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Provisional Peer-Reviewed Toxicity Values for

delta-Hexachlorocyclohexane
(CASRN 319-86-8)

U.S. EPA Office of Research and Development
Center for Public Health and Environmental Assessment

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Center for Public Health and Environmental Assessment
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Questions regarding the contents of this PPRTV document may be directed to the U.S. EPA Office of Research and Development (ORD) Center for Public Health and Environmental Assessment (CPHEA) website at https://ecomments.epa.gov/pprtv.

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COMMONLY USED ABBREVIATIONS AND ACRONYMS

α2u-g	alpha 2u-globulin
ACGIH	American Conference of Governmental Industrial Hygienists
AIC	Akaike's information criterion
ALD	approximate lethal dosage
ALT	alanine aminotransferase
AR	androgen receptor
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
BMC	benchmark concentration
BMCL	benchmark concentration lower confidence limit
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
BW	body weight
CA	chromosomal aberration
CAS	Chemical Abstracts Service
CASRN	Chemical Abstracts Service Registry Number
CBI	covalent binding index
CHO	Chinese hamster ovary (cell line cells)
CL	confidence limit
CNS	central nervous system
CPHEA	Center for Public Health and Environmental Assessment
CPN	chronic progressive nephropathy
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DEN	diethylnitrosamine
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
ER	estrogen receptor
FDA	Food and Drug Administration
FEV_1	forced expiratory volume of 1 second
GD	gestation day
GDH	glutamate dehydrogenase
GGT	γ-glutamyl transferase
GSH	glutathione
GST	glutathione S transferase
Hb/g A	animal blood gas partition coefficient
Hb/g H	human blood gas partition coefficient
HEC	human equivalent concentration
HED	human equivalent dose
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IVF	in vitro fertilization
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
MN	micronuclei
MNPCE	micronucleated polychromatic erythrocyte
MOA	mode of action
MTD	maximum tolerated dose
NAG	*N*-acetyl-β-D-glucosaminidase
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
NZW	New Zealand White (rabbit breed)
OCT	ornithine carbamoyl transferase
ORD	Office of Research and Development
PBPK	physiologically based pharmacokinetic
PCNA	proliferating cell nuclear antigen
PND	postnatal day
POD	point of departure
POD_{ADJ}	duration adjusted POD
QSAR	quantitative structure-activity relationship
RBC	red blood cell
RDS	replicative DNA synthesis
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	regional gas dose ratio
RNA	ribonucleic acid
SAR	structure-activity relationship
SCE	sister chromatid exchange
SD	standard deviation
SDH	sorbitol dehydrogenase
SE	standard error
SGOT	glutamic oxaloacetic transaminase, also known as AST
SGPT	glutamic pyruvic transaminase, also known as ALT
SSD	systemic scleroderma
TCA	trichloroacetic acid
TCE	trichloroethylene
TWA	time-weighted average
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_C	composite uncertainty factor
UF_D	database uncertainty factor
UF_H	intraspecies uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
U.S.	United States of America
WBC	white blood cell

Abbreviations and acronyms not listed on this page are defined upon first use in the PPRTV assessment.

DRAFT PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR DELTA-HEXACHLOROCYCLOHEXANE (CASRN 319-86-8)

BACKGROUND

A Provisional Peer-Reviewed Toxicity Value (PPRTV) is defined as a toxicity value derived for use in the Superfund program. PPRTVs are derived after a review of the relevant scientific literature using established U.S. Environmental Protection Agency (U.S. EPA) guidance on human health toxicity value derivations.

The purpose of this document is to provide support for the hazard and dose-response assessment pertaining to chronic and subchronic exposures to substances of concern, to present the major conclusions reached in the hazard identification and derivation of the PPRTVs, and to characterize the overall confidence in these conclusions and toxicity values. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of this substance.

Currently available PPRTV assessments can be accessed on the U.S. EPA's PPRTV website at https://www.epa.gov/pprtv. PPRTV assessments are eligible to be updated on a 5-year cycle and revised as appropriate to incorporate new data or methodologies that might impact the toxicity values or affect the characterization of the chemical's potential for causing toxicologically relevant human-health effects. Questions regarding nomination of chemicals for update can be sent to the appropriate U.S. EPA eComments Chemical Safety website at https://ecomments.epa.gov/chemicalsafety/.

QUALITY ASSURANCE

This work was conducted under the U.S. EPA Quality Assurance (QA) program to ensure data are of known and acceptable quality to support their intended use. Surveillance of the work by the assessment managers and programmatic scientific leads ensured adherence to QA processes and criteria, as well as quick and effective resolution of any problems. The QA manager, assessment managers, and programmatic scientific leads have determined under the QA program that this work meets all U.S. EPA quality requirements. This PPRTV assessment was written with guidance from the CPHEA Program Quality Assurance Project Plan (PQAPP), the QAPP titled *Program Quality Assurance Project Plan (PQAPP) for the Provisional Peer-Reviewed Toxicity Values (PPRTVs) and Related Assessments/Documents (L-CPAD-0032718-QP)*, and the PPRTV assessment development contractor QAPP titled *Quality Assurance Project Plan—Preparation of Provisional Toxicity Value (PTV) Documents (L-CPAD-0031971-QP)*. As part of the QA system, a quality product review is done prior to management clearance. A Technical Systems Audit may be performed at the discretion of the QA staff.

All PPRTV assessments receive internal peer review by at least two CPHEA scientists and an independent external peer review by at least three scientific experts. The reviews focus on whether all studies have been correctly selected, interpreted, and adequately described for the purposes of deriving a provisional reference value. The reviews also cover quantitative and qualitative aspects of the provisional value development and address whether uncertainties associated with the assessment have been adequately characterized.

DISCLAIMERS

The PPRTV document provides toxicity values and information about the toxicologically relevant effects of the chemical and the evidence on which the value is based, including the strengths and limitations of the data. All users are advised to review the information provided in this document to ensure that the PPRTV used is appropriate for the types of exposures and circumstances at the site in question and the risk management decision that would be supported by the risk assessment.

Other U.S. EPA programs or external parties who may choose to use PPRTVs are advised that Superfund resources will not generally be used to respond to challenges, if any, of PPRTVs used in a context outside of the Superfund program.

This document has been reviewed in accordance with U.S. EPA policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

QUESTIONS REGARDING PPRTVs

Questions regarding the content of this PPRTV assessment should be directed to the U.S. EPA ORD CPHEA website at https://ecomments.epa.gov/pprtv.

1. INTRODUCTION

delta-Hexachlorocyclohexane (δ-hexachlorocyclohexane; δ-HCH; delta-HCH), CASRN 319-86-8, is a discrete organic chemical consisting of a chlorinated six-membered cycloaliphatic with defined stereochemistry. δ-HCH is one of the HCH isomers that comprises the technical-grade HCH pesticide, which also contains lindane (gamma-HCH; γ-HCH) and other isomers of HCH. γ-HCH production ceased in 1976 in the United States; however, imported γ-HCH is available in the United States for insecticide use as a dust, powder, liquid, or concentrate. It is also available as a prescription medicine (lotion, cream, or shampoo) to treat and/or control scabies (mites) and head lice in humans (ATSDR, 2024). HCH is produced via photochlorination of benzene, which results in an isomeric mixture of alpha- (α-), beta- (β-), γ-, δ-, and epsilon- (ε-) HCH (NCBI, 2022a). δ-HCH is listed on the U.S. EPA's Toxic Substances Control Act (TSCA) public inventory, the Comprehensive Environmental Response, Compensation, and Liability (CERCLA) Act list of hazardous substances, the Superfund Amendments and Reauthorization Act (SARA), and the Clean Water Act (CWA) Priority Pollutant list (U.S. EPA, 2022c).

The empirical formula for δ-HCH is $C_6H_6Cl_6$; its structure is shown in Figure 1. The physicochemical properties for δ-HCH are provided in Table 1. δ-HCH is a colorless solid. It has moderate water solubility of 31.4 mg/L and low vapor pressure of 3.52×10^{-5} mm Hg at 25°C. Its low vapor pressure indicates that it will not volatilize from dry soil surfaces and will exist in both the vapor and particulate phase in air. In the atmosphere, vapor-phase δ-HCH has an estimated half-life of 18.7 days, based on rate of reaction with photochemically-produced hydroxyl radicals (U.S. EPA, 2022a). The measured soil adsorption coefficient (K_{oc}) values for δ-HCH indicate moderate potential for sorption to soil and low to slight mobility in soils (NCBI, 2022a; U.S. EPA, 2012). Experimental data for the hydrolysis of δ-HCH are not readily available. However, based on hydrolysis half-lives of 1.2 and 0.8 years at pH values of 7 and 8, respectively, for the related isomer, α-HCH, hydrolysis is expected to be slow (NCBI, 2022a).

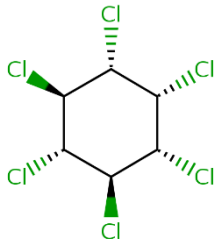


Figure 1. δ-Hexachlorocyclohexane (CASRN 319-86-8) Structure

Table 1. Physicochemical Properties of δ-HCH (CASRN 319-86-8)

Property (unit)	Value[a]
Physical state	Solid
Boiling point (°C)	60 (at 0.34 mm Hg)[b]
Melting point (°C)	141.5[b]
Density (g/cm^3 at 25°C)	1.59 (predicted average)
Vapor pressure (mm Hg at 25°C)	3.52×10^{-5} [b]
pH (unitless)	NA
Acid dissociation constant (pKa) (unitless)	NA
Solubility in water (mg/L)	31.4 (at 25°C)[b]
Octanol-water partition coefficient (log K_{ow})	4.14[b]
Henry's law constant (atm-m^3/mol)	NV[b]
Soil adsorption coefficient K_{oc} (L/kg)	2.7×10^3 (predicted average)
Atmospheric OH rate constant (cm^3/molecule-sec at 25°C)	1.66×10^{-13} (predicted average)
Atmospheric half-life (d)	18.7[b,c]
Relative vapor density (air = 1)	NV
Molecular weight (g/mol)	290.81
Flash point (°C)	145 (predicted average)

[a]Data were extracted from the U.S. EPA CompTox Chemicals Dashboard: δ-HCH, CASRN 319-86-8; https://comptox.epa.gov/dashboard/chemical/properties/DTXSID5024134; accessed February 7, 2024. Data presented are experimental averages unless otherwise noted.
[b]Data were obtained from the PhysProp database: δ-HCH, CASRN 319-86-8; https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface; accessed November 4, 2021.
[c]Half-life = 0.6931/k[OH]; calculated assuming k[OH] = 5.73×10^{-13}, 12-hour day, and 1.5OH/cm^3.

HCH = hexachlorocyclohexane; NA = not applicable; NV = not available.

A summary of available toxicity values for δ-HCH from the U.S. EPA and other agencies/organizations is provided in Table 2.

Table 2. Summary of Available Toxicity Values and Qualitative Conclusions Regarding Carcinogenicity for δ-HCH (CASRN 319-86-8)

Source/Parameter[a,b]	Value (applicability)	Notes	Reference[c]
Noncancer			
IRIS	NV	No RfD or RfC derived	U.S. EPA (2003)
HEAST	NV	Data inadequate for quantitative risk assessment	U.S. EPA (1997)
DWSHA	NV	NA	U.S. EPA (2018a)
ATSDR	NV	NA	(ATSDR, 2024)
WHO	NV	NA	IPCS (2020)
CalEPA	NV	NA	CalEPA (2022, 2020)

Table 2. Summary of Available Toxicity Values and Qualitative Conclusions Regarding Carcinogenicity for δ-HCH (CASRN 319-86-8)

Source/Parameter[a,b]	Value (applicability)	Notes	Reference[c]
OSHA	NV	NA	OSHA (2020, 2017a, 2017b)
NIOSH	NV	NA	NIOSH (2018)
ACGIH	NV	NA	ACGIH (2022)
TCEQ (RfD)	0.0003 mg/kg-d	Basis for RfD not specified; value developed with TCEQ's protocol	TCEQ (2021, 2015)
Cancer			
IRIS	Group D, not classifiable as to human carcinogenicity	Based on no human data and inadequate data from animal bioassays	U.S. EPA (2003)
HEAST	NV	NA	U.S. EPA (1997)
DWSHA	NV	NA	U.S. EPA (2018a)
NTP (WOE for HCH isomers including technical-grade HCH)	Reasonably anticipated to be human carcinogens	Based on sufficient evidence of carcinogenicity from studies in experimental animals	NTP (2021)
IARC (WOE for HCHs)	Group 2B, possibly carcinogenic to humans	Based on inadequate evidence for carcinogenicity in humans; sufficient evidence for carcinogenicity in animals for technical-grade HCH and α-HCH; and limited evidence for carcinogenicity in animals for β-HCH and γ-HCH	IARC (1987)
CalEPA	NV	NA	CalEPA (2022, 2020)
ACGIH	NV	NA	ACGIH (2022)
TCEQ (WOE)	B2, probable human carcinogen with inadequate evidence of carcinogenicity	Basis not specified; value developed with TCEQ's protocol	TCEQ (2021, 2015)
TCEQ (OSF)	1.8 $(mg/kg\text{-}d)^{-1}$	Basis not specified; value developed with TCEQ's protocol	TCEQ (2021, 2015)
TCEQ (IUR)	0.00051 $(\mu g/m^3)^{-1}$	Basis not specified; value developed with TCEQ's protocol	TCEQ (2021, 2015)

[a]Sources: ACGIH = American Conference of Governmental Industrial Hygienists; ATSDR = Agency for Toxic Substances and Disease Registry; CalEPA = California Environmental Protection Agency; DWSHA = Drinking Water Standards and Health Advisories; HEAST = Health Effects Assessment Summary Tables; IARC = International Agency for Research on Cancer; IRIS = Integrated Risk Information System; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; TCEQ = Texas Commission of Environmental Quality; WHO = World Health Organization.
[b]Parameters: IUR = inhalation unit risk factor; OSF = oral slope factor; RfC = reference concentration; RfD = reference dose; WOE = weight of evidence.
[c]Reference date is the publication date for the database and not the date the source was accessed.

HCH = hexachlorocyclohexane; NA = not applicable; NV = not available.

Non-date-limited literature searches were conducted in July 2019 and most recently updated in May 2024 for studies pertinent to understanding potential human health hazards of δ-HCH, CASRN 319-86-8. Searches were conducted using the U.S. EPA's Health and Environmental Research Online (HERO) database of scientific literature. HERO searches the following databases: PubMed, TOXLINE (including TSCATS1)[1], Scopus, and Web of Science. The National Technical Reports Library (NTRL) was searched for government reports from 2018 through July 2023[2]. The following resources were searched outside of HERO for health-related values: American Conference of Governmental Industrial Hygienists (ACGIH), Agency for Toxic Substances and Disease Registry (ATSDR), California Environmental Protection Agency (CalEPA), Defense Technical Information Center (DTIC), European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), European Chemicals Agency (ECHA), the U.S. EPA Chemical Data Access Tool (CDAT), U.S. EPA's ChemView, the U.S. EPA Integrated Risk Information System (IRIS), the U.S. EPA Health Effects Assessment Summary Tables (HEAST), the U.S. EPA Office of Water (OW), International Agency for Research on Cancer (IARC), the U.S. EPA TSCATS2/TSCATS8e, the U.S. EPA High Production Volume (HPV), Chemicals via International Programme on Chemical Safety (IPCS) INCHEM, Japan Existing Chemical Data Base (JECDB), Organisation for Economic Co-operation and Development (OECD) Screening Information Data Sets (SIDS), OECD International Uniform Chemical Information Database (IUCLID), OECD HPV, National Institute for Occupational Safety and Health (NIOSH), National Toxicology Program (NTP), Occupational Safety and Health Administration (OSHA), and World Health Organization (WHO).

