UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
Center for Public Health and Environmental Assessment
Superfund Health Risk Technical Support Center

OFFICE OF
RESEARCH AND DEVELOPMENT

April 07, 2021

MEMORANDUM

SUBJECT: Evaluation of the use of chlordane as a surrogate for cis- and trans-chlordane. (ORD-041306)

FROM: Superfund Technical Support Center
Center for Public Health and Environmental Assessment (CPHEA)
Office of Research and Development (ORD)

TO: EPA Region 2

CC: Office of Superfund Remediation and Technology Innovation (OSRTI)
Office of Land and Emergency (OLEM)

Problem Statement:

U.S. EPA Region 2 office contacted the Superfund Technical Support Center (STSC) to inquire as to whether technical chlordane (CASRN 12789-03-6) could be used as a surrogate for *cis*- and *trans*-chlordane (CASRN 5103-71-9 and 5103-74-2, respectively) for the evaluation of oral noncancer toxicity. Center for Public Health and Environmental Assessment (formerly EPA's National Center for Environmental Assessment) scientists applied an expert-driven read-across approach to determine the suitability of the proposed surrogate based on three similarity contexts (structure, toxicokinetics,and toxicity) as described in Wang et al. (2012). This assistance request to U.S. EPA Region 2 was originally developed in 2018 and provided to the Region to support site-specific risk management decisions.

This memorandum contains scientific evaluation provided in response to a request for site-specific technical support with limited scope. The evaluations herein are intended to address specific scientific questions posed to researchers and/or consultants with applicable experience. The observations provided are intended to assist U.S. EPA regional risk accessors with relevant and innovative science to help meet site-specific environmental goals. The observations are provided in good faith, and due to the limited scope of technical support requests, include potential uncertainty. This memorandum is not to be considered the only source of information for decision making, nor should the information provided here be parsed. It would be advisable to consider this memorandum in conjunction with multiple lines of evidence including history, experience of site managers, and other pertinent information available to U.S. EPA risk assessors that retain the duties and responsibilities of all context-specific decisions and regulatory actions.

Methods:

Structural similarity evaluations were conducted using ChemIDplus, and information on toxicokinetics(i.e., absorption, metabolism, distribution, and excretion) and toxicities were obtained from existing toxicological profiles from EPA and Center for Disease Control (CDC), and updated by literature searches using PubMed on January 3, 2018, and updated on February 5, 2018. Search terms in PubMed were used for Chlordane (*cis*- and *trans*-, Alpha- and Gamma-, Technical and Analytical) and some notable metabolites (i.e., oxychlordane and heptachlor epoxide) and impurities (i.e., heptachlor). The majority of the studies identified in the search were exposure related, methods related, or performed in non-mammalian species, and not considered further. Studies were further excluded if not by oral route of exposure (inhalation, dermal and injection studies not considered further). Information from the relevant literature pertaining to each of the threeprimary similarity contexts was evaluated using a weight of evidence approach as described in Wang et al. (2012).

Background:

The term chlordane is used to describe several mixtures of cyclodiene insecticides. The first use of theterm chlordane is for the $C_{10}Cl_8$ product itself, which consists of two isomers (*cis*- and *trans*-), sometimes (erroneously) referred to as alpha and gamma (beta). In this document, the two principal isomers of chlordanewill be referred to as *cis*- and *trans*-chlordane. Most historical toxicity studies regarding chlordane were conducted with the technical mixture, a few with analytical grade, and very few with individual isomers. Thus, the available databases for individual chlordane isomers represent a small fraction of the overall chlordane database (ATSDR, 1994; U.S. EPA, 1997). Technical chlordane is a complex mixture. Roughly 60-85% of technical chlordane consists of the *cis*- and *trans*- isomers ($C_{10}Cl_8$). Nonachlor ($C_{10}Cl_9$) and Heptachlor ($C_{10}Cl_7$) are the primary contaminants (Buchert et al, 1989). Dearth and Hites (1991a) identified147 different compounds by GCMS; in a preparation of technical chlordane, the 12 most abundant included *cis*-chlordane (15%); *trans*-chlordane (15%); *trans*-nonachlor (9.7%); octachlordane (3.9%); heptachlor (3.8%); *cis*-nonachlor (2.7%); "compound K," (2.6%); dihydrochlordene (2.2%); nonachlor III (2%); and three stereoisomeric dihydroheptachlors totaling 10.2%. These 12 compoundscomprise 67% of the mixture, and the remaining 33% of the mixture consists of 135 other compounds.

