1957). Survival of rats was not adversely affected by chronic dietary exposure to hexanedioic acid, and the occurrence of clinical signs of toxicity was comparable in treated and control rats. Body weights of males exposed to 3% or 5% hexanedioic acid were less than controls throughout the 2-year exposure period (17% and 32% less than controls at Week 8, and remaining below controls throughout the study, with terminal deficits of 9% and 18%, respectively). Food consumption of males exposed to 5% was slightly ($\approx 6\%$) lower than controls. Body weights and food intake of all other male and female treated groups were similar to controls throughout the exposure period. There was no effect on organ weights or the incidence of tumors or nonneoplastic gross or microscopic lesions in any of the tissues examined at any dose. Based on the body weight changes, this study identified a LOAEL of 3% (1500 mg/kg-day) and NOAEL of 1% (470 mg/kg-day). Although this study appears to have been conducted at appropriate doses, with the high dose of 5% approaching the MTD (maximum tolerated dose), it was limited as a carcinogenicity bioassay by the small number of animals evaluated.

Table 1. Mean Body Weight, Survival, and Feed Consumption of Male and Female Rats Exposed to Hexanedioic Acid in the Diet for 2 Years (Horn et al., 1957)					
Concentration	Dose (mg/day)	Dose (mg/kg-day)^a	Avg Body Wt (Weeks 0-104)^a	Survival^b	Mean Daily Food Intake (g)
<i>Males</i>					
0%	0	0	368.9	82.5%	16.8
0.1%	17	44	382.7	87.7%	17.0
1%	175	470	374.1	94.7%	17.5
3%	505	1500	332.5	94.5%	16.8
5%	814	2800	291.5	97.2%	15.8
<i>Females</i>					
0%	0	0	259.6	98.9%	14.2
1%	158	630	251.1	96.3%	15.8
^a Calculated for the current review using body weight data reported in Table II of the publication.					
^b Reported by the researchers to account for both number of survivors and length of survival.					



There is evidence that toxicity of hexanedioic acid is enhanced by oral gavage administration compared with dietary exposure. Short-term gavage studies found severe effects, including death, at doses comparable to the subchronic/chronic dietary NOAELs/LOAELs. In a range-finding study for their chronic study, Horn et al. (1957) gave male albino mice (13/dose) a single dose of hexanedioic acid (6% suspension in methylcellulose) at 1500, 2000, or 2500mg/kg-day. Mortality was observed at all doses and increased with dose (3/13, 8/13, and 9/13 in the low- mid- and high-dose groups, respectively). Animals that died showed distention of the stomach and small intestine, irritation and hemorrhage of the intestine, and spastic contractions of the cecum. An LD₅₀ of 1900 mg/kg-day was calculated. In another study, treatment of male rats with 3600, 4000, 5000, or 5600 mg/kg-day by oral gavage for 5 days resulted in mortality at all doses (3/6, 5/6, 6/6, 6/6, and 6/6, respectively), and a calculated LD₅₀ of 3615 mg/kg-day (Litton Bionetics, 1974). Clinical signs of toxicity included depression, labored respiration, ataxia, and convulsions in all dose groups. No lesions were found at necropsy. Dietary exposure studies found no clinical signs of toxicity and no effect on survival, but did find gastrointestinal lesions, at comparable doses.

Results from several short-term repeated-dose studies were reported in a review by Informatics (1974). The original study reports were not available for review, and very few details on the experimental designs or results were provided in these summaries; therefore, study adequacy and reliability cannot be independently assessed. These data are summarized in Table 2.