[1]TOXLINE was retired in December 2019. Searches of this database were conducted through July 2019.
[2]NTRL was a subset of TOXLINE until December 2019 when TOXLINE was discontinued. Searches of NTRL were conducted starting in 2018 to ensure that references were not missed due to delays in importing items into the database.

2. REVIEW OF POTENTIALLY RELEVANT DATA (NONCANCER AND CANCER)

Table 3 provides an overview of the relevant noncancer database for δ-HCH and includes all potentially relevant chronic studies. No repeated-dose short-term-, subchronic-, or reproductive and developmental toxicity studies for δ-HCH- in humans or animals exposed by oral or inhalation routes adequate for derivation of provisional toxicity values were identified. The phrase "statistical significance," and term "significant," used throughout the document, indicates a *p*-value- of <0.05 unless otherwise specified.

Table 3. Summary of Potentially Relevant Noncancer Data for δ-HCH (CASRN 318-96-8)

Category[a]	Number of Male/Female, Strain Species, Study Type, Reported Doses, Study Duration	Dosimetry (mg/kg-d)[b]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference (comments)	Notes[c]
Human							
ND							
Animal							
1. Oral (mg/kg-d)							
Chronic	20 M, dd mouse, diet, 24 wk Reported treatment: 0, 100, 250, or 500 ppm	0, 18.6, 47.1, 93.1	Significantly increased absolute and relative liver weights (20 and 23%, respectively); slight hepatocellular hypertrophy (incidence not reported).	47.1	93.1	Ito et al. (1973) Only mortality, body weight, and hepatic endpoints were evaluated.	PR
Chronic	5−8 M, Wistar rat, diet, 24 or 48 wk Reported treatment: 0, 500, or 1,000 ppm	0, 44.02, 89.12 (24-wk groups) 0, 42.16, 82.80 (48-wk groups)	None identified.	NDr	NDr	Ito et al. (1975) Only mortality, body weight, and hepatic endpoints were evaluated; there was no appropriate matched control because untreated animals were sacrificed after 72 wk, while treated animals were sacrificed at 24 or 48 wk.	PR
2. Inhalation (mg/m^3)							
ND							

[a]Duration category is defined as follows: chronic = repeated exposure for >10% life span for humans (>~90 days to 2 years in typically used laboratory animal species) (U.S. EPA, 2002).
bDosimetry: Doses are presented as ADDs (mg/kg-day) for oral noncancer effects. All NOAELs/LOAELs were identified by the U.S. EPA unless noted otherwise.
cNotes: PR = peer reviewed.

ADD = adjusted daily dose; HCH = hexachlorocyclohexane; LOAEL = lowest-observed-adverse-effect level; ND = no data; NDr = not determined; NOAEL = no-observed-adverse-effect level.

2.1. HUMAN STUDIES

Pi et al. (2020)

In a published, case-control study, 103 cases of newborns or fetuses (including stillbirths or terminations) with orofacial clefts (OFC) were evaluated for placental concentrations of organochlorine pesticides (i.e., HCH [including δ-HCH], dichlorodiphenyltrichloroethane, aldrin, and isodrin), and compared with 103 controls matched for sex, location, and maternal menstrual cycle (Pi et al., 2020). The association between placental concentrations and OFCs was evaluated using logistic regression and Bayesian kernel machine regression. Median (P_{25}–P_{75} quartiles) placental concentration levels of δ-HCH were 4.83 (3.82−6.3) ng/g in cases and 5.74 (4.05−7.6) ng/g in controls. There was a statistically significant association between placental concentrations of δ-HCH and reduced odds of OFCs in the logistic regression analysis and no association in the Bayesian kernel machine regression. Although quantitative biomonitoring data were reported, information does not exist to support the calculation of direct δ-HCH exposure (i.e., external dose) from reported exposure biomarker concentrations (i.e., physiologically based pharmacokinetic [PBPK] models were not identified for δ-HCH). Therefore, this study is inadequate for quantitative dose-response analysis.

No other human studies were identified.

2.2. ANIMAL STUDIES

2.2.1. Oral Exposures

No short-term, subchronic, or reproductive/developmental toxicity studies of δ-HCH were identified. Two chronic oral studies that focused on cancer endpoints (Ito et al., 1975; Ito et al., 1973) are described below.

Chronic Studies

Ito et al. (1973)

In a published, peer-reviewed study, male dd strain mice (20/group) were administered δ-HCH (purity >99%) at concentrations of 0, 100, 250, or 500 ppm (corresponding to adjusted daily doses [ADDs] of 0, 18.6, 47.1, and 93.1 mg/kg-day[3]) in the diet for 24 weeks. Additional groups were administered other isomers of HCH (α-, β-, γ-) alone or in combination with each other. Throughout the treatment period, animals were evaluated for mortality and weekly body-weight measurements. After treatment, surviving animals were fasted for 18 hours prior to sacrifice. Animals were weighed at necropsy and macroscopically evaluated for liver tumors and metastases (tissues examined were not specified). Livers were excised and weighed, and sections were analyzed by light and electron microscopy. Statistical analyses were not performed by the study authors. Statistical analysis of continuous data sets was conducted by the U.S. EPA for this review using Student's *t*-test.

[3]Dose estimates were calculated using reported body weights (BWs) and allometric equations to relate food consumption to BW (U.S. EPA, 1988). Averages of initial and final BWs in the mouse study (Ito et al., 1973) were 0.0289, 0.0280, and 0.0289 kg in the 100-, 250-, and 500-ppm groups, respectively. Food intake rates estimated from these BWs were 0.00538, 0.00527, and 0.00538 kg/day, respectively. A representative calculation is as follows: dose (mg/kg-day) = concentration in diet (mg/kg diet) × food consumption rate (kg diet/day) ÷ estimated average BW (kg): 100 mg/kg diet × 0.00538 kg diet/day ÷ 0.0289 kg BW = 18.6 mg/kg-day.

No mortality or changes in body weights were observed at any dose. Absolute and relative liver weights were significantly increased by 20 and 23%, respectively, at 93.1 mg/kg-day compared to control animals (see Table B-1, statistical test performed for this review). No change in liver weights was observed at 47.1 mg/kg-day. Slight hepatocellular hypertrophy was observed at 93.1 mg/kg-day (incidence not reported). No other histopathological lesions were observed, including liver tumors. Ultrastructural examination revealed increased smooth endoplasmic reticulum in the cytoplasm in δ-HCH-treated animals (dose not specified). No other ultrastructural changes were observed.

A lowest-observed-adverse-effect level (LOAEL) of 93.1 mg/kg-day was identified in this study based on slight hepatocellular hypertrophy and increased liver weights (absolute and relative increases of 20 and 23%, respectively). A no-observed-adverse-effect level (NOAEL) of 47.1 mg/kg-day was identified. The administered doses of 0, 18.6, 47.1, and 93.1 mg/kg-day correspond to human equivalent doses (HEDs) of 2.65, 6.65, or 13.3 mg/kg-day[4].

Ito et al. (1975)

In a published, peer reviewed study, male Wistar rats (5−8/group) were administered δ-HCH (purity >99%) at concentrations of 0, 500, or 1,000 ppm via the diet. Separate groups were exposed for 24 or 48 weeks. Doses were estimated to be 44.02 and 89.12 mg/kg-day in the 500- and 1,000-ppm groups, respectively, exposed for 24 weeks and 42.16 and 82.80 mg/kg-day in the corresponding groups exposed for 48 weeks[5]. Controls were sacrificed after 72 weeks and animals in the treatment groups were sacrificed after 24 or 48 weeks. Mortality and body weights were measured. At necropsy, animals were evaluated for grossly observable tumors, and livers were excised, weighed, and examined for histopathological changes. Statistical analysis was not described by the study authors.

No mortality was reported. Body weights, absolute and relative liver weights, and histopathology findings are summarized in Table B-2. However, since the control animals were sacrificed after 72 weeks vs. 24 or 48 weeks for the treated animals, a direct comparison could not be made. Slight hepatocellular hypertrophy was observed in animals of the 1,000-ppm group that were sacrificed at 48 weeks (incidence not reported); however, no control animals were sacrificed at this time point. Control animals sacrificed at 72 weeks exhibited no hepatic histopathological changes. It was reported that changes were not remarkable in any other organs, but no further details were provided. No tumors were observed in the exposed or control groups. Effect levels for noncancer endpoints could not be determined in the absence of an appropriate control. The administered doses of 0, 44.02, or 89.12 mg/kg-day (24-week groups) correspond to HEDs of 0, 10.90, and 21.89 mg/kg-day; the administered doses of 0, 42.16, and

[4]ADDs were converted to HEDs of 0, 2.65, 6.65, and 13.3 mg/kg-day in males using dosimetric adjustment factors (DAFs) of 0.14, where HED = ADD × DAF. The DAFs were calculated as follows: DAF = $(BW_a \div BW_h)^{1/4}$, where BW_a = animal BW and BW_h = human BW. Averages of initial and final BWs in the mouse study (Ito et al., 1973) were 0.0289, 0.0280, and 0.0289 kg in the 100-, 250-, and 500-ppm groups, respectively. For humans, the reference value of 70 kg was used for BW, as recommended by the U.S. EPA (1988).

[5]Dose estimates were calculated using reported BWs and allometric equations to relate food consumption to BW (U.S. EPA, 1988). Averages of initial and final BWs were 0.2635 and 0.2547 kg in the 500- and 1,000-ppm groups, respectively, exposed for 24 weeks and 0.2977 and 0.3140 kg in the corresponding groups exposed for 48 weeks. Food intake rates estimated from these BWs were 0.0232, 0.0227, 0.0251, and 0.0260 kg, respectively. A representative calculation is as follows: dose (mg/kg-day) = concentration in diet (mg/kg diet) × food consumption rate (kg diet/day) ÷ estimated average BW (kg): 500 mg/kg diet × 0.0232 kg diet/day ÷ 0.2635 kg BW = 44.02 mg/kg/d.

82.80 mg/kg-day (48-week groups) correspond to HEDs of 0, 10.77, and 21.43 mg/kg-day[6], respectively.

2.2.2. Inhalation Exposures

No inhalation studies of δ-HCH were identified in the available literature.

2.3. OTHER DATA (SHORT-TERM TESTS, OTHER EXAMINATIONS)

2.3.1. Genotoxicity

Genotoxicity data for δ-HCH are limited to a single study of deoxyribonucleic acid (DNA) binding. When male NMRI mice (two per group) were administered ~7 mg/kg [^{3}H]δ-HCH via gavage followed by [^{14}C]thymidine injection, δ-HCH exhibited a low level of binding to hepatic DNA based on measured radioactivity (Sagelsdorff et al., 1983). No additional genotoxicity studies were identified.

2.3.2. Supporting Animal Studies

Table 4 provides an overview of other supporting studies on δ-HCH. Acute-duration studies of rats given δ-HCH showed no effects on body weight, food consumption, body temperature, or brain serotonin or dopamine levels at single oral doses up to 30 mg/kg (Artigas et al., 1988b; Camón et al., 1988). Unlike its stereoisomer γ-HCH, δ-HCH did not induce convulsions after single oral doses up to 100 mg/kg in rats (Barrón et al., 1995) or up to 300 mg/kg in mice (Tusell et al., 1993). In rats given 100 mg/kg δ-HCH by gavage and sacrificed 4 hours later, no changes in brain histopathology were noted (Barrón et al., 1995). Mice exposed once to δ-HCH by intraperitoneal (i.p.) injection exhibited reduced motor activity in the first hour after doses ≥240 mg/kg (Fishman and Gianutsos, 1988, 1987).

[6]ADDs were converted to HEDs of 0, 10.90, and 21.89 mg/kg-day (24-week groups) and 0, 10.77, and 21.43 mg/kg-day (48-week groups) using DAFs of 0.25 and 0.26 (24- and 48-week groups, respectively), where HED = ADD × DAF. The DAFs were calculated as follows: DAF = $(BW_a \div BW_h)^{1/4}$, where BW_a = animal BW and BW_h = human BW. Average of initial and final BWs in the rat study (Ito et al., 1975) were 0.2635 and 0.2547 kg in the 500- and 1,000-ppm groups, respectively, exposed for 24 weeks and 0.2977 and 0.3140 kg in the corresponding groups exposed for 48 weeks. For humans, the reference value of 70 kg was used for BW, as recommended by the U.S. EPA (1988).

Table 4. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Supporting studies in animals following oral exposure				
Acute (oral)	Male Wistar rats (7−10/group) were administered a single dose of 0 or 30 mg/kg δ-HCH in olive oil. Animals were sacrificed and brains were excised and dissected by region for evaluation of tissue levels of serotonin, dopamine, noradrenaline, and their metabolites 1 or 5 h after dosing.	No effects were observed on serotonin or dopamine in any region. Results for noradrenaline and metabolites were not reported for δ-HCH.	δ-HCH did not affect neurotransmitter levels in any region of the brain.	Artigas et al. (1988b)
Acute (oral)	Male Wistar rats (eight per sex per group) were administered a single dose of 0 or 30 mg/kg δ-HCH (99.5% purity) (vehicle not reported). Animals were observed for 5 h. Endpoints evaluated included body weight, food consumption, and body temperature.	No changes in body weight, food consumption, or body temperature were observed in animals exposed to δ-HCH.	δ-HCH did not affect body weight, food consumption, or body temperature under the conditions of this study.	Camón et al. (1988)
MOA/mechanistic studies				
Acute (oral)	Male Wistar rats (10/group) were administered 100 mg/kg δ-HCH, 30 mg/kg γ-HCH, 100 mg/kg δ-HCH intragastrically followed by i.p. administration of 30 mg/kg γ-HCH 2 h later, or a convulsant (60 mg/kg PTZ). HCH isomers were given via gavage in olive oil, while PTZ was administered by i.p. injection. Animals were observed for 4 h postdosing for convulsions. Brain tissue was excised and evaluated for histopathology and calmodulin (CaMI and CaMII) mRNA expression.	No convulsions were observed in the groups treated with δ-HCH (alone or with γ-HCH). There were no histopathological lesions, cell damage, or changes in cell number in the cortex or hippocampus in the groups treated with δ-HCH. Decreases in CaMI mRNA and increases in CaMII mRNA were observed in δ-HCH-treated animals compared to controls.	δ-HCH did not induce convulsions or histopathological changes in the brain under the conditions of the study. δ-HCH is a strong depressant that decreases motor activity and inhibits the convulsive effect of γ-HCH. The effects of δ-HCH on convulsant activity may be related to intracellular calcium levels.	Barrón et al. (1995)

