

# TOXICITY CRITERION FOR GAMMA- HEXACHLOROCYCLOHEXANE

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The logo for Integral Consulting Inc. features the word "integral" in a blue, lowercase, sans-serif font. A thin, curved line starts from the bottom of the letter "l" and loops around to the right, ending under the word "consulting inc." which is written in a smaller, lowercase, sans-serif font.  
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## ACRONYMS AND ABBREVIATIONS

ATSDR	Agency for Toxic Substances and Disease Registry
BMD	benchmark dose
CHO	Chinese hamster ovary
DNA	deoxyribonucleic acid
EPA	U.S. Environmental Protection Agency
FAO	Food and Agricultural Organization
HCB	hexachlorobenzene
HCH	hexachlorocyclohexane
HEAST	Health Effects Assessment Summary Table
Integral	Integral Consulting Inc.
IRIS	Integrated Risk Information System
LOAEL	lowest-observed-adverse-effect level
MF	modifying factor
mg/kg-day	milligram per kilogram per day
MRL	minimal risk level
MTD	maximum tolerated dose
NDEP	Nevada Division of Environmental Protection
NHL	non-Hodgkins Lymphoma
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
OC	organochlorine
PB	Phenobarbital
PD	Parkinson's Disease
PFC	plaque forming cell
POD	point of departure
ppm	parts per million
RfD	reference dose
SCE	sister chromatid exchange

UDS	unscheduled DNA synthesis
UF	uncertainty factor
WHO	World Health Organization
WOE	weight of evidence

## EXECUTIVE SUMMARY

Integral Consulting Inc. (Integral) has developed an updated toxicity criterion for gamma-hexachlorocyclohexane (gamma-[HCH]). Gamma-HCH has previously been regulated as a potential human carcinogen by the Nevada Division of Environmental Protection (NDEP) using a toxicity criterion housed in U.S. Environmental Protection Agency's (EPA) Health Effects Assessment Summary Tables (HEAST; USEPA 1997)<sup>1</sup>. This project was initiated by Integral on behalf of Syngenta Crop Protection and Stauffer Management Company to update the NDEP toxicity criterion for gamma-HCH by incorporating 1) recent advances in the approach to carcinogenic risk assessment recommended by the USEPA (2005a) and 2) new data on the potential toxicity of gamma-HCH that have been published since the original toxicity criterion was developed.

The collective evidence indicates that gamma-HCH is not carcinogenic in animals or humans. In accordance with USEPA (2005a) guidance, the following weight of evidence (WOE) cancer classification was determined for gamma-HCH: **"not likely to be carcinogenic in humans."**

For non-cancer effects, immunological, hepatic, and neurological effects were observed in animals exposed to gamma-HCH at doses less than 1 mg/kg-day. Human data were insufficient to inform the non-cancer sensitivity evaluation. Overall, the body of evidence indicates that the immune system is the most sensitive target system for toxicity. Considering these findings and following USEPA (2000) guidance, a reference dose (RfD) was developed. The recommended oral RfD for gamma-HCH is 0.00001 mg/kg-day. The value is based upon a point of departure (POD) of 0.012 mg/kg-day for effects measured in immunoassays (including delayed-type hypersensitivity reaction, lymphocyte transformation, and haemolytic plaque-forming cell [PFC] assays) and a total uncertainty factor (UF) of 1,000 (10 each to account for intra- and inter-species extrapolation, and 10 for extrapolation from a lowest-observed-adverse-effect level [LOAEL] to no-observed-adverse-effect level [NOAEL]).

For perspective, the recommended RfD is equal to the intermediate minimal risk level (MRL) established by the Agency for Toxic Substances and Disease Registry (ATSDR) and more than two orders of magnitude less than the oral chronic RfD proposed by EPA. The intermediate oral MRL proposed by ATSDR is based on an identical POD for immunotoxicity and cumulative UF as those applied as the components of the RfD recommended here. In their 2002 *Reregistration Eligibility Decision for Lindane*, EPA established an oral RfD of 0.0047 mg/kg-day based on hepatic toxicity. The Integrated Risk Information System (IRIS) additionally houses a

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<sup>1</sup> The toxicity criterion included in EPA's HEAST was derived using data from Thorpe and Walker (1973). The Thorpe and Walker study suffers from multiple limitations including high mortality rates and high incidence of spontaneous tumors in untreated control animals and EPA later dismissed the Thorpe and Walker (1973) study as unreliable for classifying the compound's carcinogenicity (USEPA 2001).

chronic oral RfD of 0.0003 mg/kg-day, however the criterion was last updated in 1988 and is based upon renal effects observed in rats that are no longer considered relevant to humans (USEPA 1991).