Table 2. Summary of Selected Repeated-Dose Studies Reported by Informatics, 1974				
Study Duration	Exposure Route	Species	NOAEL/ LOAEL	Citation
4 Weeks	Adipic acid by oral gavage (vehicle not specified)	Rat (young)	NOAEL: 243 mg/d (1350 mg/kg-d) LOAEL: None	Enders, 1941, as cited in Informatics, 1974
4 Weeks	Adipic acid by oral gavage (vehicle not specified)	Rat (adult)	NOAEL: 730 mg/d (2433 mg/kg-d) LOAEL: None	Enders, 1941, as cited in Informatics, 1974
5 Weeks	Adipic acid by oral gavage in ethanol	Rats (adult)	NOAEL: 200 mg/d (610 - 922 mg/kg-d) LOAEL: None	NAS, 1943 as cited in Informatics, 1974
1 Week	Adipic acid by oral capsule	Guinea Pig	NOAEL: 400 mg/d (682-942 mg/kg-d) LOAEL: None	NAS, 1943 as cited in Informatics, 1974
5 Weeks	Adipic acid by oral capsule	Guinea Pig	NOAEL: 600 mg/d (1032-1739 mg/kg-d) LOAEL: None	NAS, 1943 as cited in Informatics, 1974
9 Weeks	Sodium adipate by unspecified route	Rats (young)	NOAEL: None LOAEL: 199 mg/d (638-1332 mg/kg-d) Reduced body weight	NAS, 1943 as cited in Informatics, 1974

Hexanedioic acid was the subject of a series of teratology studies in rats, mice, hamsters, and rabbits conducted on behalf of the U.S. Food and Drug Administration (FDRL, 1972, 1974). These studies followed similar protocols; hexanedioic acid was administered via oral gavage to the following:

1. 25-31 mated CD-1 mice/dose on gestation days (GD) 6-15 at 0, 2.6, 12.0, 56.0, or 263 mg/kg-day;
2. 24-28 mated Wistar rats/dose on GDs 6-15 at 0, 2.9, 13.0, 62.0, or 288 mg/kg-day;
3. 25-27 mated Golden hamsters/dose on GDs 6-10 at 0, 2.0, 9.5, 44.0, or 205 mg/kg-day; and
4. 13-20 artificially inseminated Dutch-belted rabbits on GDs 6-18 at 0, 2.5, 12, 54, and 250 mg/kg-day.

In each study, body weights were determined on gestation day (GD) 0, at one or two intervals before treatment ended, on the last day of hexanedioic acid treatment, and at terminal sacrifice. Appearance, behavior, and food consumption were evaluated daily. Two to 11 days after the final hexanedioic acid administration, pregnant animals were subjected to Cesarean section and the following parameters were recorded: number of corpora lutea (rabbits only), implantation sites, resorption sites, and live and dead fetuses. A detailed examination of the urogenital tract of each pregnant female was performed, fetal weights of live pups were recorded, and a gross examination for external congenital abnormalities was performed on all fetuses (survival of neonatal rabbits was evaluated after placing live fetuses in an incubator for 24 hours). All surviving rabbit fetuses were dissected and examined for visceral abnormalities, then cleared, stained, and examined for skeletal defects. For mice, rats, and hamsters, approximately one-third of the fetuses of each litter were subjected to visceral examination, the rest were stained and evaluated for skeletal defects. Aspirin or 6-aminonicotinamide was used as a positive control in each study. Key parameters of each study design are summarized in Table 3.

Hexanedioic acid treatment did not affect implantation of the conceptus into the uterus, maternal or fetal survival, or the incidence of soft tissue or skeletal tissue abnormalities. The NOAEL in rats, mice, hamsters, and rabbits was 288, 263, 205, and 250 mg/kg-day, respectively, the highest dose evaluated in each study. Although hexanedioic acid did not induce developmental toxicity in any of the species tested, the positive control substance did not clearly induce developmental toxicity in mice or hamsters; therefore, it is not clear that the assay was adequately sensitive to detect positive responses in these species. Also, maternal toxicity was not achieved in any of the studies; therefore, a definitive conclusion cannot be made regarding the ability of hexanedioic acid to induce developmental toxicity. These data do, however, support the conclusion that teratogenicity is not likely a sensitive toxicological endpoint for hexanedioic acid.