Table 4. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Acute (oral)	Male Wistar rats (number not reported) were given a single gavage administration of δ-HCH at doses of 0, 20, 40, 80, or 100 mg/kg. At 2 h postdosing, a convulsant (20 mg γ-HCH/kg, 60 mg PTZ/kg, or 1.5 mg PIC/kg) was administered via i.p. injection. Animals were sacrificed and brain tissue was extracted for c-Fos mRNA expression by northern blot and in situ hybridization. The protooncogene, c-Fos, was considered an early indicator of neurotoxicity.	δ-HCH alone did not induce c-Fos expression in the brain. Pretreament with δ-HCH blocked c-Fos induction by γ-HCH, as shown both by northern blot and in situ hybridization. δ-HCH did not block c-Fos induction but reduced the levels of mRNA induced by PTZ. In contrast, δ-HCH did not affect c-Fos induction by PIC.	δ-HCH blocked the induction of c-Fos mRNA expression by γ-HCH, and reduced the c-Fos induction by PTZ, but did not affect the induction of c-Fos expression by PIC.	Vendrell et al. (1992a, 1992b)
Acute (oral)	Wistar rats (five females/group) were administered a single dose of δ-HCH (purity 98−99%) at 200 mg/kg via gavage in olive oil followed by i.v. PTZ (a convulsant). Concentrations of δ-HCH in the brain were measured. The relationship between brain concentration of δ-HCH and PTZ-induced seizure was evaluated by linear regression.	δ-HCH increased the dose of PTZ required to induce convulsions, when compared with PTZ alone. The minimally effective concentration of δ-HCH for PTZ antagonistic action was 12−14 μg/g wet brain weight. In contrast to the other HCH isomers, the inhibition by δ-HCH was short-lived; the PTZ threshold returned to normal within 2 d after dosing.	δ-HCH inhibited PTZ-induced convulsions under the conditions of this study.	Vohland et al. (1981)
Acute (oral)	Male OF1 mice (10/group) were given a single administration of δ-HCH at doses of 100, 200, or 300 mg/kg via gavage in olive oil followed after 0.5 h by administration of a convulsant: γ-HCH (100 mg/kg) via gavage; or BAY-K-8644 (calcium channel agonist, 5 mg/kg), PTZ (GABAergic antagonist, 60 mg/kg), PIC (GABAergic antagonist, 4 mg/kg), NDMA (excitatory amino acid receptor agonist, 160 mg/kg), kainic acid (excitatory acid amino receptor agonist, 80 mg/kg), or Ro-5-4864 (atypical benzodiazepine, 40 mg/kg) via i.p. injection. Endpoints evaluated included mortality and convulsions.	In animals treated with δ-HCH alone, no mortality was reported, and no convulsions were observed at any dose. δ-HCH inhibited γ-HCH- and BAY-K-8644-induced convulsions and partially inhibited PTZ-induced convulsions. δ-HCH did not alter the convulsant effects of NDMA, kainic acid, or Ro-5-4864. δ-HCH administration potentiated PIC-induced convulsions. The incidences of convulsions induced by PIC (3 mg/kg) were 50, 80, and 100% at 0, 100, and 300 mg/kg δ-HCH, respectively; the incidences of PIC-induced mortality were 0, 30, and 60% at 0, 100, and 300 mg/kg δ-HCH, respectively.	δ-HCH alone did not cause death or convulsions. δ-HCH inhibited convulsions induced by γ-HCH, BAY-K-8644, and PTZ, and potentiated convulsions induced by PIC.	Tusell et al. (1993)

Table 4. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Acute (i.p.)	Male CD-1 mice (six per group) were administered 0, 40, 80, 240, or 400 mg/kg δ-HCH (purity 98%) in corn oil via i.p injection and observed for 1 h prior to sacrifice. Other groups were injected with 400 mg/kg δ-HCH and then 0 or 120 mg/kg γ-HCH 30 min later, followed by sacrifice 1 h after dosing with γ-HCH. At sacrifice, brains were excised, and cerebella were dissected for evaluation of cGMP. In a separate experiment, inhibition of TBOB (a ligand for the $GABA_a$-receptor-linked chloride channel) binding was examined using mouse cerebellar membranes cultured in vitro.	Motor activity was reduced in treated mice at 240 and 400 mg/kg. No convulsions were observed. In contrast to the substantial increase in cGMP induced by γ-HCH, exposure to ≥80 mg/kg δ-HCH resulted in decreased cerebellar cGMP. Co-exposure to δ- and γ-HCH resulted in a significant decrease in cGMP accumulation. In vitro experiments showed that δ-HCH inhibited TBOB binding with an IC_{50} of 19.4 μM; its affinity for TBOB was much lower than that of γ-HCH.	δ-HCH administration reduced motor activity in mice and cGMP accumulation in the mouse brain. In vitro, δ-HCH inhibited binding of TBOB, a ligand for the $GABA_a$-receptor-linked chloride channel.	Fishman and Gianutsos (1987)
Acute (i.p.)	Male CD-1 mice (8−10/treated, 24 control) were administered 0, 40, 80, 240, or 400 mg/kg δ-HCH (purity 98%) via i.p. injection in corn oil. Animals were placed in the box for locomotor activity for 20 min postinjection for acclimation then removed for 1 h and returned for 1-h observation. Additional groups were administered convulsant inducers, PTZ (50 mg/kg, i.p.) or PIC (20 mg/kg, i.p.), 1 h following δ-HCH. Endpoints evaluated include locomotor activity, convulsions, and startle response. In separate experiments, TBOB binding and $GABA_a$-stimulated ^{36}Cl uptake were measured in mouse cerebellar membranes in vitro.	Motor activity was reduced in treated mice at 400 mg/kg. No effect was observed on startle response, tremors, or hyperexcitability. Pretreatment with δ-HCH resulted in a dose-related decrease in the mean severity of seizures induced by PTZ, but increased the severity of seizures induced by PIC, compared to treatment with the convulsants alone. In vitro studies showed that δ-HCH decreased TBOB binding and ^{36}Cl uptake, but with much less potency than PTZ or PIC, respectively. Co-exposure to δ-HCH inhibited the decrease in ^{36}Cl uptake induced by PTZ, and potentiated the decrease induced by PIC in vitro. These results suggest that δ-HCH may influence convulsant activity via effects on $GABA_a$-linked chloride uptake.	δ-HCH alone reduced motor activity in mice. Pretreatment with δ-HCH inhibited convulsions induced by PTZ but increased convulsions induced by PIC.	Fishman and Gianutsos (1988)

Table 4. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Metabolism/toxicokinetic studies				
Acute ADME	Male Wistar rats (seven per group) were administered 30 mg/kg δ-HCH via gavage followed by 5-h observation period. The brain was excised and dissected by region for measurement of δ-HCH and its metabolites.	Of all the HCH isomers, δ-HCH had the highest concentrations of metabolites in the brain and the shortest half-life. Concentrations of metabolites in the cerebellum following δ-HCH administration were as follows: 3,5/4,6-pentachlorocyclohexane: 1,453 ng/L; pentachlorobenzene: 3.3 ng/L; and hexachlorobenzene: 0.5 ng/L. In addition to the above metabolites, 3,6/4,5-pentachlorocyclohexane and HCH were reported to be below the detection or quantitation limit of 5 ng/L. The concentration of unchanged δ-HCH was 9.6 ng/L.	δ-HCH produced the following cerebellar metabolites in rats: 3,5/4,6-pentachlorocyclo-hexane, pentachlorobenzene, and hexachlorobenzene. δ-HCH was rapidly removed from the brain via metabolism.	Artigas et al. (1988a)
Acute ADME	Weanling female Sprague Dawley rats (four per group) were administered 0 (vehicle control) or 2 mg/d δ-HCH via gavage for 7 d. Rat 24-h urine samples were collected daily for metabolite analysis using gas chromatography.	The chlorophenol metabolites of δ-HCH detected in urine were 2,4,6- and 2,4,5-TCP. Mean daily excretion of 2,4,6-TCP was ~8 times higher than mean daily excretion of 2,4,5-TCP.	In rats, urinary metabolites of δ-HCH were 2,4,6- and 2,4,5-TCP, with 2,4,6-TCP predominating.	Chadwick and Freal (1973)
Acute ADME	Wistar rats (five females/group) were administered single doses (200 mg/kg) of δ-HCH (purity 98−99%) via gavage. Concentrations in the brain, blood, and fat were measured at sacrifice 24 h after dosing. In another group of rats, concentrations in the brain were measured over 12 d after a dose of 200 mg/kg. A dose-response experiment was conducted in another group of rats; in this group, brain concentrations were measured 24 h after single oral doses of up to 1,000 µmol/kg.	Concentrations of δ-HCH in the blood ranged from 1.4 to 6.5 µg/g. Tissue:blood ratios varied with dose (brain:blood 3:1−5.5:1; fat:blood 123:1−213:1). The concentration of δ-HCH in the brain at 24 h increased in a linear and dose-dependent manner. The peak brain concentration of δ-HCH occurred between 12 and 24 h after dosing. Clearance of δ-HCH was faster than that of other isomers; the half-life was estimated to be 0.5 d.	Tissue concentrations, in order of greatest to least, were fat > brain > blood. The peak brain concentration of δ-HCH was reached by 24 h after dosing, and the half-life in the brain was 0.5 d.	Vohland et al. (1981)

Table 4. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Subchronic ADME	Technical-grade HCH containing ~4% δ-HCH was applied to the skin of male Wistar rats (6/group) at doses of 0, 50, or 100 mg/kg-d for 60 d. Animals were sacrificed the day after the last dose after overnight fasting. Testes were excised and dissected for isolation of the plasma membrane, which was analyzed for δ-HCH concentration.	δ-HCH concentration in the testicular plasma membrane was significantly increased at both doses compared to control animals.	δ-HCH was distributed to the testicular membrane after dermal exposure to technical-grade HCH.	Srivastava et al. (1995)

ADME = absorption, distribution, metabolism, and excretion; CAMI = calmodulin I; CAMII = calmodulin II; cGMP = cyclic GMP; GABA = γ-aminobutyric acid; HCH = hexachlorocyclohexane; IC_{50} = median inhibitory concentration; i.p. = intraperitoneal; i.v. = intravenous; MOA = mode-of-action; mRNA = messenger ribonucleic acid; NDMA = *N*-methyl-D-aspartate; PIC = picrotoxin; PTZ = pentylenetetrazol; TBOB = t-[3H]butylbicycloorthobenzoate; TCP = trichlorophenol.

2.3.3. Mode-of-Action/Mechanistic Studies

In rat and mouse studies examining the effect of δ-HCH pretreatment (by oral or i.p. administration) on the incidence and severity of convulsions induced by other compounds, δ-HCH administration was shown to inhibit convulsions induced by the γ-aminobutyric acid (GABA) antagonists, γ-HCH (Tusell et al., 1993) and pentylenetetrazol (PTZ) (Tusell et al., 1993; Fishman and Gianutsos, 1988; Vohland et al., 1981), and also by the voltage sensitive calcium channel agonist, BAY-K-8644 (Tusell et al., 1993). In contrast, δ-HCH pretreatment potentiated the effects of the GABA antagonist, picrotoxin (PIC), increasing the incidences and severity of seizures (Tusell et al., 1993; Fishman and Gianutsos, 1988).

Several studies (see Table 4) have examined the mechanistic basis for δ-HCH's disparate effects on convulsants (Tusell et al., 1993; Fishman and Gianutsos, 1988, 1987; Vohland et al., 1981). The divergent effects of δ-HCH on GABA antagonists, PTZ and PIC, were also seen in vitro in experiments using mouse brain membranes. In these experiments, co-exposure to δ-HCH inhibited the decrease in GABA-linked chloride uptake induced by PTZ, but potentiated the decrease induced by PIC (Fishman and Gianutsos, 1988). Barrón et al. (1995) observed that oral administration of δ-HCH to rats resulted in decreased expression of CaM I (calmodulin I, an intracellular calcium binding protein) messenger RNA (mRNA) and increased expression of CaM II mRNA in the brain. Vendrell et al. (1992a, 1992b) showed that δ-HCH pretreatment blocked the induction of c-Fos mRNA expression by γ-HCH and reduced the c-Fos induction by PTZ, but did not affect the induction of c-Fos expression by PIC. The expression of c-Fos protooncogene is induced by increases in intracellular calcium, and its expression is considered a marker of neuronal activity (Vendrell et al., 1992b). The c-Fos changes seen with δ-HCH exposure prior to convulsant administration paralleled its effects on convulsant activity, suggesting that c-Fos expression (possibly in conjunction with changes in intracellular calcium) may play a role in the effects of δ-HCH on convulsant activity.

2.3.4. Metabolism/Toxicokinetic Studies

Absorption of δ-HCH is inferred from detection of the compound in blood, adipose, and semen of exposed humans and in the blood, adipose, and brain following oral administration in animals (ATSDR, 2024). In female rats given single oral doses of δ-HCH, tissue concentrations showed preferential accumulation in adipose tissues, followed by brain and blood (Vohland et al., 1981). Distribution to the brain was dose-dependent and peaked within 24 hours of dosing, with an estimated half-life for clearance from the brain of 0.5 days (Vohland et al., 1981). The primary metabolite measured in brain (cerebellar) tissue of male rats 5 hours after a single oral dose of δ-HCH was 3,5/4,6-pentachlorocyclohexene, with much smaller concentrations of pentachlorobenzene and hexachlorobenzene (Artigas et al., 1988a). Compared with other HCH isomers tested, δ-HCH had the highest concentrations of metabolites in the brain and the lowest half-life, suggesting that metabolism is the main mechanism for clearance of δ-HCH from the brain (Artigas et al., 1988a). Dermal exposure of male rats to technical-grade HCH (~4% δ-HCH) for 60 days was shown to result in detectable δ-HCH in the testes (Srivastava et al., 1995). 2,4,6-Trichlorophenol was the major urinary metabolite of δ-HCH in weanling female rats exposed orally; lesser amounts of 2,4,5-trichlorophenol were also excreted in the urine (Chadwick and Freal, 1973).

3. DERIVATION OF PROVISIONAL VALUES

3.1. DERIVATION OF PROVISIONAL REFERENCE DOSES

The available studies of oral exposure to δ-HCH are limited to two chronic studies (Ito et al., 1975; Ito et al., 1973) evaluating only mortality, body weight, and liver endpoints in male mice and rats. Because these studies did not evaluate comprehensive health endpoints, and the rat study (Ito et al., 1973) did include concurrent controls at 24- and 48-week time points, these data are not adequate to derive provisional reference doses (p-RfDs) for δ-HCH. Instead, subchronic and screening chronic p-RfDs are derived in Appendix A using an alternative analogue approach.

3.2. DERIVATION OF PROVISIONAL REFERENCE CONCENTRATIONS

No studies were located regarding toxicity of δ-HCH to humans or animals via inhalation exposure. Due to the lack of inhalation toxicity data for δ-HCH, subchronic and chronic provisional reference concentrations (p-RfCs) were not derived. An alternative analogue approach to derivation of inhalation toxicity values was attempted, but a suitable analogue was not identified (see Appendix A).

3.3. SUMMARY OF NONCANCER PROVISIONAL REFERENCE VALUES

The noncancer screening provisional reference values for δ-HCH are summarized in Table 5.

Table 5. Summary of Noncancer Reference Values for δ-HCH (CASRN 318-96-8)

Toxicity Type (units)	Species	Critical Effect	p-Reference Value	POD Method	POD (HED/HEC)	UF_C	Principal Study
Screening subchronic p-RfD (mg/kg-d)	Rat	Electrophysiology changes in offspring	6×10^{-8}	NOAEL	0.000017 (based on analogue[a] POD)	300	Sauviat et al. (2005) as cited in ATSDR (2024)
Screening chronic p-RfD (mg/kg-d)	Rat	Electrophysiology changes in offspring	6×10^{-8}	NOAEL	0.000017 (based on analogue[a] POD)	300	Sauviat et al. (2005) as cited in ATSDR (2024)
Subchronic p-RfC (mg/m^3)	NDr						
Chronic p-RfC (mg/m^3)	NDr						

[a]γ-HCH was selected as a suitable source analogue for δ-HCH as described in Appendix A.

HCH = hexachlorocyclohexane; HEC = human equivalent concentration; HED = human equivalent dose; NDr = not determined; POD = point of departure; p-RfC = provisional inhalation reference concentration; p-RfD = provisional oral reference dose; UF_C = composite uncertainty factor.

3.4. CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR AND PROVISIONAL CANCER RISK ESTIMATES

A cancer assessment was not performed because a cancer WOE is available on the U.S. EPA's IRIS database (U.S. EPA, 2003). δ-HCH was determined to be not classifiable as to human carcinogenicity due to no human data and inadequate data from animal bioassays. No newer cancer data were located, precluding the derivation of cancer risk estimates for δ-HCH.