# 1 INTRODUCTION

Integral Consulting Inc. (Integral) has developed an updated toxicity criterion for gamma-hexachlorocyclohexane (gamma-[HCH]). Gamma-HCH has previously been regulated as a potential human carcinogen by the Nevada Division of Environmental Protection (NDEP) using a toxicity criterion housed in U.S. Environmental Protection Agency's (EPA) Health Effects Summary Tables (HEAST; USEPA 1997). This project was initiated by Integral on behalf of Syngenta Crop Protection and Stauffer Management Company to update the NDEP toxicity criterion for gamma-HCH by incorporating 1) recent advances in the approach to carcinogenic risk assessment recommended by the USEPA (2005a) and 2) new data on the potential toxicity of gamma-HCH that have been published since the original toxicity criterion was developed. This report presents a summary of the methods and results of the toxicological review and presents a recommended toxicity criterion for adoption by NDEP into its regulatory programs.

## 2 METHODOLOGY

The available toxicological data were compiled and reviewed to assess the potential carcinogenicity and non-cancer effects of gamma-HCH. USEPA's *Guidelines for Carcinogen Risk Assessment* (2005a) provided the over-arching framework for the evaluation and assessment of potential carcinogenic effects. Approaches and principles outlined in EPA guidance for dose-response modeling (USEPA 2000) and EPA's review of the reference dose (RfD) process also were applied (USEPA 2002).

Key steps in the assessment were: literature summary and quality assessment; hazard assessment; and dose-response assessment and criterion derivation. The methods utilized for each of these steps are discussed briefly below.

### 2.1 LITERATURE SUMMARY AND QUALITY ASSESSMENT

A comprehensive literature search was conducted to identify relevant literature with which to support the evaluation. Data related to the assessment of oral exposures were the focus of the review as this is a principal pathway for current human exposures to ambient gamma-HCH. EPA and Agency for Toxic Substances and Disease Registry (ATSDR) reviews of HCH toxicity (ATSDR 2005; USEPA 1987, 2001) provided the starting point for identification of literature to be evaluated. Original studies identified in these documents were obtained for review. In addition, literature searches were conducted to identify more recent toxicity literature relevant to cancer and non-cancer endpoints.

All studies were reviewed and basic information characterizing study design, findings, and dose-response was compiled in a Microsoft Access database. In addition, each study was critically reviewed to assess quality and reliability using criteria developed from Klimisch et al. (1997), USEPA (2005a), and Durda and Preziosi (2000). Evaluation criteria included:

- Study is conducted using standard methods. Test substance purity and origin are described.
- Controls are included.
- Statistical power is appropriately included in the study design.
- Study design controls for potential confounders. Data on secondary effects which may influence the result are described.
- Methods and results are clearly and completely documented.

- Animal mortality and/or viability of the test system are described.

A summary of each paper and the data quality ranking assigned as a result of the critical review was compiled in a Microsoft Access database. The database is provided as Attachment A. The database additionally includes definitions for the criteria used in ranking each study and notes regarding the rank assigned for each study.

Poor quality and/or unreliable data were excluded from further technical evaluation and from use in the derivation of a toxicity criterion. Data of intermediate quality were used to support qualitative evaluations of toxicity (i.e., hazard assessment). Only high quality data were considered appropriate and utilized for quantitative dose-response modeling.

## 2.2 HAZARD ASSESSMENT

Studies of acceptable quality were further reviewed collectively to assess overall human carcinogenic potential and non-cancer effects. The outcomes of this step were a determination of the potential human carcinogenicity of gamma-HCH and the identification of the most sensitive target organ/system to be used as the basis of the toxicity criterion.