Strain	Rat	Mouse	Hamster	Rabbit
		Wistar	Albino CD-1 outbred	Golden
Number of Animals Dosed	24 to 28 per dose	25 to 31 per dose	25 to 27 per dose	13 to 20 per dose
Number of Animals Evaluated (pregnant)	20-24 per dose	20-24 per dose	21-24 per dose	10-14 per dose
Doses	2.9, 13, 62, 288 mg/kg-day	2.6, 12, 56, 263 mg/kg-day	2, 9.5, 44, 205 mg/kg-day	2.5, 12, 54, 250, mg/kg-day
Dosing Volume	1-2 mL/kg	10 mL/kg	1 mL/kg	1 mL/kg
Dosing Schedule	GD 6-15	GD 6-15	GD 6-10	GD 6-18
Sacrifice Day	GD 20	GD 17	GD 14	GD 29
Parameters Evaluated	Number of corpora lutea (rabbits only), implantation sites, resorption sites, and live and dead fetuses, urogenital tract normality of dams or does, fetal weight, external congenital abnormalities (gross examination), visceral abnormalities, and skeletal defects.			
Negative Control	Water	Water	Water	Water
Positive Control	Aspirin (150 mg/kg)	Aspirin (250 mg/kg-day)	Aspirin (250 mg/kg-day)	6-Aminonicotinamide (2.5 mg/kg, Day 9)

Inhalation Exposure. Only one study was located regarding the inhalation toxicity of hexanedioic acid in animals. Alderley Park specific-pathogen-free rats (two per sex, weighing approximately 200 grams) were exposed to powdered hexanedioic acid at 126 mg/m³ via inhalation in a dynamic chamber 6 hours/day, 5 days/week for three weeks (Gage, 1970). Body weight and clinical signs of toxicity were monitored throughout the exposure period. Rats were sacrificed after 3 weeks of exposure and the following parameters were evaluated: unspecified urinalysis and hematology parameters, gross pathology, and microscopic pathology of 5 tissues (lungs, liver, kidneys, spleen and adrenals). The study report also indicated that the heart, jejunum, ileum, and thymus were “occasionally” examined. No effects on any endpoint were observed, making the 126 mg/m³ concentration used in this study a free standing NOAEL.

Other Studies

Hexanedioic acid was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, or TA1538 or in *Escherichia coli* (WP2uvrA), with or without addition of exogenous metabolic activation (Prival et al., 1991). Results were also negative in tests for mutagenicity in *S. typhimurium* strains TA1530 or G46 without activation *in vitro* and in host-mediated assays with these strains in mice given either a single dose or five consecutive daily doses of hexanedioic acid (Litton Bionetics, 1974). Hexanedioic acid did not cause a significant increase in *Saccharomyces cerevisiae* D3 recombinants *in vitro* and produced little or no response in host-mediated assays with this strain in mice (Litton Bionetics, 1974). Hexanedioic acid did not induce chromosomal aberrations in human embryonic lung cultures (WI-38) *in vitro* or in rat bone marrow *in vivo*, and did not induce dominant lethal mutations in an *in vivo* assay in male rats (Litton Bionetics, 1974). The compound also did not induce chromosomal nondisjunction in *Drosophila* (Ramel and Magnusson, 1979). A cell transformation assay in Syrian hamster embryo (SA7/SHE) cells was negative (Heidelberger et al., 1983).

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC RfDs FOR HEXANEDIOIC ACID

No relevant data were located regarding the subchronic or chronic toxicity of hexanedioic acid to humans following oral exposure. The most widely reported and most sensitive effect in animal feeding studies was reduced body weight gain, which was observed at doses of 1500-2800 mg/kg-day with chronic exposure (Horn et al., 1957) and 1800-5000 mg/kg-day with subchronic exposure (Lang and Bartsch, 1953; Hazleton, 1950; Litton Bionetics, 1974). In one of the subchronic studies, the reduction in weight gain was accompanied by marked chronic inflammation of the intestinal mucosa, as well as slight liver and kidney lesions (Lang and Bartsch, 1953). No intestinal (or other) lesions were seen in the chronic study (Horn et al., 1957). Horn et al. (1957) did, however, observe intestinal irritation and hemorrhage at ≥ 1500 mg/kg in an acute range-finding study in mice exposed by oral gavage in methylcellulose. This suggests that hexanedioic acid is irritating to the intestinal mucosa by concentrated bolus exposure, and that differences in dietary composition or feeding regimen may explain the different findings in the subchronic and chronic studies. However, gastrointestinal lesions were not directly related to the effect on body weight gain, which was found with or without the lesions. A series of gestational exposure studies found no evidence to suggest that the developing fetus is a sensitive target for hexanedioic acid (FDRL, 1972, 1974).