APPENDIX A. NONCANCER SCREENING PROVISIONAL VALUES

Due to the lack of evidence described in the main Provisional Peer-Reviewed Toxicity Value (PPRTV) assessment, it is inappropriate to derive a provisional toxicity value for delta-hexachlorocyclohexane (δ-HCH). However, some information is available for this chemical, which although insufficient to support derivation of a provisional toxicity value under current guidelines, may be of limited use to risk assessors. In such cases, the Center for Public Health and Environmental Assessment (CPHEA) summarizes available information in an appendix and develops a "screening value." Appendices receive the same level of internal and external scientific peer review as the provisional reference values to ensure their appropriateness within the limitations detailed in the document. Users of screening toxicity values in an appendix to a PPRTV assessment should understand that there could be more uncertainty associated with deriving an appendix screening toxicity value than for a value presented in the body of the assessment. Questions or concerns about the appropriate use of screening values should be directed to the CPHEA.

APPLICATION OF AN ALTERNATIVE ANALOGUE APPROACH (METHODS)

The analogue approach allows for the use of data from related compounds to calculate screening values when data for the compound of interest are limited or unavailable. Details regarding searches and methods for analogue analysis are presented in Wang et al. (2012) and Lizarraga et al. (2023) and chemical-specific parameters can be found in Appendix C. Candidate analogues are identified on the basis of three similarity categories (structure, toxicokinetics [metabolism] and toxicodynamics [toxicity and mode of action; MOA]) to facilitate the final analogue chemical selection. The analogue approach may or may not be route-specific or applicable to multiple routes of exposure. All information is considered together as part of the final WOE approach to select the most suitable source analogue.

In this assessment, an expanded analogue identification approach was utilized to collect an augmented set of candidate analogues for the target chemical. As described below, this approach applies a variety of tools and methods for identifying candidate analogues that are similar to the target chemical based on structural features; metabolic relationships; or related toxic effects and mechanisms of action. The application of a variety of different tools and methods to identify candidate analogues minimizes the impact of limitations of any individual tool or method on the pool of chemicals included, chemical fragments considered, and methods for assessing similarity. Further, the inclusion of techniques to identify analogues based on metabolism and toxicity or bioactivity expands the pool of candidates beyond those based exclusively on structural similarity. The specific tools described below used for the expanded analogue searches were selected because they are publicly available, supported by U.S. and Organisation for Economic Co-operation and Development (OECD) agencies, updated regularly, and widely used.

To identify structurally related compounds, an initial pool of analogues is identified using automated tools, including ChemIDplus[7] (NLM, 2022), the CompTox Chemicals Dashboard[8] (U.S. EPA, 2022a), and the OECD Quantitative Structure-Activity Relationship (QSAR) Toolbox[9] (OECD, 2022). Additional analogues identified as ChemIDplus-related substances, mixtures, and CompTox "related substances"[10] are also considered. CompTox General Read-Across (GenRA)[11] analogues are collected using the methods deployed on the publicly available GenRA Beta version, which may include Morgan fingerprints, Torsion fingerprints, ToxPrints and the use of ToxCast, Tox21, and ToxRef data (Patlewicz and Shah, 2023). For compounds that have very few analogues identified by structural similarity using a similarity threshold of 0.8 or 80%, substructure searches may be performed in the QSAR Toolbox, or similarity searches may be re-run using a reduced similarity threshold (e.g., <80%). Structural analogues are clustered using the Chemical Assessment Clustering Engine (ChemACE)[12] (U.S. EPA, 2011b) based on chemical fragments to support expert-driven refinement of the candidate pool. The ChemACE output is reviewed by an experienced chemist, who narrows the list of structural analogues based on expert judgment of multiple lines of evidence including known or expected structure-activity relationships, reactivity, and known or expected metabolic pathways. Initially, candidate analogues are screened for structural and chemical similarity to confirm that the analogues have the same reactive functional groups and similar overall size and structural features as the target chemical. Chemicals lacking key functionality or bearing additional functionality relative to the target are less desirable as analogues and are not selected as structural analogues. The selection may be expanded to include chemicals expected to be part of a metabolic series (either as metabolic precursors or as metabolites) of the target chemical. Chemicals that produce metabolites in common with the target may also be selected if the metabolite is known or suspected to be part of the mechanism of action. All candidate analogues

[7]ChemIDplus is a free, web search system that provides access to the structure and nomenclature authority files used for the identification of chemical substances cited in National Library of Medicine (NLM) databases, including the TOXNET system. The database contains over 350,000 chemical records, of which over 80,000 include chemical structures, and allows users to draw a chemical structure to search for similar substances using PubChem Substructure fingerprints (NLM, 2009; Liwanag et al., 2000). NLM retired ChemIDplus in December 2022.

[8]The U.S. EPA's CompTox Chemicals Dashboard provides publicly-accessible chemistry, toxicity, and exposure information for over one million chemicals (Williams et al., 2017). Using EPAM's Bingo fingerprints, the "Similar Compounds" tab provides a list of chemicals that are similar in structure to the selected chemical, based on the Tanimoto similarity search metric with a minimum similarity factor threshold of 0.8 (EPAM, 2024).

[9]The OECD QSAR Toolbox is a software application intended to be used by government, industry, and other stakeholders to fill gaps in data needed for assessing the hazards of chemicals. The application allows users to search for analogues based on structure similarity criteria and input similarity thresholds (OECD, 2017). It also contains metabolism simulators which are simplified versions of the simulators in CATALOGIC and TIMES and consist of hierarchically ordered molecular transformations (Yordanova et al., 2019).

[10]The CompTox Chemicals Dashboard "Related Substances" tab provides a chemical list of all chemicals related to the queried chemical through mapped relationships underlying the database. Relationships include searched chemical (self-relationship), salt form, monomer, polymer, predecessor component, component, Markush parent, Markush child, transformation parent, and transformation product (Williams et al., 2021).

[11]Operationalized within the CompTox Chemicals Dashboard, GenRA is an algorithmic approach that makes read-across predictions on the basis of a similarity weighted activity of source analogues (nearest neighbors). GenRA gives users the ability to identify candidate analogues based on structural and bioactivity information (U.S. EPA, 2022b).

[12]ChemACE clusters chemicals into groups based on structural features and a reasonable presumption that toxicity may be influenced by such structural characteristics (e.g., structural alerts, toxicophores). ChemACE identifies structural diversity in a large chemical inventory and highlights analogous clusters for potential read across. In the expanded analogue approach, clustering with ChemACE supports expert refinement of the candidate analogue pool. The ChemACE methodology is based on logic implemented in the Analog Identification Methodology (AIM) tool (http://aim.epa.gov) that identifies analogues based on the presence of common fragments using a tiered approach (U.S. EPA, 2011a).

are then screened for structural features that can influence their activity relative to the target. Examples of such features include steric influences of bulky substituent groups, branching, rigidity, presence of blocking groups on a functional group, and differing substitution patterns on aromatic rings. Finally, key physical and chemical properties of the candidate analogues are compared with the target to confirm that they can be expected to have similar bioavailability, similar transport, and similar abiotic transformation properties.

Toxicokinetic studies tagged as potentially relevant supplemental material during screening are used to identify metabolic analogues (metabolites and metabolic precursors). Metabolites are also identified from two OECD QSAR Toolbox metabolism simulators (in vivo rat metabolism simulator and rat liver S9 metabolism simulator). Targeted PubMed searches are conducted to identify metabolic precursors and other compounds that share any of the observed or predicted metabolites identified for the target chemical.

In vivo toxicity data for the target chemical (if available) are evaluated to determine whether characteristic effects associated with a particular mechanism of toxicity are observed (e.g., cholinesterase inhibition, inhibition of oxidative phosphorylation). In addition, in vitro mechanistic data tagged as potentially relevant supplemental material during screening or obtained from tools including GenRA, ToxCast/Tox21[13], and Comparative Toxicogenomics Database (CTD)[14] (CTD, 2022) are also evaluated for this purpose. ToxCast/Tox21 data available from the CompTox Chemicals Dashboard are collected for the target chemical to determine bioactivity in in vitro assays that may indicate potential mechanism(s) of action. The GenRA tool is used to search for analogues using Morgan, Torsion and ToxPrints fingerprint similarities and activity in ToxCast/Tox21 in vitro assays or ToxRef data (10 analogues collected from each neighbors dataset). Using the ToxCast/Tox21 bioactivity data, nearest neighbors identified may be considered potential candidate analogues. The CTD is searched to identify compounds with gene interactions similar to those induced by the target chemical; compounds with gene interactions similar to the target chemical (similarity index >0.5) may be considered potential candidate analogues.

Candidate analogues identified on the basis of the structural, metabolic, and toxicodynamic similarity contexts are interrogated through the CompTox Chemicals Dashboard, where QSAR-ready simplified molecular-input line-entry system (SMILES) are collected and toxicity value availability is determined (e.g., from the Agency for Toxic Substances and Disease Registry [ATSDR], California Environmental Protection Agency [CalEPA] Office of Environmental Health Hazard Assessment [OEHHA], the U.S. EPA Integrated Risk Information System [IRIS], PPRTV assessments). Analogues that have subchronic or chronic toxicity data or toxicity values available from other public health agencies are flagged for potential consideration as supportive evidence.

Analogue Search Results for δ-Hexachlorocyclohexane (CASRN 319-86-8) – Oral and Inhalation Routes

Candidate analogues for δ-HCH were identified as described above. Details of analogue search results are provided below. Settings of analogue search tools are provided in Appendix C.

[13] ToxCast and Tox21 are publicly available databases containing high-throughput assay endpoints covering a range of high-level cell responses (Thomas et al., 2018; U.S. EPA, 2018b).

[14] The CTD is a publicly available database that provides manually curated information about chemical–gene/protein interactions, chemical–disease and gene–disease relationships. The CTD allows users to identify chemicals that induce gene interactions similar to those induced by the target chemical (Davis et al., 2021).

Identification of Structural Analogues with Established Toxicity Values

δ-HCH is not a member of an existing OECD or New Chemical category. Candidate structural analogues for δ-HCH were identified using similarity searches in the OECD QSAR Toolbox (OECD, 2022), the U.S. EPA CompTox Chemicals Dashboard (U.S. EPA, 2022a), and ChemIDplus tools (NLM, 2022). A total of 160 unique structural analogues were identified for δ-HCH in the Dashboard, OECD QSAR Toolbox, and ChemIDplus (80% similarity threshold). Candidate analogues selected for inclusion were limited to stereoisomers of δ-HCH because these compounds are structurally identical to the target (100% similarity) and the toxicity of several stereoisomers has been well studied.

Using these criteria, a total of eight candidate structural analogues for δ-HCH were identified, as shown in Table A-1. Oral toxicity values were identified for three candidate structural analogues (bold). No inhalation toxicity values were identified for any candidate structural analogues.

Table A-1. Candidate Structural Analogues Identified for δ-HCH (CASRN 319-86-8) based on Tools and Expert Judgment

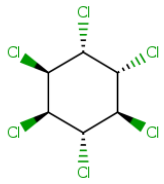
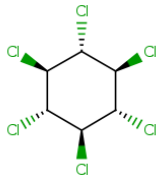
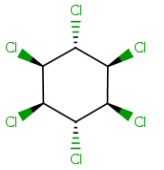
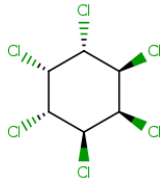
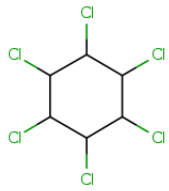
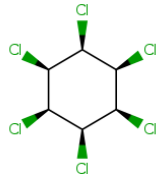
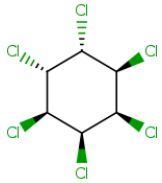
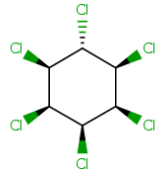
Tool (method)[a]	Analogue (CASRNs) Selected for Toxicity Value Searches[b]	Structure
Dashboard (Tanimoto), OECD QSAR Toolbox, and ChemIDplus (method not described)	**α-Hexachlorocyclohexane (CASRN 319-84-6)**	
	β-Hexachlorocyclohexane (CASRN 319-85-7)	
	γ-Hexachlorocyclohexane (lindane) (CASRN 58-89-9)	
	ε-Hexachlorocyclohexane (CASRN 6108-10-7)	
	1,2,3,4,5,6-Hexachlorocyclohexane (mixed isomers) (CASRN 608-73-1)	

Table A-1. Candidate Structural Analogues Identified for δ-HCH (CASRN 319-86-8) based on Tools and Expert Judgment

Tool (method)[a]	Analogue (CASRNs) Selected for Toxicity Value Searches[b]	Structure
ChemIDplus (method not described)	ζ-Hexachlorocyclohexane (CASRN 6108-11-8)	
	η-Hexachlorocyclohexane (CASRN 6108-12-9)	
	θ-Hexachlorocyclohexane (CASRN 6108-13-0)	

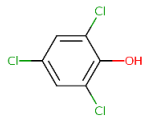
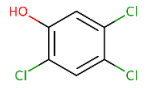
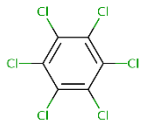
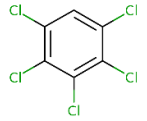
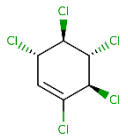
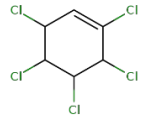
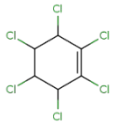
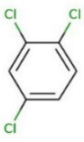
[a]80% similarity threshold was applied.
[b]**Bold** shows compounds with oral toxicity values.

HCH = hexachlorocyclohexane; OECD = Organisation for Economic Co-operation and Development; QSAR = quantitative structure-activity relationship.

Identification of Toxicokinetic Precursors or Metabolites with Established Toxicity Values

Experimental studies in the scientific literature (Artigas et al., 1988a; Chadwick and Freal, 1973) identified five urinary or brain metabolites of δ-HCH, and the OECD QSAR Toolbox (OECD, 2022) identified six additional predicted metabolites. The experimental and predicted metabolites of δ-HCH are shown in Table A-2.

Table A-2. Experimental and Predicted Metabolites of δ-HCH

Source	Metabolite Name	Structure
Experimental[a]	2,4,6-Trichlorophenol (CASRN 88-06-2)	
	2,4,5-Trichlorophenol (CASRN 95-95-4)	
	Hexachlorobenzene (CASRN 118-74-1)	
	Pentachlorobenzene (CASRN 608-93-5)	
	Cyclohexene, 1,3,4,5,6-pentachloro-, (3alpha,4beta,5alpha,6beta), also known as 3,5/4,6-pentachlorocyclohexene (CASRN 643-15-2)	
Predicted[b] (only)	1,3,4,5,6-Pentachlorocyclohexene (CASRN 1890-40-0)[c]	
	2,3,5-Trichloro-7-oxabicyclo[4.1.0]hepta-2,4-diene[d]	Not provided
	1,2,3,4,5,6-Hexachloro-1,3-cyclohexadiene[d]	Not provided
	1,2,3,4,5,6-Hexachlorocyclohexene (CASRN 1890-41-1)	
	1,3,5,6-Tetrachloro-1,3-cyclohexadiene[d]	Not provided
	1,2,4-Trichlorobenzene (CASRN 120-82-1)	

[a]Artigas et al. (1988a); Chadwick and Freal (1973).
[b]OECD QSAR Toolbox (OECD, 2022).
[c]Stereochemistry not defined by the OECD QSAR Toolbox profilers.
[d]CASRN not available for this metabolite; consequently, chemical structure is not provided.

HCH = hexachlorocyclohexane; OECD = Organisation for Economic Co-operation and Development; QSAR = quantitative structure-activity relationship.