### 2.2.1 Cancer Assessment

A weight of evidence (WOE) approach was taken to determine the carcinogenic potential of gamma-HCH, following USEPA's *Guidelines for Carcinogen Risk Assessment* (2005a). Under the WOE approach, the available data on carcinogenicity, including epidemiological studies, animal bioassays, and *in vitro* assays were critically reviewed. Generally accepted causation criteria (Bradford Hill 1965), including strength, specificity, and consistency of the association, evidence for a dose-response relationship, temporal association between exposure and effect, and biological plausibility, were considered as part of the overall WOE evaluation.

The carcinogenic potential in humans was summarized into a WOE narrative, following USEPA (2005a) guidance. EPA classifies potential human carcinogens using the following hazard classification categories:

- Carcinogenic to humans
- Likely to be carcinogenic to humans
- Suggestive evidence of carcinogenic potential
- Inadequate information to assess carcinogenic potential
- Not likely to be carcinogenic to humans.

## 2.2.2 Non-Cancer Assessment

For non-cancer effects, studies exploring toxic response for non-cancer endpoints in all organ systems were reviewed. Relative potency to target organs based on animal data and the potential for increased susceptibility in human subpopulations were evaluated. The evaluation of relative potency focused on animal studies that considered effects associated with low doses<sup>2</sup> delivered during subchronic or chronic exposure durations, because these types of exposure scenarios are most relevant for human health risk assessment (USEPA 1992a). Low dose animal studies of reproductive and developmental endpoints were also included, regardless of the exposure duration, as recommended by USEPA (2005b). The potential for increased susceptibility of human subpopulations was evaluated considering lifestage (e.g., age, pregnancy), gender, underlying disease, genetic polymorphisms, and lifestyle factors (e.g., nutrition, smoking).

## 2.3 DOSE-RESPONSE ASSESSMENT AND CRITERION DEVELOPMENT

The toxicity criterion was derived consistent with the general principles and procedures outlined in USEPA's *Benchmark Dose Technical Guidance Document* (2000) and *A Review of the Reference Dose and Reference Concentration Processes* (2002). First, a point of departure (POD) for the critical effect<sup>3</sup> was selected. The POD is the dose-response point that marks the beginning of a low-dose extrapolation. The point can be the lower bound on dose for an estimated incidence or a change in response level from a dose-response model, or a no-observed-adverse-effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) for an observed incidence, or change in level of response (USEPA 2011).

The POD was determined by first identifying the endpoints that appropriately reflect, or are tightly related to, the critical effect and then selecting the most sensitive. Both a traditional RfD approach, and benchmark dose (BMD) modeling were explored for developing the appropriate toxicity criterion. Uncertainty factors (UFs) and/or modifying factors (MFs) were applied to the POD to account for uncertainties associated with the available data and variability between the test species and sensitive human populations.

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<sup>2</sup> Based on the experimental literature, these were defined as studies with one or more oral doses less than or equal to 10 mg/kg-day.

<sup>3</sup> For the purposes of developing toxicity criteria, EPA defines a critical effect as the first adverse effect, or its known precursor, that occurs to the most sensitive species as the dose rate of an agent increases (USEPA 2011). EPA defines an adverse effect as a biochemical change, functional impairment, or pathological lesion that affects the performance of the whole organism, or reduces an organism's ability to respond to an additional environmental challenge (USEPA 2011). It is recognized that the distinction between adverse effects and non-adverse effects is not always clear cut, and best professional judgment is required in making that distinction (Bogdanffy et al. 2001; HERA 2004).

### 3 FINDINGS – HAZARD ASSESSMENT

The collective evidence indicates that gamma-HCH is not carcinogenic in animals or humans. Following USEPA (2005a) guidance, the following WOE cancer classification was determined for gamma- HCH: **“not likely to be carcinogenic in humans.”**

For non-cancer effects, the body of evidence suggests that the immune system is the most sensitive target system for toxicity. A summary of the review completed for this determination is provided below.

#### 3.1 CARCINOGENICITY REVIEW

A summary of the human, animal bioassay, and *in vitro* data reviewed to develop the finding for carcinogenic potential is presented below.