Structural Similarity:

Structural and physicochemical properties of chlordane compounds are shown in Table A-1. The chlordane-related compounds have strikingly similar structures (isomers); they share a bicyclic ring that is heavily chlorinated. The *cis*- and *trans*- isomers of chlordane have similar properties to the technical and the analytical mixtures, including comparable molecular weight, melting points, water solubility, and LogP. The Henry's law constants and the vapor pressure of the *cis*- and *trans*- isomers are somewhat higher and thereforethese isomers may be less persistent in the environment as they are more likely to volatilize from soil or water.

Toxicokinetic Similarity:

Animal studies indicate that single doses of chlordane administered orally are well absorbed, extensively metabolized, and distributed throughout the body. These products are almost completely eliminated in 7 days, but leave persistent residues in several tissues, predominately fat (U.S. EPA, 1997). Data related to the absorption, distribution, metabolism,

and excretion of chlordanes are shown in Table A-2.

Chlordane and its metabolites are persistent pollutants that are fat soluble and readily absorbed (Ewing etal., 1985). The compounds are widely distributed, with the highest levels found in fat, followed by liver, kidney, brain, and muscle when fed either *cis*- or *trans*-chlordane (Barnett and Dorough, 1974). The principalmetabolites in mammals are cytochrome P450 (CYP2B family) oxidation products including oxides and epoxides (Kania-Korwel and Lehmier, 2013). In almost all mammals, technical chlordane is metabolized to two persistent epoxides: heptachlor epoxide (with 7 chlorines) and oxychlordane (with 8 chlorines) (Barnett and Dorough, 1974; Dearth and Hites, 1991b). The primary oxidative metabolite of both technical chlordane and *cis*- and *trans*-chlordane is the epoxide oxychlordane, which is environmentally persistent, bioaccumulative, and the ultimate toxic metabolite (Bondy, et al., 2000; 2004; U.S. EPA, 1997). Other components of technical chlordane, in particular *cis*- and *trans*-nonachlor, are also metabolized to oxychlordane in rats (Tashiro and Matsummura, 1978). Other oxidative metabolites include chlordene chlorhydrin, and monohydroxylated dihydrochlordene (Tashiro and Matsumura, 1978). Dechlorination occursin the environment, however, very slowly, with half-lives spanning decades (Stewart and Chisholm, 1971).

Oxidative metabolites are slowly excreted in urine, while the non-metabolized parent molecules and dechlorination products are excreted in feces and milk of mammals (Barnett and Dorough, 1974; Newsome and Ryan, 1999; Taguchi and Yakushiji, 1988, as cited in U.S. EPA, 1997). Due to the long retention time inadipose tissue, oxychlordane is believed to be more toxic than its parent isomers, which are eliminated relatively rapidly from the body (Satoh and Kikawa, 1992); therefore, oxychlordane may be the major contributor to chlordane toxicity.

Toxicity Similarities:

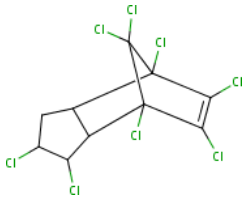
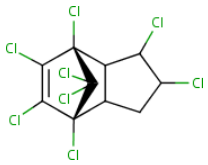
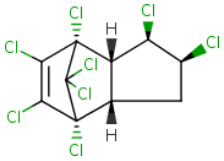
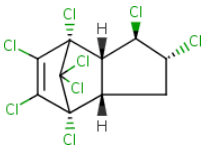
Nearly all existing in vivo animal studies were conducted with technical chlordane. There is limitedinformation on specific isomers, including *cis*- and *trans*-chlordane. Reported toxicities across the chlordane-related compounds are summarized in Table A-3. Mechanistically, all of these chemicals are known to act as insecticides via antagonism of the gamma-aminobutyric acid (GABA) receptor (Deng et al, 1991). Specifically, the $GABA_a$ receptor is a chloride channel, and these polychlorinated compounds compete for the chloride-binding motif of the receptor. Oxychlordane is thought to be the primary toxicant in liver tissue for non-acute exposures (U.S. EPA, 1997). Among the available repeat-dose toxicity studies that evaluate the chlordanes, and persistent metabolites such as oxychlordane and heptachlor-epoxide, the liver is the most commonly observed target organ (Bondy et al., 2000; 2003). Liver toxicity has been observed at all examined durations (including short-term, subchronic, and chronic). For example, both Kacew and Singhai (1973), with *cis*-chlordane and Truhart et al (1975) with technical chlordane, observed both liver andkidney effects at the same dose (200 mg/kg). Hepatic effects also were noted in inhalation studies in rats (Khasawinah et al., 1989a) indicating that the liver is a common target organ regardless of route of chlordaneadministration. Heptachlor, a component of technical chlordane with similar structure (95% structural similarity), has the same point of departure (POD) (and oral reference dose [RfD]) as technical chlordane (1.5×10^{-1} mg/kg-d) in the same target organ (liver). Oral median lethal dose (LD_{50}) values, while not necessarily predictive of relative toxicity for longer-duration exposures, do show similar toxicities among theisomers. For

example, in mice, the LD_{50} for analytical chlordane is 145 mg/kg, for *cis*-chlordane it is 125 mg/kg, while trans-chlordane is 275 mg/kg (See Table A-3). These data support a general toxicologicalsimilarity of the chlordane-related compounds.