PubMed searches (searching "δ-Hexachlorocyclohexane" or "319-86-8" and "metabolite") were conducted to identify metabolic precursors to δ-HCH. No metabolic precursors were identified. PubMed was also searched to identify other compounds that are metabolized to any of the observed or predicted metabolites of δ-HCH (searching the metabolite name or [CASRN if available] and "metabolite"). Four compounds that share at least one metabolite with δ-HCH were identified in these searches: gamma-HCH (γ-HCH), beta-HCH (β-HCH), β-1,3,4,5,6-pentachlorocyclohexane [3,4,6/5-PCCH], and prochloraz. The candidate analogues that share a metabolite with δ-HCH, along with the common metabolite and the reference(s) supporting the relationship are shown in Table A-3.

Table A-3. Candidate Analogues that Share a Common Metabolite with δ-HCH

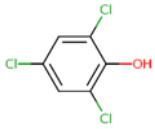
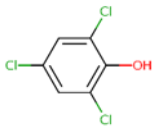
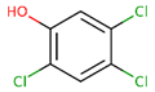
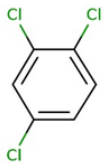
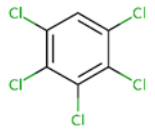
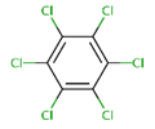
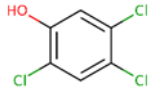
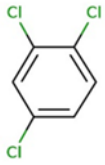
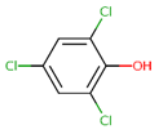
Candidate Analogue (parent compound)	δ-HCH Metabolite Shared by Candidate	Metabolite Structure	Reference
β-HCH	2,4,6-Trichlorophenol (CASRN 88-06-2)		Coosen and van Velsen (1989)
γ-HCH (lindane)	2,4,6-Trichlorophenol (CASRN 88-06-2)		Fitzloff et al. (1982)
	2,4,5-Trichlorophenol (CASRN 95-95-4)		Fitzloff and Pan (1984)
	1,2,4-Trichlorobenzene (CASRN 120-82-1)		Fitzloff and Pan (1984)
	Pentachlorobenzene (CASRN 608-93-5)		Pompa et al. (1994); Fitzloff et al. (1982); Engst et al. (1976)
	Hexachlorobenzene (CASRN 118-74-1)		Gopalaswamy and Aiyar (1986); Chadwick and Copeland (1985)

Table A-3. Candidate Analogues that Share a Common Metabolite with δ-HCH

Candidate Analogue (parent compound)	δ-HCH Metabolite Shared by Candidate	Metabolite Structure	Reference
β-1,3,4,5,6-pentachlorocyclohexane (3,4,6/5-PCCH) (CASRN 54083-25-9)	2,4,5-Trichlorophenol (CASRN 95-95-4)		Fitzloff and Pan (1984)
	1,2,4-Trichlorobenzene (CASRN 120-82-1)		Fitzloff and Pan (1984)
Prochloraz	2,4,6-Trichlorophenol (CASRN 88-06-2)		Laignelet et al. (1992)

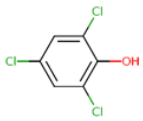
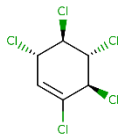
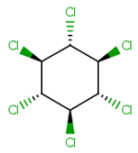
HCH = hexachlorocyclohexane.

Due to the large number of potential metabolic analogues (five experimental metabolites, six predicted metabolites, and four compounds that share at least one metabolite with δ-HCH), further investigation of the available data was performed to determine whether minor metabolites of δ-HCH or compounds that share minor metabolites with δ-HCH could be ruled out from consideration. Metabolites of δ-HCH measured in rat urine were 2,4,6- and 2,4,5-trichlorophenol, with excretion of 2,4,6-trichlorophenol predominating (ninefold higher than 2,4,5-trichlorophenol) (Chadwick and Freal, 1973). As the predominant urinary metabolite, 2,4,6-trichlorophenol was retained as a candidate metabolic analogue and 2,4,5-trichlorophenol was not considered further. In rat brain tissue, metabolites of δ-HCH included 3,5/4,6-pentachlorocyclohexene, pentachlorobenzene, and hexachlorobenzene (Artigas et al., 1988a). The primary metabolite of δ-HCH was 3,5/4,6-pentachlorocyclohexene, while pentachlorobenzene and hexachlorobenzene were identified as minor metabolites. Thus, 3,5/4,6-pentachlorocyclohexene was retained as a candidate metabolic analogue, while hexachlorobenzene and pentachlorobenzene were not considered further. Finally, given the availability of high-quality structural analogues (stereoisomers of δ-HCH) and in vivo metabolite information, predicted metabolites of δ-HCH were not considered further as candidate metabolic analogues.

Prochloraz, identified in the searches for compounds that share metabolites with δ-HCH, is metabolized to ethanol and acetic acid derivatives, with only minor amounts of 2,4,6-trichlorophenol (the metabolite shared with δ-HCH) produced (JMPR, 2001). Therefore, this compound was not considered further. β-1,3,4,5,6-Pentachlorocyclohexane, another compound that shares metabolites with the candidate, is metabolized to 2,4,5-trichlorophenol, which is only a minor metabolite of δ-HCH, and 1,2,4-trichlorobenzene, which is a predicted, but not experimentally observed, metabolite of δ-HCH; it does not share the predominant metabolite of δ-HCH (2,4,6-trichlorophenol) and was therefore not considered further.

Table A-4 summarizes the selected candidate metabolic analogues for δ-HCH. Relevant oral toxicity values were identified (in bold) for the following candidate metabolic analogues: 2,4,6-trichlorophenol (CASRN 88-06-2), γ-HCH, and β-HCH. None of the candidate metabolic analogues had a relevant inhalation toxicity value.

Table A-4. Candidate Metabolic Analogues of δ-HCH

Relationship to δ-HCH	Compound[a]	Structure	References
Metabolic precursor	None identified		
Metabolite	**2,4,6-Trichlorophenol (CASRN 88-06-2)**		U.S. EPA (2007)
	Cyclohexene, 1,3,4,5,6-pentachloro-, (3alpha,4beta,5alpha,6beta) (CASRN 643-15-2)[b]		
Shares common metabolite(s)	**γ-Hexachlorocyclohexane (CASRN 58-89-9)**		ATSDR (2024); U.S. EPA (1987)
	β-Hexachlorocyclohexane (CASRN 319-85-7)		ATSDR (2024)

[a]**Bold** shows compounds with oral toxicity values.
[b]Also known as 3,5/4,6-pentachlorocyclohexene.

HCH = hexachlorocyclohexane.

Identification of Analogues on the Basis of Toxicity/Mechanistic/MOA Information and Established Toxicity Values

No mechanistic analogues for δ-HCH were identified using the methods outlined above.

Available toxicity and mechanistic data for δ-HCH, described in the main PPRTV assessment above, were reviewed to determine whether characteristic effects associated with a particular mechanism of toxicity were observed (e.g., cholinesterase inhibition, inhibition of oxidative phosphorylation) that could be used to identify candidate analogues. In general, very few health effects from exposure to δ-HCH have been evaluated; thus, there is limited toxicity information to make a comparison with other analogues. The available animal studies (Ito et al., 1975; Ito et al., 1973) were focused on hepatic carcinogenicity, and reported only changes in liver weight and increased incidences of hepatocellular hypertrophy in male mice and rats. These effects do not suggest a specific mechanism of toxicity for δ-HCH.

δ-HCH was queried for bioactivity in assays reported in the U.S. EPA CompTox Chemicals Dashboard (U.S. EPA, 2022a). The compound was active in 89 out of 462 ToxCast assays (invitrodb version 3.4; accessed on December 6, 2021). There were no bioactive PubChem assays reported (accessed on December 6, 2021). The GenRA option within the Dashboard offers an option to search for analogues based on similarities in activity in ToxCast in vitro assays. Using the ToxCast bioactivity data, none of the nearest neighbors identified by GenRA had a similarity index ≥0.5 (beta version; accessed on January 21, 2022). Thus, no candidate analogues were identified from bioactivity data on the basis of toxicodynamic similarity.

The CTD identified several compounds with gene interactions similar to interactions induced by δ-HCH (Davis et al., 2021). In the CTD, similarity is measured by the Jaccard index, calculated as the size of the intersection of interacting genes for chemical A and chemical B divided by the size of the union of those genes (range 0 [no similarity] to 1 [complete similarity]). Among the compounds with gene interactions similar to δ-HCH, the numbers of common gene interactions ranged from 4 to 9 and similarity indices ranged from 0.19 to 0.35; the compound with the highest similarity index (0.35) was picrotoxinin (PIC; CASRN 17617-45-7). There were no compounds with a similarity index >0.5. No candidate analogues were identified from gene interaction data on the basis of toxicodynamic similarity.

Candidate Analogues Moving Forward for Evaluation

Searches for metabolic, structural, and toxicity/mechanistic analogues for δ-HCH yielded a total of 10 unique candidate analogues: 2 metabolites (2,4,6-trichlorophenol and 3,5/4,6-pentachlorocyclohexene), 2 compounds with common metabolites (β-HCH and γ-HCH) that were also identified as structural analogues, and 6 other structural analogues (α-HCH, ε-HCH, ζ-HCH, η -HCH, θ-HCH, and mixed HCH). Of the candidates, four compounds have available oral toxicity values (α-, β -, and γ-HCH and 2,4,6-trichlorophenol) and none of the candidates has any available inhalation toxicity values.

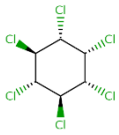
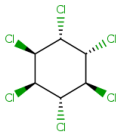
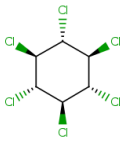
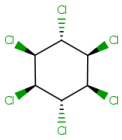
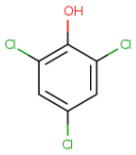
Structural Analogues

Four compounds were identified as candidate analogues of δ-HCH with oral toxicity values. Among these, three stereoisomers of δ-HCH were identified as structural analogues, with β-HCH and γ-HCH also identified as compounds that share a common metabolite with δ-HCH. The stereoisomers are structurally identical to δ-HCH, differing only in spatial orientation of the chlorines. The fourth candidate analogue is the primary metabolite of δ-HCH, 2,4,6-trichlorophenol. These analogues were carried forward for the read-across analysis.

The physicochemical properties of δ-HCH and the candidate analogues are summarized in Table A-5. δ-HCH and the candidate analogues are all chlorinated compounds. δ-HCH and the HCH isomers share the same molecular weight, 290.81 g/mol, while the molecular weight of 2,4,6-trichlorophenol is lower (197.44 g/mol). All compounds are solids at room temperature based on their experimental melting points. α-, δ- and γ-HCH have moderate vapor pressures and are expected to exist in both the gas and particulate phases in the atmosphere. 2,4,6-Trichloro-phenol has a high vapor pressure and is expected to exist mostly in the gas phase, whereas β-HCH has low measured vapor pressure, indicating low potential for inhalation exposure as gases or vapors. Slight to moderate volatilization from water to air is expected for all the compounds, based on their Henry's law constants. 2,4,6-Trichlorophenol is moderately soluble in water (>10-fold higher than δ-HCH). All other compounds have low solubility in water, with measured water solubility values <31.4 mg/L. Based on the octanol-water partition coefficient (log K_{ow}) values ranging between 3.69 and 3.72, δ-HCH and the candidate analogues are

lipophilic and are likely to partition to fat compartments in the body following absorption. Based on physicochemical and structural properties, the α-, β-, and γ-HCH stereoisomers are more similar to δ-HCH than is 2,4,6-trichlorophenol.

Table A-5. Physicochemical Properties of δ-HCH (CASRN 319-86-8) and Candidate Analogues

Property	Target Chemical	Candidate Analogues			
	δ-HCH (target)[a]	α-HCH[a]	β-HCH[a]	γ-HCH[a]	2,4,6-Trichloro-phenol[b]
Structure					
CASRN	319-86-8	319-84-6	319-85-7	58-89-9	88-06-2
Molecular weight (g/mol)	290.81	290.81	290.81	290.81	197.44
Melting point (°C)	141.5	159.5	314.5	112.5	67.0
Boiling point (°C)	60 (at 0.34 mm Hg)	288	60 (at 0.58 mm Hg)	323.4	246
Vapor pressure (mm Hg)	3.52×10^{-5} (at 25°C)	4.5×10^{-5} (at 25°C) extrapolated	3.6×10^{-7} (at 20°C)	4.2×10^{-5} (at 20°C)	8.0×10^{-3}
Henry's law constant (atm-m^3/mol)	NA	6.7×10^{-6} (at 23°C)	4.4×10^{-7} (at 25°C)	5.14×10^{-6} (at 25°C)	NA
Water solubility (mg/L)	31.4 (at 25°C)	2 (at 25°C)	0.24 (at 25°C)	7.3 (at 25°C)	1,579
Octanol-water partition coefficient (log K_{ow})	4.14	3.80	3.78	3.72	3.69

[a]Data were obtained from the PhysProp database: δ-HCH, CASRN 319-86-8; https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface; accessed April 7, 2022.
[b]Data were extracted from the U.S. EPA CompTox Chemicals Dashboard: 2,4,6-trichlorophenol, CASRN 88-06-2; https://comptox.epa.gov/dashboard/chemical/properties/DTXSID5021386; accessed February 7, 2024. Data presented are experimental averages unless otherwise noted.

Relevant structural alerts and toxicity predictions for noncancer health effects were identified using computational tools from the OECD (2022) QSAR Toolbox profilers, OCHEM (2022) ToxAlerts, and IDEAconsult (2018) Toxtree.

The model results for δ-HCH and its analogue compounds are shown in Figure A-1. Concerns for protein binding, hepatotoxicity, developmental and/or reproductive toxicity, and cytochrome P450 (CYP) metabolism of δ-HCH and its analogues were indicated by models within the predictive tools. Because the HCH isomers are structurally identical to δ-HCH, differing only in stereochemistry, the structural alerts for the other HCH isomers (α HCH, β-HCH, and γ-HCH) are identical to those for δ-HCH, as shown in Figure A-1.

Structural Category[a]	Compounds (CASRN)					
	Target Chemical	Candidate Analogues				
	δ-HCH (319-86-8)	α-HCH (319-84-6)	β-HCH (319-85-7)	γ-HCH (58-89-9)	2,4,6-Trichlorophenol (88-06-2)	Source
Protein Binding						
Protein binding (based on SN2 episulfonium ion formation for 1,2-dihaloalkanes and SN2 reaction at sp3 carbon atom for alkyl halides); protein binding by OECD						OECD QSAR Toolbox
Protein binding (based on nucleophilic aliphatic substitution SN2 alert)						Toxtree
Protein binding (based on Michael acceptor alert)						Toxtree
Hepatotoxicity						
Hepatotoxicity (based on halogenated aliphatic compounds alert); HESS model						OECD QSAR Toolbox
Developmental/Reproductive Toxicity						
Reproductive and developmental toxic potential (based on known precedent polychlorinated mono- or fused/bridged-cyclic compounds); DART model						OECD QSAR Toolbox
Reproductive and developmental toxic potential (based on known precedent polyhalogenated benzene derivatives); DART model						OECD QSAR Toolbox
Metabolism/Reactivity						
Energy metabolism dysfunction (based on nitrophenols/ halophenols)						OECD QSAR Toolbox
CYP-mediated drug metabolism predicted (based on sp3 hybridized carbon atoms)						ToxAlerts
CYP-mediated drug metabolism predicted (based on sp2 hybridized carbon atoms)						ToxAlerts

Model results or structural alerts indicating concern for noncancer toxicity/endpoint of interest.
Model results or structural alert indicating no concern for noncancer toxicity/endpoint of interest.
[a]Models with results are presented in the heat map (models without results indicate that the queried chemical fell outside of the applicability domain and are omitted).

CYP = cytochrome P450; DART = Developmental and Reproductive Toxicity; HCH = hexachlorocyclohexane; HESS = Hazard Evaluation Support System; OECD = Organisation of Economic Co-operation and Development; QSAR = quantitative structure-activity relationship.