##### 3.1.1 Human Data

Table 1 summarizes the study designs, findings, and overall quality of the human data reviewed for evaluating the potential carcinogenicity of gamma-HCH. Few epidemiological studies specifically evaluating gamma-HCH were available; however, several studies measuring a potential association between Lindane and cancer endpoints were reviewed. Findings of studies evaluating Lindane, however, are not necessarily relevant. The technical make-up of Lindane changed in the mid 1970s; in 1973 the Food and Agricultural Organization (FAO) required that Lindane must contain greater than 99 percent gamma-HCH (WHO 1991). Prior to this time, the makeup of Lindane was not standardized and contained greater amounts of isomers other than gamma-HCH. Any epidemiologic study that ascertained information on Lindane exposure prior to the mid-1970s, or spanning this time period, was considered to be limited by confounding factors.

Epidemiological studies evaluating gamma-HCH or Lindane and cancer types including breast cancer, non-Hodgkins Lymphoma (NHL), and prostate cancer were reviewed. In addition, a study evaluating incidence of an array of cancer types with Lindane exposure was also reviewed (Purdue et al. 2006).

The available epidemiologic studies determined sporadic and, when present, weak associations between Lindane exposure and various cancer types (see Table 1). The mostly weak associations, coupled with significant methodological issues in study design (e.g., non-specific exposure metrics, exposure misclassification, and poor measurement and control for potential confounders) do not allow for the available studies to lend credible insight to the carcinogenicity classification for gamma-HCH. Although there are limitations associated with

the collective body of evidence, the epidemiological data do not indicate that gamma-HCH is carcinogenic in humans.

### 3.1.2 Animal Bioassays

Overall, the animal bioassay data do not indicate that gamma-HCH is carcinogenic in animals. Table 2 summarizes the study designs, findings, and overall data quality of the animal bioassays reviewed for the evaluation of gamma-HCH<sup>4</sup> carcinogenic potential.

Fourteen studies in two species, and multiple strains for each, were reviewed. Three of the reviewed studies (Fitzhugh et al. 1950; Goto et al. 1972; Thorpe and Walker 1973) were considered to be inconclusive for the carcinogenicity evaluation due to severe limitations of the studies, as described in Table 2. Several remaining studies evaluated effects following chronic or lifetime dietary exposure to gamma-HCH. Although study design limitations reduce the confidence in the findings of each individual study, the collective data indicate that gamma-HCH is not carcinogenic in animals. In a lifetime bioassay conducted by the National Toxicology Program (NTP), no consistent increase in liver tumors was observed in gamma-HCH-treated rats or mice (NTP 1977). Liver tumors were observed in low-dose male mice, but not in high dose male mice or in female mice (NTP 1977). Multiple additional studies support that subchronic or chronic exposure to gamma-HCH does not result in tumor formation in rats or mice (Herbst et al. 1975; Ito et al. 1973a,b, 1975; USEPA 1983, 1989; Weisse and Herbst 1977). Liver tumor formation was observed in one rat study, but only at the highest dose and that dose clearly exceeded the maximum tolerated dose (MTD; USEPA 1992b).

Hepatic foci formation has been observed in gamma-HCH treated mice and rats and has been shown to be significantly augmented by treatment with known initiators and/or by partial hepatectomy (Hanada et al. 1973; Pereira et al. 1982; Schroter et al. 1987). Importantly, gamma-HCH is not itself a tumor initiator: no hepatic foci were observed in partially hepatectomized rats given a single dose of gamma-HCH followed by 15 weeks of dietary Phenobarbital (PB) (Schroter et al. 1987). Collectively, these data suggest that atypical foci can form in gamma-HCH-treated animals, particularly after initiation, and that the response is threshold-based. However, the data from longer-term studies with gamma-HCH, together with knowledge of hepatic tumor formation in general (e.g., Narama et al. 2003), suggest that foci formation is not predictive of carcinogenic potential.