Weight of Evidence Summary:

cis- and *trans*-chlordane are isomers of chlordane, and major components of the technical and analytical chlordane mixtures. These compounds share common metabolites; oxychlordane is the major toxicand bioaccumulative metabolite, for both technical and analytical chlordane mixtures, and the *cis*- and *trans*- isomers. The technical mixture and individual isomers share similar toxicities and target organs, and exert similar acute effects at similar doses. In addition, other structurally-related chlorinated compounds including major components in the chlordane mixture (i.e., nonachlor, heptachlor) show similar target organ toxicities. As such, technical chlordane can be considered a suitable surrogate for screening-level assessmentsof *cis*- and *trans*-chlordane.

Appendix A. Data Tables

Table A-1. Physicochemical Properties of Chlordane Isomers and Mixtures				
Name	**Chlordane Technical (A complex Mixture)**	**Chlordane Analytical (*cis*-/*trans*-)**	***cis*-Chlordane**	***trans*-Chlordane**
Structure				
CASRN	12789-03-6	57-74-9	5103-71-9	5103-74-2
Molecular weight	409.78	409.78	409.78	409.78
Melting point (°C)	ND	106	106	104
Boiling point (°C)	ND	351.09	ND	ND
Vapor pressure (mm Hg at 25°C)	9.98×10^{-6}	9.75×10^{-6}	3.6×10^{-5}	5.03×10^{-5}
Henry's law constant (atm-m^3/mole at 25°C)	7.0×10^{-5}	4.86×10^{-5}	3.47×10^{-4}	4.84×10^{-4}
Water solubility (mg/L)	0.013 mg/L	0.056 mg/L	0.056 mg/L	0.056 mg/L
LogP (K_{ow})	6.16	6.16	6.1	6.22
pKa	NA	NA	NA	NA

Data were gathered from ChemIDplus for each respective compound unless otherwise

specified.NA = not applicable; ND = no data

Table A-2. Toxicokinetic Data of Chlordanes

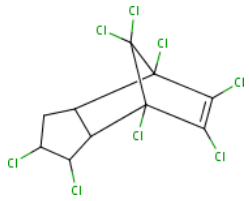
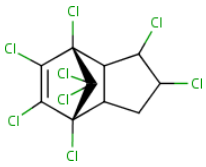
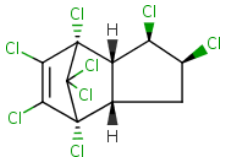
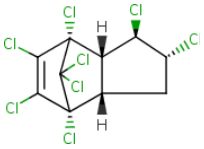
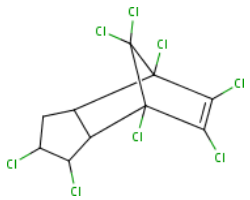
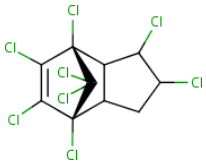
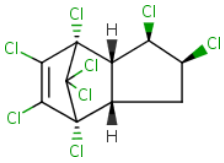
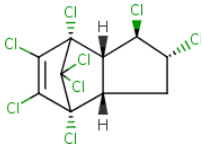
Name	**Chlordane Technical (A complex Mixture)**	**Chlordane Analytical (Iso-*cis*-/*trans*-)**	***cis*-Chlordane**	***trans*-Chlordane**
Structure				
Absorption	Readily Absorbed	Readily Absorbed	Readily Absorbed	Readily Absorbed
Distribution	After a fatal human exposure, concentrations were, 59.93 ug/g in liver, 23.27 ug/g in in brain, 22 ug/g in fat, 19.15 ug/g in spleen and 14.12 ug/g in kidney (Kutz, et al, 1983, as cited by ATSDR, 1994. After 28-day exposure, relative concentration in fat and liver were oxychlordane > trans-nonachlor > *cis*-nonachlor > *trans*-chlordane > heptachlor (Bondy et al, 2000)	Liver, kidney, brain and muscle levels were 1/8, 1/10, 1/25, and 1/50 of the concentrations in the feed (Barnett and Dorough, 1974). Oxychlordane major metabolite 32-35% total residue in kidney, 53-63% in fat (Barnett and Dorough, 1974)	Peak concentrations reached at 4 h after oral exposure in rats and mice, except brain, liver, and muscle, which peaked at 2 h. Highest concentrations were in liver, followed by fat, kidney, lungs, brain, testes, and muscle (Ewing et al, 1985).	In 28-day rat study, residues found with males/female ratios of Fat (7/13); Liver (0.3/1); Kidney (7.5/2.8) (Bondy et al., 2005)
Metabolism	Oxychlordane represents 41% of the total chlordane in human milk (Newsome and Ryan, 1999).	The primary persistent metabolic product of analytical chlordane (3:1 *cis*-/*trans*-) is Oxychlordane (Barnett et al., 1974)	The primary persistent metabolic product of radiolabeled *cis*-chlordane is Oxychlordane (Barnett et al., 1974)	The primary persistent metabolic product of radiolabeled Trans-Chlordane is Oxychlordane (Barnett et al., 1974, Street and Blau, 1972) Metabolized quickly in mice to Oxychlordane with a half-life of one day. The Oxychlordane half-life was over 100 d. (Satoh and Kiiawa, 1992.)
Excretion	Fecal concentration of 719 ppm on D 2, and 105 ppm on D 3 following technical chlordane ingestion by human female, shows rapid excretion (Aldrich and Holms, 1969).	In rats, a 3:1 mixture of *cis*- and *trans*-chlordane was >90% eliminated in 7 d, mostly by fecal route. (Barnett and Dorough, 1974).	In rats, a 3:1 mixture of *cis*- and *trans*-chlordane was >90% eliminated in 7 d, mostly by fecal route. (Barnett and Dorough, 1974). 83% of labelled *cis*-Chlordane excreted in feces in rats and mice by 3 d. (Ewing, et al., 1985)	In rats, a 3:1 mixture of *cis*- and *trans*-chlordane was >90% eliminated in 7 d, mostly by fecal route. (Barnett and Dorough, 1974). More than 90% of labelled trans-chlordane excreted in feces by D 7 (Barnet and Dorough, 1985).