Figure A-1. Structural Alerts for δ-Hexachlorocyclohexane and Candidate Analogues

Alerts for protein binding were identified for δ-HCH and the HCH stereoisomer candidate analogues, based on nucleophilic aliphatic substitution SN2 (IDEAconsult, 2018), SN2 episulfonium ion formation for 1,2-dihaloalkanes, and SN2 reaction at sp3 carbon for alkyl halides (OECD, 2022). Another structural alert for protein binding was identified for 2,4,6-trichlorophenol (based on Michael acceptor alert) (IDEAconsult, 2018).

The OECD (2022) QSAR Toolbox Hazard Evaluation Support System (HESS) model showed concern for hepatotoxicity for δ-HCH and the other HCH stereoisomers based on the halogenated aliphatic compounds alert. No alert for hepatotoxicity was identified for 2,4,6-trichlorophenol.

The OECD (2022) QSAR Toolbox Developmental and Reproductive Toxicity (DART) model showed concern for developmental and/or reproductive toxicity for δ-HCH and the other HCH stereoisomers, based on known precedent polychlorinated mono- or fused/bridged-cyclic compounds. Another structural alert for developmental and/or reproductive toxicity was identified for 2,4,6-trichlorophenol (based on known precedent polyhalogenated benzene derivatives alert).

An alert for energy metabolism dysfunction was identified for 2,4,6-trichlorophenol, based on nitrophenols/halophenols (OECD, 2022). Alerts for CYP-mediated drug metabolism were identified for δ-HCH, the other HCH stereoisomers (based on sp3 hybridized carbon atoms) and 2,4,6-trichlorophenol (based on sp2 hybridized carbon atoms) (OCHEM, 2022).

In summary, structural alerts for protein binding, reproductive and developmental toxicity, and CYP-mediated metabolism were identified for all HCH isomers and 2,4,6-trichlorophenol. A liver toxicity structural alert was identified for the HCH isomers, but not for 2,4,6-trichlorophenol. The liver toxicity alert for the HCH isomers is consistent with toxicity data for the candidate analogues (see Toxicodynamic Analogues below).

Metabolic Analogues

Absorption, distribution, metabolism, and excretion (ADME) data for δ-HCH and the candidate analogues are presented in Table A-6. Absorption of δ-HCH and the α- and β- isomers is inferred from detection of the compound in blood, adipose, and semen of exposed humans and laboratory animals (ATSDR, 2024). No data are available from humans or animals to quantify the extent or rate of absorption. Data for γ-HCH suggest rapid absorption from the gastrointestinal tract and dermal absorption that is dependent on the application vehicle and the dose (ATSDR, 2024). Oral absorption is also rapid and extensive for 2,4,6-trichlorophenol (ATSDR, 2021).

Table A-6. Comparison of ADME Data for δ-HCH (CASRN 319-86-8) and Candidate Analogues

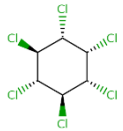
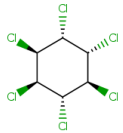
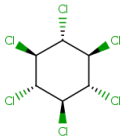
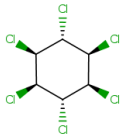
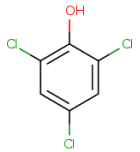
Type of Data	δ-HCH (target)	α-HCH	β-HCH	γ-HCH	2,4,6-Trichlorophenol
Structure					
CASRN	319-86-8	319-84-6	319-85-7	58-89-9	88-06-2
Absorption					
Rate and extent of absorption	**Humans:** • Absorption inferred from detection in blood, adipose, and semen of exposed persons. **Laboratory animals (oral):** • Absorption inferred from detection in the blood, adipose, and brain.	**Humans:** • Absorption inferred from detection in blood, adipose, and semen of exposed persons.	**Humans:** • Absorption inferred from detection in blood, adipose, and semen of exposed persons.	**Humans:** • High blood concentrations after oral exposure demonstrate absorption. • Absorption after dermal application depends on vehicle (ranging from 5% when applied in acetone to 60% in white spirit). **Laboratory animals (oral):** • Readily absorbed via GI tract of fasted animals. • Fractional dermal absorption inversely related to dose.	**Laboratory animals (oral):** • Oral absorption is extensive (>82% based on urinary excretion of radioactivity over 5 d).

Table A-6. Comparison of ADME Data for δ-HCH (CASRN 319-86-8) and Candidate Analogues

Type of Data	δ-HCH (target)	α-HCH	β-HCH	γ-HCH	2,4,6-Trichlorophenol
Distribution					
Extent of distribution	**Humans:** • Detected in placenta and umbilical cord. **Laboratory animals:** • Detected in rat testicular plasma membrane after dermal exposure. • Tissue concentrations in rats, in order of greatest to least, were fat > brain > blood after oral exposure.	**Humans:** • Detected in adipose tissue of workers and general population. • Detected in placenta and umbilical cord. • Detected in breast milk of women exposed to technical-grade HCH. **Laboratory animals (oral):** • Preferential accumulation in brain white matter as opposed to gray matter.	**Humans:** • Detected in adipose tissue of workers and general population. • Detected in placenta and umbilical cord. • Detected in breast milk of women exposed to technical-grade HCH. **Laboratory animals (all routes):** • Greatest distribution to adipose, followed by kidney, lungs, liver, and muscle.	**Humans (oral, dermal):** • Distributed to CNS. • Detected in placenta and umbilical cord. • Detected in breast milk of women exposed to technical-grade HCH. **Laboratory animals (inhalation, oral):** • Greatest distribution to adipose, followed by brain, kidney, muscle, and lungs. • Detected in amniotic fluid, placenta, and fetal tissues.	**Laboratory animals (i.p.):** • Peak concentration in blood observed 30 min after exposure. • Highest concentrations in kidney, followed by blood, liver, fat, muscle, and brain. **In vitro:** • Strong binding to serum proteins.

Table A-6. Comparison of ADME Data for δ-HCH (CASRN 319-86-8) and Candidate Analogues

Type of Data	δ-HCH (target)	α-HCH	β-HCH	γ-HCH	2,4,6-Trichlorophenol
Metabolism					
Primary reactive metabolites	**Laboratory animals (oral):** • Urinary metabolites include 2,4,6- and 2,4,5-trichlorophenol (with 2,4,6-trichlorophenol as the primary metabolite in urine, see Table A-7). • Metabolites detected in the cerebellum were 3,5/4,6-pentachlorocyclohexene, pentachlorobenzene, and hexachlorobenzene (see Table A-8).	**Laboratory animals (oral):** • Urinary metabolites include 2,4,6- and 2,4,5-trichlorophenol (see Table A-7). • Metabolites detected in the cerebellum were 3,6/4,5-hexachlorocyclohexene, pentachlorobenzene, and hexachlorobenzene (see Table A-8).	**Laboratory animals (oral):** • Little metabolism of β-HCH occurs. • Urinary metabolite is 2,4,6-trichlorophenol (see Table A-7). • Metabolites detected in the cerebellum were hexachlorobenzene and pentachlorobenzene (see Table A-8).	**Humans (dermal):** • Urinary metabolites include glucuronide and sulfate conjugates of 2,4,6-, 2,4,5-, and 2,3,5-trichlorophenols. **Laboratory animals (oral):** • Urinary metabolites include 2,4,6-, 2,4,5-, and 2,3,5-trichlorophenols; pentachlorophenol; and 2,3,4,6- and 2,3,4,5-tetrachlorophenols (see Table A-7). • Urinary metabolites conjugated with mercapturic acid, glucuronide, and sulfate. • Metabolites detected in the cerebellum were 3,6/4,5 pentachlorocyclohexene, 3,6/4,5 hexachlorocyclohexene, pentachlorobenzene, and hexachlorobenzene (see Table A-8).	**Humans:** • Sulfate conjugates detected in urine of exposed workers. **Laboratory animals (oral):** • Urinary metabolites are trichlorophenol isomers and glucuronic acid conjugates. **In vitro (rat liver microsomes):** • Metabolites included 2,6-dichloro-1,4-hydroquinone, two isomers of hydroxypentachlorodiphenyl ether, and the 2,6-dichloro-1,4-semi-quinone free radical.

Table A-6. Comparison of ADME Data for δ-HCH (CASRN 319-86-8) and Candidate Analogues

Type of Data	δ-HCH (target)	α-HCH	β-HCH	γ-HCH	2,4,6-Trichlorophenol
Excretion					
Elimination half-time; route of excretion	**Laboratory animals (oral):** • Half-life for clearance from brain was 0.5 d after 200 mg/kg dose.	**Laboratory animals (oral):** • Half-life for clearance from brain was 6 d after 200 mg/kg dose.	**Humans:** • Half-life in whole blood of workers was 7.2 yr. **Laboratory animals (oral):** • Half-life for clearance from brain was 20 d after 200 mg/kg dose.	**Humans:** • Elimination half-life ranged between 18 and 111 h after controlled dermal application. **Laboratory animals (oral):** • Major route of elimination is urine. • Some excreted via breast milk. • Half-life for clearance from brain was 1.5 d after 60 mg/kg dose.	**Laboratory animals (oral):** • Primarily excreted in urine (82−93% of administered dose). • Fecal excretion is low (6−22%).
References	ATSDR (2024); Srivastava et al. (1995); Artigas et al. (1988a); Vohland et al. (1981); Chadwick and Freal (1973)	ATSDR (2024); Artigas et al. (1988a); Chadwick and Freal (1973)	ATSDR (2024); Artigas et al. (1988a); Chadwick and Freal (1973)	ATSDR (2024); Artigas et al. (1988a); Chadwick and Freal (1973)	ATSDR (2021)

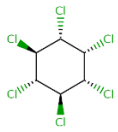
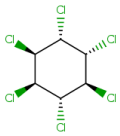
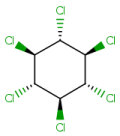
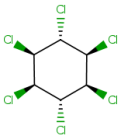
ADME = absorption, distribution, metabolism, and excretion; CNS = central nervous system; GI = gastrointestinal; i.p. = intraperitoneal.

The distribution of HCH isomers in humans and animals is primarily to the adipose tissue but also to the brain, kidney, muscle, liver, lung, blood, and other tissues (ATSDR, 2024). Distribution for the 2,4,6-trichlorophenol is similar to the HCH isomers, with uptake in the fat, liver, kidneys, blood, muscle, and brain (ATSDR, 2021). HCH isomers have been measured in the placenta and umbilical cord blood of humans (and animals for γ-HCH), indicating that transplacental exposure to fetuses is likely to occur. HCH isomers have also been detected in human breast milk (ATSDR, 2024). There are no studies reporting 2,4,6-trichlorophenol concentrations in placenta, umbilical cord blood, or breast milk in humans or animals (ATSDR, 2021).

In an oral study evaluating rat brain concentration and clearance of HCH isomers (α-, β-, δ-, and γ-HCH), there was a linear, dose-dependent increase in brain concentrations of all isomers, in order of greatest to least: α > δ > γ > β (Vohland et al., 1981). Peak brain concentration was reached 12−24 hours after administration of a single dose of all isomers and clearance rates (half-life in days) were as follows: α = 6, β = 20, δ = 0.5, and γ = 1.5 (it should be noted that the doses administered were 60 mg/kg for γ-HCH, due to toxicity, and 200 mg/kg for all other HCH isomers).

Metabolites of δ-HCH measured in rat urine were 2,4,5- and 2,4,6-trichlorophenol. For the other HCH isomers, chlorophenols were also the primary urinary metabolites (Chadwick and Freal, 1973). Approximate mean daily excretion rates of chlorophenols in rats exposed to the HCH isomers are shown in Table A-7. Very little β-HCH was metabolized, and γ-HCH was metabolized to several compounds that were not detected after exposure to δ-HCH. δ-HCH exposure yielded much higher excretion of 2,4,6-trichlorophenol than did the other isomers.

Table A-7. Approximate Mean Daily Urinary Excretion[a] (µg/day) of Chlorophenols in Female Sprague Dawley Rats Exposed to HCH Isomers via Seven Daily Gavage Doses of 2 mg/Rat[b]

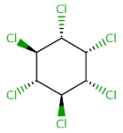
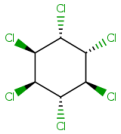
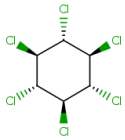
Metabolite	δ-HCH (target)	α-HCH	β-HCH	γ-HCH
Structure				
CASRN	319-86-8	319-84-6	319-85-7	58-89-9
2,4,6-Trichlorophenol	160	60	20	40
2,4,5-Trichlorophenol	20	40	ND	30
2,3,5-Trichlorophenol	ND	ND	ND	60
2,3,4,6-Tetrachlorophenol	ND	ND	ND	40
2,3,4,5-Tetrachlorophenol	ND	ND	ND	20
2,3,4,5,6-Pentachloro-2-cyclohexen-1-ol	ND	ND	ND	60

[a]Estimated by visual inspection of mean (n = 4) presented graphically.
[b]Chadwick and Freal (1973).

HCH = hexachlorocyclohexane.

In rat brain tissue, metabolites of δ-HCH included 3,5/4,6-pentachlorocyclohexene, pentachlorobenzene, and hexachlorobenzene. For the other HCH isomers, metabolites in brain tissue included 3,6/4,5-pentachlorocyclohexene, 3,6/4,5-hexachlorocyclohexene, pentachlorobenzene, and hexachlorobenzene (Artigas et al., 1988a). Relative amounts of each metabolite in the brains of rats exposed to each of the four HCH isomers are provided in Table A-8. The primary metabolite of δ-HCH (3,5/4,6-pentachlorocyclohexene) in the brain was not detected as a metabolite of the other stereoisomers. Pentachlorobenzene and hexachlorobenzene were identified as metabolites of all the isomers, albeit at very low levels. Based on the metabolite quantities, there appears to be greater metabolism of δ-HCH in rat brain than of the other HCH isomers, and very little metabolism of β-HCH.

Table A-8. Metabolites of HCH Isomers in Cerebellum of Male Wistar Rats Exposed via Single Gavage Dose of 30 mg/kg[a]

Measured Compound	δ-HCH (target)	α-HCH	β-HCH	γ-HCH
Structure				
CASRN	319-86-8	319-84-6	319-85-7	58-89-9
Parent compound (µg/g)	9.6 ± 2.0[b]	17.2 ± 4.7	4.2 ± 0.6	5.1 ± 0.9
Half-life in the brain (days)[c]	0.5	6	20	1.5
Metabolite (ng/g)				
3,6/4,5-Pentachlorocyclohexene	<5	<5	<5	250 ± 67
3,5/4,6-Pentachlorocyclohexene	1,453 ± 471	<5	<5	<5
3,6/4,5-Hexachlorocyclohexene	<5	96 ± 19	<5	107 ± 14
Pentachlorobenzene	3.3 ± 1.5	1.2 ± 0.4	0.7 ± 0.2	6.0 ± 3.1
Hexachlorobenzene	0.5 ± 0.1	0.7 ± 0.1	1.2 ± 0.6	1.2 ± 0.3

[a]Artigas et al. (1988a).
[b]Mean ± SD of seven animals.
[c]Vohland et al. (1981)

HCH = hexachlorocyclohexane; SD = standard deviation.

γ-HCH metabolites are primarily excreted in urine as mercapturic acid, glucuronide, and sulfate conjugates. Some excretion also occurs in breast milk. 2,4,6-Trichlorophenol is also primarily excreted in urine, either unchanged or as sulfate or glucuronide conjugates (ATSDR, 2024, 2021).

In summary, absorption by the oral route and distribution appear to be similar for δ-HCH and all the candidate analogues. Metabolism of β-HCH appears limited, compared with

metabolism of δ-HCH. Limited metabolism of β-HCH may be a partial explanation for its long half-life in the brain. 2,4,6-Trichlorophenol is the major urinary metabolite of δ-HCH and is a significant metabolite of α- and γ-HCH, but not of β-HCH (relative to other HCH isomers). Available data indicate that the α- and γ-HCH isomers and 2,4,6-trichlorophenol are the most suitable toxicokinetic analogues for δ-HCH.