Lung tumors were observed in two mice studies (Wolff et al. 1987; USEPA 2001); however, the strains of mice tested were genetically susceptible to lung tumors. The incidence of tumors in these strains may potentially be mediated by a pulmonary adenoma susceptibility gene that is

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<sup>4</sup> Within the available animal bioassays the names Lindane and gamma-HCH are to a large extent used interchangeably. For the purpose of the evaluation, for animal bioassays, the composition of Lindane was assumed to be gamma-HCH except when noted otherwise. Failure to evaluate and describe the isomer make-up, however, was considered as a limitation to the study in the data quality review.

present in multiple mouse strains (Manenti et al. 2003). Moreover, an additional potential genetic determinant of lung tumor susceptibility in mice has recently been identified (Liu et al. 2009). Therefore, the relevance of these effects to human health is inconclusive.

The collective data suggest that tumors form in gamma-HCH-treated animals only in association with increased mortality or underlying genetic susceptibility.

Among the reliable studies, two lifetime bioassays demonstrated lack of tumorigenicity at appropriate dose levels for an appropriate duration (NTP 1977; USEPA 1992b). These findings strongly support the conclusion that gamma-HCH does not cause liver tumors in rats or mice. Also supporting this conclusion are several other negative studies (Herbst et al. 1975; Ito et al. 1973a,b, 1975; USEPA 1983, 1989; and Weisse and Herbst 1977). The only studies in which tumors were observed were limited in design, reporting, or potential underlying genetic susceptibility, as described above.

Taken together, the data show that gamma-HCH results in tumor formation only under conditions of high-dose, extremely toxic exposure or when underlying genetic susceptibility specific to particular laboratory animal strains is present. The overall WOE suggests that gamma-HCH does not cause tumors in laboratory animals.

### 3.1.3 Mutagenicity and Genotoxicity Assays

Overall, the available evidence for gamma-HCH suggests that it is not mutagenic. Table 3 summarizes the short term mutagenicity and genotoxicity assays of gamma-HCH.

Two *in vitro* gene mutation assays, completed at a range of concentrations both with and without metabolic activation, were reviewed. While Gopaldaswamy and Nair (1992) reported positive results for the ability of gamma-HCH to induce gene mutations, Pool-Zobel et al. (1993) reported negative results. The positive finding was in a single bacterial test strain (Gopaldaswamy and Nair 1992).

Two *in vitro*, and three *in vivo* DNA binding assays, conducted in a variety of systems showed that gamma-HCH displays weak binding activity to deoxyribonucleic acid (DNA) (Gopaldaswamy and Nair 1992; Iverson et al. 1984; Sagelsdorff et al. 1983). Sagelsdorff et al. (1983) characterized the level of binding as "minute DNA binding", stating that "the level of binding is more than three orders of magnitude lower than would be expected if the mechanism of tumor induction was genotoxicity." Gopaldaswamy and Nair (1992) additionally evaluated the potential for hexachlorobenzene (HCB), a metabolite of HCH, to bind DNA. The binding efficiency of the metabolite was lower than for the parent compound.

Eight studies measuring DNA damage or fragmentation, or repair of such damage, via the comet assay, micronucleus assay, and assays measuring unscheduled DNA synthesis (UDS) were reviewed. Overall the studies showed mixed results over a large array of test systems and

test conditions. Importantly several of the studies reported positive findings only under a subset of test conditions, including at higher doses and with co-exposure to inhibitors of DNA repair (Kalantzi et al. 2004; Martin et al. 1999).

Pool-Zobel et al. (1993) conducted *in vitro* and *in vivo* assays for sister chromatid exchange (SCE) in Chinese hamster ovary (CHO) and Chinese hamster bone marrow cells respectively. The findings for both cell types were negative.

Although some evidence of mutagenic potential has been observed, there was a lack of a consistent positive response among the short term bioassays that were conducted in a variety of *in vitro* and *in vivo* systems and evaluated a variety of endpoints associated with DNA damage. The body of evidence for gamma-HCH is not supportive of mutagenicity.

### 3.1.4 Summary of Carcinogenicity and Uncertainties for the Weight of Evidence

The collective WOE indicates that gamma-HCH is not carcinogenic in humans or animals. The only two studies, in which potentially reliable tumorigenic effects were observed, used mice that are or may be genetically susceptible to tumor formation in multiple organs (USEPA 2001; Wolff et al. 1987). The relevance of the liver and lung tumor findings for humans is therefore questionable, particularly because multiple other strains of mice do not show a tumorigenic response to gamma-HCH (e.g., B6C3F1, NMRI, DD mice). Because the relevance of potential genetic susceptibility is unclear, the confidence with which it can be concluded that gamma-HCH is not a carcinogen is slightly reduced.