The chronic study by Horn et al. (1957), which found both the lowest LOAEL for hexanedioic acid and a corresponding NOAEL, is suitable for both subchronic and chronic RfD derivation. In this study, male rats were maintained on a diet supplemented with 0, 0.1%, 1%, 3%, or 5% hexanedioic acid (approximately 0, 44, 470, 1500, or 2800 mg hexanedioic acid/kg-day). Females were only exposed at 0% or 1% (approximately 0 or 630 mg/kg-day, respectively). The only effect observed in this study was decreased body weight at the 3% and 5% dietary concentrations (≈ 1500 and 2800 mg/kg-day). Body weights at these concentrations were substantially reduced at all reported time intervals (\geq Week 8). The use of the 2-year study as the basis for the subchronic study is justified because the observed effect (decreased body

weight) occurred at subchronic durations (≥ 8 weeks) and persisted for the entire 2-year study period. The study included gross necropsy, weight determinations for the major organs, and microscopic examination of 14 tissues. The NOAEL was 470 mg/kg-day. Application of a composite uncertainty factor of 300 (10 for extrapolation from rats to humans, 10 to protect sensitive individuals, and 3 for deficiencies in the database, including absence of reproduction toxicity studies) to the NOAEL of 470 mg/kg-day yields provisional **subchronic and chronic RfDs of 2 mg/kg-day** for hexanedioic acid, as follows:

$$\begin{aligned} \text{subchronic p-RfD/p-RfD} &= \text{NOAEL} / \text{UF} \\ &= 470 \text{ mg/kg-day} / 300 \\ &= 2 \text{ mg/kg-day or } 2\text{E-}0 \text{ mg/kg-day} \end{aligned}$$

Confidence in the critical study is medium. The study included investigations of key systemic endpoints in multiple dose groups and identified a NOAEL and LOAEL. The sensitivity of the test may have been somewhat compromised by the lack of clinical chemistry or hematology evaluations and the limited number of tissues microscopically examined. Confidence in the database is medium because of the lack of reproductive toxicity studies and the lack of available data on some commonly evaluated toxicological parameters (e.g., clinical chemistry). Confidence in the subchronic and chronic p-RfDs for hexanedioic acid is, therefore, medium.

FASEB (1976) estimated per capita consumption of hexanedioic acid in the U.S. to be about 0.8 mg/kg-day, based on the quantity of the chemical used in foods in 1970. This estimate was considered to be high because wastage and other losses were not taken into account. Estimated intake based on market surveys was as high as 8 mg/kg-day for chronic exposure, but these data were considered to be less reliable (FASEB, 1976). On the basis of more recent production data, WHO (2000) estimated per capita daily intake of about 18 mg (0.26 mg/kg-day) for hexanedioic acid from its use as a flavoring agent in the U.S. Based on the most recent and most reliable data available, intake of hexanedioic acid in the U.S. is less than 0.8 mg/kg-day, and probably less than 0.26 mg/kg-day. The provisional RfD of 2 mg/kg-day, therefore, is at least 2-10 fold higher than the best estimate of chronic intake of hexanedioic acid in the U.S.

FEASIBILITY OF DERIVING PROVISIONAL SUBCHRONIC AND CHRONIC RfCs FOR HEXANEDIOIC ACID

Short-term (3 weeks) inhalation exposure to hexanedioic acid powder at a concentration of 126 mg/m³ resulted in no observed toxic effects in male or female rats (Gage, 1970). However, the limitations of this study (e.g., use of only 2 rats/sex, evaluation of few toxicological endpoints, microscopic evaluation of only 5 tissues, inclusion of only a single dose level, short exposure duration) preclude its use for p-RfC derivation. No other repeated-dose inhalation toxicity studies were located. The lack of inhalation data precluded the derivation of non-cancer inhalation toxicity values.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR HEXANEDIOIC ACID

Weight-of-evidence Classification

No evidence of carcinogenicity was observed in a 2-year dietary exposure study in rats (Horn et al., 1957). The study, however, was limited by the small number of animals evaluated. A fairly broad array of genotoxicity studies found no evidence that hexanedioic acid is a genetic toxicant. Overall, there is *inadequate information to assess carcinogenic potential* of hexanedioic acid under the Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005).

Quantitative Estimates of Carcinogenic Risk

Derivation of quantitative estimates of cancer risk for hexanedioic acid is precluded by the lack of data demonstrating carcinogenicity associated with hexanedioic acid exposure.

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