Toxicodynamic Analogues

No candidate analogues with inhalation toxicity values were identified. Oral toxicity values for δ-HCH and candidate analogues are presented in Table A-9.

Table A-9. Comparison of Available Oral Toxicity Values for δ-HCH (CASRN 319-86-8) and Candidate Analogues

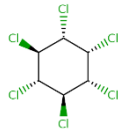
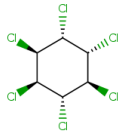
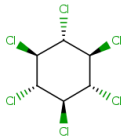
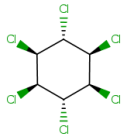
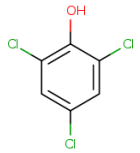
Type of Data	δ-HCH (target)	α-HCH	β-HCH	γ-HCH (lindane)	2,4,6-Trichlorophenol
Structure					
CASRN	319-86-8	319-84-6	319-85-7	58-89-9	88-06-2
Subchronic oral toxicity values					
POD (mg/kg-d)	ND	2	0.18	7.6×10^{-5}	0.46
POD type	ND	NOAEL	LOAEL	NOAEL	NOAEL
Subchronic UF_c	ND	100 (UF_A, UF_H) 10 MF	300 (UF_A, UF_H, UF_L)	100 (UF_A, UF_H)	100 (UF_A, UF_H)
Subchronic p-RfD or intermediate-duration MRL (mg/kg-d)	ND	0.002 (MRL)	6×10^{-4} (MRL)	8×10^{-7} (MRL)	5×10^{-3} (MRL)
Critical effects	ND	Increased liver weight and histopathology	Hyalinization of centrilobular liver cells	Altered cardiac electrophysiology in offspring	Increased absolute liver weight
Species	ND	Rat	Rat	Rat	Rat
Duration	ND	28 d	13 wk	13 wk	From conception through weaning and for additional 12 wk
Route (method)	ND	Gavage	Oral (diet)	Oral (water)	Oral (water)
Source	NA	ATSDR (2024)	ATSDR (2024)	ATSDR (2024)	ATSDR (2021)

Table A-9. Comparison of Available Oral Toxicity Values for δ-HCH (CASRN 319-86-8) and Candidate Analogues

Type of Data	δ-HCH (target)	α-HCH	β-HCH	γ-HCH (lindane)	2,4,6-Trichlorophenol
Chronic oral toxicity values					
POD (mg/kg-d)	ND	0.9	ND	0.33	3
POD type	ND	NOAEL	ND	NOAEL	NOAEL
Chronic UF_c	ND	100 (UF_A, UF_H) 10 MF	ND	1,000 (UF_A, UF_H, UF_S)	3,000 (UF_A, UF_D, UF_H, UF_S)
Chronic RfD/p-RfD (mg/kg-d)	ND	9×10^{-4} (MRL)	ND	3×10^{-4}	1×10^{-3}
Critical effects	ND	Increased liver weight and histopathology	ND	Liver and kidney toxicity	Decreased litter size
Species	ND	Rat	ND	Rat	Rat
Duration	ND	107 wk	ND	12–18 wk	One generation
Route (method)	ND	Oral (diet)	ND	Oral (diet)	Oral (water)
Source	NA	ATSDR (2024)	NA	U.S. EPA (1987)	U.S. EPA (2007)
Acute oral lethality data					
Rat oral LD_{50} (mg/kg)	ND	ND	ND	88−91	820
Toxicity at rat LD_{50}	ND	ND	ND	ND	ND
Source	NA	NA	NA	ATSDR (2024)	NCBI (2022b)

HCH = hexachlorocyclohexane; LD_{50} = median lethal dose; MF = modifying factor; MRL = Minimal Risk Level; NA = not applicable; ND = no data; NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfD = provisional reference dose; RfD = reference dose; UF_A = interspecies uncertainty factor; UF_C = composite uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor; UF_L = LOAEL-to-NOAEL uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor.

Oral toxicity information on δ-HCH is limited to two chronic studies focused on limited liver toxicity and carcinogenicity in male mice and rats (Ito et al., 1975; Ito et al., 1973). These studies also tested the candidate analogues, α-, β-, and γ-HCH. Tables A-10 and A-11 compare the noncancer findings of these studies across the four stereoisomers. Based on limited available data, δ-HCH is most similar to γ-HCH with respect to liver toxicity. In the mouse study (see Table A-10), the liver weight changes seen with δ-HCH exposure (23% increases) were consistent with those observed with γ-HCH exposure (33% increases) at 500 ppm, while larger increases in liver weights were seen at lower doses with both α- and β-HCH. Similarly, liver histopathology findings in both mice and rats were nonexistent or equivocal at all doses of both δ- and γ-HCH, in contrast to α- and β-HCH (see Tables A-10 and A-11). It is important to note, however, that other subchronic and chronic studies of γ-HCH have shown liver histopathology changes in Wistar rats at doses from 0.3 to 5 mg/kg-day [Fitzhugh (1950) and Zoecon Corporation (1983) as cited in U.S. EPA (1987)], and liver toxicity was a co-critical endpoint for the chronic reference dose (RfD) of γ-HCH (U.S. EPA, 1987). No other repeated-dose oral toxicity studies evaluating noncancer effects or studies designed to assess oral lethality were available for δ-HCH.

Table A-10. Comparative Toxicity of HCH Isomers in Male dd Mice Exposed for 24 Weeks[a]

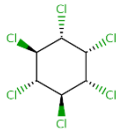
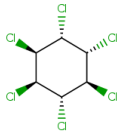
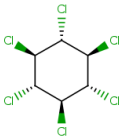
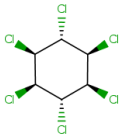
Dose (ppm) [mg/kg-d δ-HCH][b]	δ-HCH (target)	α-HCH	β-HCH	γ-HCH (lindane)
Structure				
CASRN	319-86-8	319-84-6	319-85-7	58-89-9
Body weight				
100 [18.6]	NC	NC	NC	NC
250 [47.1]	NC	NC	NC	NC
500 [93.1]	NC	NC	NC	NC
Relative liver weight[c]				
100 [18.6]	+3%	+33%	+18%	−3%
250 [47.1]	−8%	+105%	+30%	0%
500 [93.1]	+23%	+250%	+73%	+33%
Hepatocellular hypertrophy				
100 [18.6]	NC	+	NC	NC
250 [47.1]	NC	+++	+	±
500 [93.1]	±	+++	++	+
Other liver histopathology				
100 [18.6]	NC	NC	NC	NC
250 [47.1]	NC	±[d]	NC	NC
500 [93.1]	NC	±[d]	NC	NC

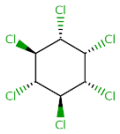
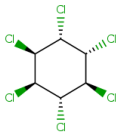
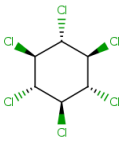
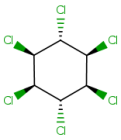
Table A-10. Comparative Toxicity of HCH Isomers in Male dd Mice Exposed for 24 Weeks[a]

Dose (ppm) [mg/kg-d δ-HCH][b]	δ-HCH (target)	α-HCH	β-HCH	γ-HCH (lindane)
Nodular hyperplasia[e]				
100 [18.6]	0/20	0/20	0/20	0/20
250 [47.1]	0/20	30/38	0/20	0/20
500 [93.1]	0/20	20/20	0/20	0/20
Hepatocellular carcinoma[e]				
100 [18.6]	0/20	0/20	0/20	0/20
250 [47.1]	0/20	10/38	0/20	0/20
500 [93.1]	0/20	17/20	0/20	0/20

[a]Ito et al. (1973).
[b]Doses are presented in ppm; doses in mg/kg-day for δ-HCH, calculated using study-specific body weight and food consumption data, are shown in brackets.
[c]Percentage change of relative liver weight (liver weight/body weight) relative to control.
[d]Oval cells and bile duct proliferation.
[e]Incidence data are presented as incidence/sample size.

HCH = hexachlorocyclohexane; NC = no significant change.

Table A-11. Comparative Liver Toxicity of HCH Isomers in Male Wistar Rats Exposed for 24−48 Weeks[a]

Duration	Dose (ppm) [mg/kg-d δ-HCH][b]	δ-HCH (target)	α-HCH	β-HCH	γ-HCH (lindane)
Structure					
CASRN		319-86-8	319-84-6	319-85-7	58-89-9
Hepatocellular hypertrophy					
24 wk	500 [44.0]	NC	±	NC	NC
	1,000 [89.1]	NC	+	+	ND
48 wk	500 [42.2]	NC	+	+	±
	1,000 [82.8]	±	++	ND	ND
Other liver histopathology					
48 wk	500 [42.2]	NC	NC	NC	NC
	1,000 [82.8]	NC	+[b]	ND	ND
Nodular hyperplasia[c]					
24 wk	500 [44.0]	0/7	0/6	0/8	0/6
	1,000 [89.1]	0/8	0/8	0/6	ND
48 wk	500 [42.2]	0/6	0/5	0/6	0/8
	1,000 [82.8]	0/5	5/12	ND	ND
Hepatocellular carcinoma[c]					
24 wk	500 [44.0]	0/7	0/6	0/8	0/6
	1,000 [89.1]	0/8	0/8	0/6	ND
48 wk	500 [42.2]	0/6	0/5	0/6	0/8
	1,000 [82.8]	0/5	0/12	ND	ND

[a]Ito et al. (1975).
[b]Doses are presented in ppm; doses in mg/kg-day for δ-HCH, calculated using study-specific body weight and food consumption data, are shown in brackets.
[c]Incidence data are presented as incidence/sample size.

HCH = hexachlorocyclohexane; NC = no significant change; ND = no data/not reported.

As Table A-9 indicates, hepatotoxicity was a critical effect for subchronic or chronic exposure for each of the candidate analogues. Points of departure (PODs) for liver effects ranged from 0.18 mg/kg-day (subchronic, β-HCH) to 2 mg/kg-day (subchronic, α-HCH). Based on the limited available data, δ-HCH did not induce liver effects in male mice exposed for 24 weeks to doses up to 93.1 mg/kg-day (500 ppm) (Ito et al., 1973) or in male rats exposed for 48 weeks to doses up to 82.8 mg/kg-day (1,000 ppm) (Ito et al., 1975).

In addition to liver toxic effects, a sensitive effect for subchronic exposure to γ-HCH was reduced activity of lymphoid follicles with prominent megakaryocytes and delayed hypersensitivity to immune challenge [Sauviat et al. (2005) as cited in ATSDR (2024)]. There is

no information on immunotoxicity of δ-HCH or α-HCH. Limited data are available on the immune system effects of β-HCH. One study showed that β-HCH reduced the lympho-proliferative response to mitogens in mice exposed in vivo [Cornacoff et al. (1988) as cited in ATSDR (2024)]. No immune system effects were observed in rats exposed to 2,4,6-trichloro-phenol in vivo. Kensler and Mueller (1978) compared the effect of HCH isomers on the mitogenic response of phytohemagglutinin (PHA)-stimulated bovine lymphocytes in vitro. δ- and γ-HCH inhibited the mitogenic response, while α-HCH was shown to enhance this response. β-HCH did not influence the mitogenic response in these cells at the exposure levels tested.

In the U.S. EPA (2007) PPRTV assessment, the critical effect for the derivation of the chronic RfD for 2,4,6-trichlorophenol was developmental toxicity (decreased litter size) in Sprague Dawley rats. There is no information on the potential developmental toxicity of δ- or α-HCH. A single study of β-HCH showed perinatal mortality in offspring of animals exposed in utero (ATSDR, 2024; Srinivasan et al., 1991). A number of animal studies have shown developmental toxicity after exposure to γ-HCH; observed effects included increased stillbirths, reduced neonatal viability, decreased pup weights, cardiac effects in offspring, and alterations in the development of the male reproductive tract (reviewed by ATSDR, 2024). The critical effect in the principal study for developmental exposure to γ-HCH was cardiac effects in offspring [Sauviat et al. (2005) as cited in ATSDR (2024)].

Of the candidate analogues, γ-HCH provides the lowest candidate POD, a no-observed-adverse-effect level (NOAEL) based on altered cardiac electrophysiology (7.6×10^{-5} mg/kg-day) in rat offspring exposed to doses of γ-HCH in drinking water for up to 13 weeks [Sauviat et al. (2005) as cited in ATSDR (2024)].

In summary, among the HCH stereoisomers, δ-HCH is most similar to γ-HCH with respect to liver toxicity, based on similarities in liver weight change and histopathology findings in in vivo isomer comparison studies in mice and rats. In addition, δ-HCH is most similar to γ-HCH with respect to immune response in vitro, as these two isomers inhibited the mitogenic response to phytohemagglutinin, while α- and β-HCH did not. There are no studies directly comparing the toxicity of δ-HCH to 2,4,6-trichlorophenol. Because the toxicity database of δ-HCH is limited to liver toxicity and immunotoxicity, a direct comparison of other systemic toxicity among δ-HCH and potential candidate analogues is not possible.

Weight-of-Evidence Approach

A WOE approach is used to evaluate information available for candidate analogues as described by Wang et al. (2012) and Lizarraga et al. (2023). Similarities between candidate analogues and the target chemical are identified across three major categories of evidence: structural/physicochemical properties; toxicokinetics (absorption, distribution, metabolism, excretion; ADME) and toxicodynamics (toxicity or MOA). Evidence of toxicological and/or toxicokinetic similarity is prioritized over evidence of similarity in structural/physicochemical properties. Candidate analogues are excluded if they demonstrate substantial differences from the pool of candidate analogues as a whole and/or the target chemical in any of the three categories of evidence. From the remaining pool of candidate analogues, the most suitable analogue (i.e., the analogue that displays the closest biological or toxicological similarity to the target chemical) with the greatest structural similarity and/or most health-protective point-of-departure is selected. Additional considerations include preference for evidence from existing U.S. EPA assessments and suitability of study duration (i.e., chronic studies are preferred over subchronic studies when selecting an analogue for the derivation of a chronic value.)

Oral Noncancer

Based on physicochemical and structural properties, the α-, β-, and γ-HCH stereoisomers are more similar to δ-HCH than is 2,4,6-trichlorophenol since they differ only in spatial orientation. Available metabolism and excretion data indicate that α-HCH, γ-HCH, and 2,4,6-trichlorophenol are the most suitable toxicokinetic/metabolic analogues for δ-HCH. The limited in vivo and in vitro studies comparing the effects of δ-HCH with those of α-, β-, and γ-HCH suggest that γ-HCH may be the most appropriate analogue based on comparative hepatotoxicity (critical effect for α- and β-HCH) and immunotoxicity.

γ-HCH is the most suitable analogue for δ-HCH based on structural similarity, physicochemical properties, and toxicological similarity for liver and immune system effects, which are sensitive endpoints identified as critical effects across candidate analogues (see Table A-9). Toxicokinetic comparisons also provide support for the selection of γ-HCH as the most appropriate analogue due to similarities in absorption and distribution, and shared metabolites. Of the candidate analogues, γ-HCH provides the most health-protective POD of 0.000076 mg/kg-day (compared to 0.18–3 mg/kg-day for other candidate analogues). The POD for γ-HCH based on cardiac effects in offspring can reasonably be expected to be protective of other observed effects such as liver and immune toxicity following exposure to δ-HCH. Therefore, γ-HCH is selected as the source analogue for δ-HCH for the oral route of exposure.

Inhalation Noncancer

None of the candidate analogues had an inhalation toxicity value, precluding derivation of screening reference concentrations for δ-HCH using the alternative analogue approach.