In 2001, EPA completed an evaluation of the carcinogenic potential of gamma-HCH (USEPA 2001) and classified gamma-HCH as having “suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential”.

## 3.2 NON-CANCER ENDPOINTS

The majority of the epidemiological data was inconclusive and was insufficient to inform the sensitivity analysis. In animals, gamma-HCH has been shown to induce a variety of toxic effects. Non-cancer effects observed following subchronic and chronic exposures to gamma-HCH include cardiovascular, hepatic, renal, immunological/lymphoreticular, neurological, behavioral, reproductive, and developmental effects (ATSDR 2005). Table 4 presents a summary of literature reviewed for non-cancer effects. Hepatic and immunological endpoints were associated with the lowest LOAELs across the endpoints evaluated in adult laboratory animals. Neurological effects associated with *in utero* exposure also were documented at relatively low doses. The collective data suggest that the immune system is the most sensitive target organ/system for gamma-HCH toxicity. The data supporting this conclusion are presented below.

### 3.2.1 Human Data

Data from epidemiological studies were not sufficient to inform either the types of toxicity or the most sensitive endpoint following gamma HCH exposures. Epidemiological studies were reviewed for neurological, reproductive, and immunological endpoints, with the following conclusions.

- Epidemiological data for neurological endpoints that evaluated HCH exposure and Parkinson's Disease (PD) were inconclusive (Corrigan et al. 2000; Hancock et al. 2008; Firestone et al. 2005; Fleming et al. 1994). If HCH does contribute to PD, it is likely to do so through a complicated multi-factorial mechanism of action involving gene-environment interaction.
- Epidemiological studies of female reproductive effects were also inconclusive. In general, body burdens of gamma-HCH were not detected sufficiently often in recent studies to support statistical analysis of reproductive outcomes. Other studies suffered from a major limitation of indirect/retrospective measurements of exposure. The evidence for a relationship between body burden of organochlorine (OC) pesticides (including Lindane) and breast cancer was inconclusive, with the majority of studies failing to demonstrate a relationship (see Table 1).
- Epidemiological studies of male reproductive effects also were inconclusive. OC pesticides have long been suspected of causing developmental effects in human males; however, the evidence for gamma-HCH was not supportive of this association. While one study found a relationship between increased tissue concentration of gamma-HCH and increased incidence of undescended testes, the relationship was not reported to be significant (Pierik et al. 2007). In addition, the results of one *in vitro* study suggested that environmentally relevant concentrations of gamma-HCH in humans may not be associated with adverse testicular effects (Pflieger-Bruss et al. 2006).
- Epidemiological studies of immunological effects (Blair et al. 1998; McDuffie et al. 2001) suffered from the same limitations as those for reproductive effects. In general, either body burdens of gamma-HCH were too low to support statistical evaluation of association with immunological conditions, such as NHL, or the measures of exposure were qualitative, indirect, and/or retrospective.

### 3.2.2 Animal Bioassays

Several animal studies were critically reviewed to assess the most-sensitive non-cancer toxic endpoint. Toxic responses observed in these studies are documented by endpoint in Tables 5 through 8. Available evidence for renal effects associated with gamma-HCH exposure indicates

that these effects occur via a MOA that is not relevant for human toxicity (USEPA 1991); therefore, the renal endpoint was not included in the sensitivity evaluation.