Table A-3. Toxicity Information on Chlordanes

Chemical Name	**Chlordane Technical (A complex Mixture)**	**Chlordane Analytical (Iso-*cis*-/*trans*-)**	***cis*-Chlordane**	***trans*-Chlordane**
Structure				
RfD (mg/kg-d) (IRIS)	5×10^{-4} mg/kg-d	3.3×10^{-5} mg/kg-d (CalEPA Child Specific chRfD)	NA	NA
POD for RfD/Effect	1.5×10^{-1} mg/kg-d (NOAEL)	NA	NA	NA
Subchronic and Chronic Target Organs	Liver, in oral chronic studies in rats (Bondy et al, 2003) and mice (Khasawinah and Grutsch, 1989). Several hepatic endpoints including liver weight, cell volume, AST, ALT, hepatic degeneration and necrosis. Liver (liver enzymes, liver histopathology) and Kidney (histopathology) in acute study in rat (Truhaut et al., 1975)	Liver, in short-term oral studies in rats. (Truhaut et al., 1975; Bondy et al., 2000) Neurological (tremors) in rats at same dose as liver tumors (NCI, 1977) Liver effects at 0.47 mg/kg-d in chronic study in mice (NCI, 1977) Liver Cancer long-term in mice at 3.9 mg/kg-d. (NCI, 1977)	Liver and kidney, in single-dose oral data only, but effects identified in rats at 200 mg/kg (Kacew and Singhai, 1973).	Short-term oral data only in mouse. Hematological effects at 8 mg/kg-d in 14-day study (Johnson 1986, as cited in ATSDR, 1994). Trans-chlordane metabolized to oxychlordane, which shows liver histopathology in 28-day study in rats, indicative of microsomal enzyme induction. Thyroid histopathology also observed (Bondy, et al., 2003).
Oral LD_{50} (mg/kg)	126 mg/kg mouse (average of 3 values cited in ATSDR. 1994) 283 mg/kg rat (ChemIDPlus) 317 mg/kg rat (average of 10 values found in ATSDR, 1994)	145 mg/kg mouse (ChemIDPlus) 100 mg/kg rabbit (ChemIDPlus) 200 mg/kg rat (ChemIDPlus)	125 mg/kg mouse (ChemIDPlus) 500 mg/kg rat (ChemIDPlus) 83 mg/kg rat (Podoski, 1979, as cited in ATSDR, 1994)	275 mg/kg mouse (ChemIDPlus)

ALT = alanine aminotransferase; AST = aspartate aminotransferase; IRIS = Integrated Risk Information System; LD_{50} = median lethal dose; NA = not applicable; NOAEL = no-observed-adverse-effect level; POD = point of departure; RfD = oral reference dose.

Appendix B. References:

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