ORAL NONCANCER TOXICITY VALUES

Derivation of a Screening Subchronic Provisional Reference Dose

Based on the overall alternative analogue approach presented in this PPRTV assessment, γ-HCH is selected as the source analogue for δ-HCH for derivation of a screening subchronic provisional reference dose (p-RfD).

There is no subchronic oral p-RfD for γ-HCH. The IRIS chronic oral RfD for γ-HCH is 3×10^{-4} mg/kg-day and was derived in 1987 based on a study by Zoecon Corp in (1983) as cited in U.S. EPA (1987). ATSDR (2024) derived a lower intermediate oral Minimal Risk Level (MRL) of 8×10^{-7} mg/kg-day based on a study by Sauviat et al. (2005) as cited in ATSDR (2024) that was not available when the IRIS assessment was completed. ATSDR did not derive a chronic oral MRL for γ-HCH. Because the ATSDR intermediate oral MRL is lower than the IRIS chronic RfD and was derived from a study that was not available at the time of the IRIS assessment, and its POD (0.000076 mg/kg-day) is lower than the POD (0.33 mg/kg-day) for liver and kidney toxicity used in U.S. EPA (1987) assessment, the ATSDR intermediate oral MRL is selected as the basis for p-RfD derivation for δ-HCH.

The study used for the ATSDR intermediate-duration oral MRL value for γ-HCH is a 13-week reproductive study in rats [Sauviat et al. (2005) as cited in ATSDR (2024)]. The *Toxicological Profile for Hexachlorocyclohexane (HCH)* provided the following study summary [Sauviat et al. (2005) as cited in ATSDR (2024)][15]:

[15]Sauviat, MP; Bouvet, S; Godeau, G; et al. (2005). Electrical activity alterations induced by chronic absorption of lindane (gamma-hexachlorocyclohexane) trace concentrations in adult rat heart. Can J Physiol Pharmacol 83: 243-251 [as cited in ATSDR (2024)].

Groups of female Sprague-Dawley rats (number not reported) were administered γ-HCH via "beverage" at doses of 0.5, 1, or 2 ppb. ATSDR estimated corresponding maternal doses of 0, 0.000076, 0.00015, and 0.00030 mg/kg/day using water intake and body weight for female Sprague-Dawley rats in subchronic studies as reported in EPA (1988b). Doses were administered prior to mating for four estrous cycles (~2 weeks); throughout mating (~2 weeks), gestation (3 weeks), lactation (3 weeks), and growth (3 weeks) until pups were 6 weeks of age for a total of ~13 weeks. Exposure of the pups after weaning was not described but assumed to occur via water at the same dose as the dams. Offspring were sacrificed at 6 weeks of age.

The study authors indicated that the high-dose offspring were less sensitive to anesthesia and more sensitive to noise than other groups, but details of these assessments and findings were not provided. Body weights of pups were significantly decreased by 21% in the 0.0003 mg/kg/day group, compared to controls; no significant body weight changes were observed in other groups.

Morphometry analysis showed that hearts from pups in the 0.0003 mg/kg/day group had a 9% increase in heart width (relative to controls), but no significant change in length, with a corresponding 9% decrease in length-to-width ratio. Heart weights and total lipid content were not significantly different in the 0.0003 mg/kg/day group compared to control. At 0.0003 mg/kg/day, offspring heart morphology was described as more round and "cherry like." The study authors reported that hearts of treated offspring showed hypertrophied areas with thinning of the left ventricular wall and few developed papillary muscles. Histopathological examination in 0.0003 mg/kg/day offspring showed that the heart tissue muscle bundles and layers were unorganized and dissociated, with large hemorrhagic interspaces and dispersion of cell nuclei, destruction of fibroblasts, and dispersion and disorganization of collagen bundles, compared to control heart muscle. Incidences of changes were not reported, and these parameters were not assessed in pups from the 0.5 and 0.00015 mg/kg/day groups.

Electrophysiology changes were evident in LVPMs [16]from animals exposed to 0.00015 mg/kg/day and 0.0003 mg/kg/day γ-HCH. Action potential durations were unchanged at 0.000076 mg/kg/day, but the plateau was shortened moderately at 0.00015 mg/kg/day, and significantly shortened at 0.0003 mg/kg/day. At 0.0003 mg/kg/day, the slow repolarizing phase was also significantly shortened.

The effects at the high dose (0.0003 mg/kg/day) represent a serious LOAEL for cardiac effects (histopathology and electrophysiology changes) and significant body weight decrements (21% decrease) in the developing rat. The only effect at the middle dose (0.00015 mg/kg/day) was shortened action potential duration at the initial plateau phase (measured at 0 millivolts); similar results were not observed in the early repolarization or terminal repolarization phases (measured at 40 and 10 millivolts, respectively). However, at the high dose (0.0003 mg/kg/day), there were effects in all three phases, suggesting a

[16]LVPMs: the left ventricular papillary muscles.

dose-response relationship. There was no assessment of cardiac morphometry or histopathology in offspring from the middle dose group. The electrophysiology changes observed at 0.00015 mg/kg/day are considered to represent a minimal LOAEL. The lowest dose (0.000076 mg/kg/day) was not associated with electrophysiology changes and is considered to be a NOAEL.

The NOAEL of 0.000076 mg/kg-day was identified as the POD for γ-HCH based on electrophysiology changes in rat offspring (ATSDR, 2024). After adjusting for human equivalent dose (HED), the $NOAEL_{HED}$[17] of 0.000017 mg/kg-day is selected as the POD (HED).

The ATSDR intermediate-duration MRL for γ-HCH was derived using a composite uncertainty factor (UF_C) of 100, reflecting 10-fold uncertainty factors for interspecies extrapolation and intraspecies variability (UF_A and UF_H) (ATSDR, 2024). Wang et al. (2012) indicated that the uncertainty factors typically applied in deriving a toxicity value for the chemical of concern are the same as those applied to the analogue unless additional information is available. To derive the screening subchronic p-RfD for δ-HCH from the γ-HCH data, an interspecies extrapolation uncertainty factor (UF_A) of 3 is applied due to the adjustment of the POD to an HED and a database uncertainty factor (UF_D) of 10 is added to account for the uncertainties in the read-across approach based on an analogue chemical; the other uncertainty factors utilized by ATSDR were unchanged. Thus, the UF_C of 300 applied to the analogue $NOAEL_{HED}$, included 3-fold for UF_A, 10-fold for UF_D and UF_H, and 1-fold for lowest-observed-adverse-effect level (LOAEL) to NOAEL extrapolation (UF_L) and subchronic to chronic extrapolation (UF_S).

Screening Subchronic p-RfD = Analogue POD (HED) ÷ UF_C
= 0.000017 mg/kg-day ÷ 300
= **6×10^{-8} mg/kg-day**

Table A-12 summarizes the uncertainty factors for the screening subchronic p-RfD for δ-HCH.

[17]NOAEL (HED) = NOAEL (0.000076 mg/kg-day) × DAF (0.22). The DAF was calculated as follows: $DAF = (BW_a^{1/4} \div BW_h^{1/4})$. Reported body weights (161 g) for rats at dose level of 0.5 ppb and humans (70 kg) recommended by the U.S. EPA (1988) were used to calculate the DAF.

Table A-12. Uncertainty Factors for the Screening Subchronic p-RfD for δ-HCH (CASRN 318-96-8)

UF	Value	Justification
UF_A	3	A UF_A of 3 is applied to account for remaining uncertainty associated with extrapolating from animals to humans when a cross-species dosimetric adjustment (HED calculation) is performed.
UF_H	10	A UF_H of 10 is applied for interindividual variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of δ-HCH in humans.
UF_D	10	A UF_D of 10 is applied to reflect the absence of adequate toxicity data for δ-HCH with a database limited to liver toxicity and immunotoxicity, and an application of a read across-based analogue assessment.
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation because the POD is a NOAEL.
UF_S	1	A UF_S of 1 is applied because the analogue POD is based on a 13-wk reproductive study with a sensitive life stage exposure.
UF_C	300	Composite UF = $UF_A \times UF_H \times UF_D \times UF_L \times UF_S$.

HCH = hexachlorocyclohexane; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfD = provisional reference dose; UF = uncertainty factor; UF_A = interspecies uncertainty factor; UF_C = composite uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor; UF_L = LOAEL-to-NOAEL uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor.

Derivation of a Screening Chronic Provisional Reference Dose

γ-HCH is also selected as the source analogue for δ-HCH for derivation of the screening chronic p-RfD. The key study and calculation of the POD are described above for the screening subchronic p-RfD. In deriving the screening chronic p-RfD for δ-HCH, the same uncertainty factors used for the screening subchronic p-RfD (UF_A of 3, UF_D of 10, UF_H of 10, UF_L of 1, and UF_S of 1) are applied. No additional uncertainty factor for study duration is applied because the 13-week principal study is a reproductive study with a sensitive life stage exposure, and the database also contains a 24-week study that identified less sensitive immunotoxic effects. The screening chronic p-RfD is derived as follows:

Screening Chronic p-RfD = Analogue POD (HED) ÷ UF_C
= 0.000017 mg/kg-day ÷ 300
= **6×10^{-8} mg/kg-day**

Table A-13 summarizes the uncertainty factors for the screening chronic p-RfD for δ-HCH.

Table A-13. Uncertainty Factors for the Screening Chronic p-RfD for δ-HCH (CASRN 318-96-8)

UF	Value	Justification
UF_A	3	A UF_A of 10 is applied to account for remaining uncertainty associated with extrapolating from animals to humans when a cross-species dosimetric adjustment (HED calculation) is performed.
UF_H	10	A UF_H of 10 is applied for interindividual variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of δ-HCH in humans.
UF_D	10	A UF_D of 10 is applied to reflect the absence of adequate toxicity data for δ-HCH with a database limited to liver toxicity and immunotoxicity, and an application of a read across-based analogue assessment.
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation because the POD is a NOAEL.
UF_S	1	A UF_S of 1 is applied because the analogue POD is based on a 13-wk reproductive study with a sensitive life stage exposure. The database also contains a 24-wk study which identified a less sensitive immunotoxic effects.
UF_C	300	Composite UF = $UF_A \times UF_H \times UF_D \times UF_L \times UF_S$.

HCH = hexachlorocyclohexane; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfD = provisional reference dose; UF = uncertainty factor; UF_A = interspecies uncertainty factor; UF_C = composite uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor; UF_L = LOAEL-to-NOAEL uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor.

APPENDIX B. DATA TABLES

Table B-1. Body Weight and Liver Weight in Male Mice Exposed to δ-HCH in the Diet for 24 Weeks[a]

ADD in mg/kg-d (ppm diet)	0 (Control)	18.6 (100 ppm)	47.1 (250 ppm)	93.1 (500 ppm)
Number of animals	20	20	20	20
Initial BW (g)	19.7 ± 0.5[b]	20.0 ± 0.0 (+2%)[c]	19.6 ± 0.5 (−0%)	19.9 ± 0.4 (+1%)
Final BW (g)	37.5 ± 2.6	37.8 ± 5.3 (+1%)	36.4 ± 3.5 (−3%)	37.9 ± 1.9 (+1%)
Absolute liver weight (g)	1.5 ± 0.3	1.5 ± 0.3 (+0%)	1.4 ± 0.2 (−7%)	**1.8 ± 0.2 (+20%)***
Relative liver weight (% of BW)	4.0 ± 0.3	4.1 ± 0.6 (+2%)	3.7 ± 0.3 (−8%)	**4.9 ± 0.6 (+23%)***

[a]Ito et al. (1973).
[b]Data are means ± SD.
[c]Value in parentheses is % change relative to control = ([treatment mean − control mean] ÷ control mean) × 100.
*Significantly different from control ($p < 0.05$) by Student's *t*-test conducted for this review.

ADD = adjusted daily dose; BW = body weight; HCH = hexachlorocyclohexane; SD = standard deviation.

Table B-2. Body Weight and Liver Weight in Male Rats Exposed to δ-HCH in the Diet for 24 or 48 Weeks[a]

	Week Sacrificed				
	72	24		48	
ADD in mg/kg-d (ppm diet)	0 (Control)	44.0 (500 ppm)	89.1 (1,000 ppm)	42.2 (500 ppm)	82.8 (1,000 ppm)
Number of animals	8	7	8	6	5
Initial BW	152.3[b]	151.5	164.5	148.3	163.6
Final BW (g)	493.6	375.5	344.9	447.1	464.4
Absolute liver weight (g)	11.3	11.0	11.3	11.9	13.3
Relative liver weight (% of BW)	2.3	2.7 (17%)[c]	3.4 (48%)	2.7 (17%)	2.8 (22%)

[a]Ito et al. (1975).
[b]Data are means; SD not reported.
[c]Value in parentheses is % change relative to control = ([treatment mean − control mean] ÷ control mean) × 100. Change from control should be interpreted with caution due to the differences in sacrifice times.

ADD = adjusted daily dose; BW = body weight; HCH = hexachlorocyclohexane; SD = standard deviation.

APPENDIX C. PARAMETERS OF TOOLS USED FOR READ-ACROSS

Table C-1. Parameters of Tools Used for Read-Across of δ-HCH

Analogue Type [number identified][a]	Tool Name [number identified]	Settings/Parameters	Searched by (date)
Structural [160]	U.S. EPA CompTox Chemicals Dashboard [91]	Tanimoto similarity threshold of 0.8 and related substances	CASRN (December 15–21, 2021)
	ChemIDplus [37]	ChemIDplus similarity search (default method) with ≥80% threshold and related substances, parent (or exact structure match), salts, and mixtures[b]	
	GenRA Beta version (in the U.S. EPA CompTox Chemicals Dashboard) [51]	Collect 10 nearest neighbors by each similarity setting and combination available: • Morgan Fingerprints • Torsion Fingerprints • ToxPrints • Morg2Tor1Bio1 • CT1:Bio3 Using each of the following data sources: ToxCast, Tox 21, and ToxRef	
	OECD QSAR Toolbox [18]	Similarity search with ≥80% similarity threshold using default settings: • Dice similarity • Atom centered fragments • Hologram calculation. • All features combined • Atom characteristics: atom type, count H attached, and hybridization	
Metabolic [15]	Experimental data [5]	NA	
	OECD QSAR Toolbox Metabolism Simulators [6]	No settings or parameters; results obtained from: • Rat liver S9 metabolism simulator version 3.7 • in vivo Rat metabolism simulator version 3.5	SMILES[c] (December 2021)
	Targeted PubMed searches [4]	• Used to search for metabolic precursors and compounds with common metabolites	

Table C-1. Parameters of Tools Used for Read-Across of δ-HCH			
Analogue Type [number identified][a]	**Tool Name [number identified]**	**Settings/Parameters**	**Searched by (date)**
Mechanistic [0]	Experimental data [0]	• Evaluated to determine if data suggested specific, characteristic activity	
	GenRA beta version (in the U.S. EPA CompTox Chemicals Dashboard) [0]	Collected 10 nearest neighbors using the ToxCast similarity settings. • Nearest neighbors with a similarity index ≥0.5 considered for use as analogue	CASRN (December 2021–January 2022)
	Comparative Toxicogenomics Database (CTD) [0]	Compounds identified with gene interactions similar to those induced by δ-HCH: • Used the interacting genes comparison search • A similarity index of ≥0.5 is considered for use as a mechanistic analogue	

[a]Number of unique analogues identified using search tools.
[b]For more information, see https://www.nlm.nih.gov/pubs/techbull/ma06/ma06_technote.html.
[c]δ-HCH SMILES: C(C(C(C(C1Cl)Cl)Cl)Cl)(C1Cl)Cl (CASRN: 319-86-8).

GenRA = generalized read-across; HCH = hexachlorocyclohexane; NA = not applicable; OECD = Organisation for Economic Co-operation and Development; QSAR = quantitative structure-activity relationship; SMILES = simplified molecular input line entry system; U.S. EPA = U.S. Environmental Protection Agency.

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