As shown in Tables 5 and 6, adverse effects to both hepatic and immunological endpoints in adult rodents have been observed at doses less than 1 mg/kg-day (Matsuura et al. 2005; Meera et al. 1992, 1993; USEPA 1983). Adverse effects to both immunological and neurobehavioral endpoints were observed to occur in offspring of females exposed to doses less than 1 mg/kg-day (see Tables 5 and 7). Of all three endpoint categories, the lowest LOAEL of 0.012 mg/kg-day shown is for an immunological effect in adult mice. The LOAEL of 0.012 mg/kg-day was associated with effects on both cell-mediated immunity (based upon results of a delayed-type hypersensitivity reaction test and a lymphocyte transformation test), and humoral immunity (based upon results of a hemolytic plaque forming cell [PFC] assay). These effects were observed in female Swiss albino mice in a study of 24-week duration (Meera et al. 1992). In this same study, the effects were observed to be both dose- and time-dependent, with stimulation of the acquired immune system observed until 8-12 weeks of exposure and suppression observed between 12-24 weeks of exposure. Another study of similar exposure duration (22 weeks) identified a LOAEL of 1.5 mg/kg-day in male rats associated with significant suppression of the humoral immune response (as evidenced by decrease in serum antibody titre to tetanus toxoid and lower increases of IgG and IgM levels after tetanus toxoid) (Saha and Banerjee 1993). Only one lifetime bioassay with immunological endpoints was identified. This study reported by USEPA (1992b) assessed leukocyte counts and spleen and bone marrow histology. In this study, significant changes in relative spleen weight were observed, but they weren't dose-dependent. No significant changes in leukocyte counts or bone marrow histology were observed. The immunological endpoints tested by USEPA (1992b), however, were less sensitive than those evaluated by Meera et al (1992).

The majority of the available data were from evaluations of hepatotoxicity in rodents (Table 6). Liver effects were observed at low doses in four different studies, in both male and female rats (Matsuura et al. 2005; Parmar et al. 2003; Schroter et al. 1987; USEPA 1983). Two of the studies with hepatic LOAELs in this range were of subchronic exposure duration. The lowest hepatic LOAELs were 0.29 mg/kg-day for males and 1.7 mg/kg-day for females for liver hypertrophy observed in a subchronic study (USEPA 1983). This study had inconsistent reporting of statistical significance by specific effect; the significance of the liver hypertrophy effect was inferred from the text. The study by Matsuura et al. (2005) was a two-generation reproductive dietary bioassay with no major quality limitations. In this study, the lowest hepatic LOAEL (0.56 mg/kg-day) was associated with increased relative liver weight in F0 males. In this same study, a LOAEL of 0.74 mg/kg-day was established for the effect of centrilobular hypertrophy of hepatocytes in F1 males. The same effects were observed for females, at the next highest dose. In this study, hepatic effects were the most sensitive; immunological and neurological effects were also observed, but at higher doses. Immunological endpoints were, however, limited to organ weight and histological findings.

As shown in Table 7, neurobehavioral effects were observed in offspring of rats exposed to gamma-HCH during gestation. The lowest LOAEL for increased spontaneous locomotor activity was associated with a maternal dose of 0.125 mg/kg-day (Johri et al. 2007). The NOAEL was 0.0625 mg/kg-day (Johri et al. 2007). The study by Matsuura et al. (2005) also evaluated neurobehavioural effects in offspring exposed during gestation, but did not find significant effects except at the highest dose tested (300 ppm). This study also evaluated hepatic effects; based upon the observed LOAELs, hepatotoxicity was most sensitive effect observed in this study.

### **3.3 MOST SENSITIVE TARGET ORGAN**

Overall, the available data indicate that the immune system is the most sensitive target organ/system for gamma-HCH toxicity. The immune system is capable of triggering a compensatory mechanism when one effect or mechanism is inhibited. Thus, the range of immunotoxicity results within a species is typically wider than that of other conventional toxicology endpoints. In addition, there is substantial variability in immune responses across different species. Specifically, there are large uncertainties in the extrapolation of alterations to immune function observed in animals to human health. However, the human relevancy of certain short-term immunoassays, such as the delayed-type hypersensitivity reaction test and the PFC assay, has been established for the purpose of hazard identification (WHO 1996). The ATSDR (2005) identified the immune system as a sensitive target organ for intermediate exposure, and used the Meera et al. (1992) study as the basis of an oral minimal risk level (MRL).

## 4 TOXICITY CRITERION

A final oral RfD of 0.00001 mg/kg-day was established for gamma-HCH. The toxicity criterion is based on the LOAEL of 0.012 mg/kg-day from Meera et al. (1992) for immunotoxicity and the combined uncertainty factor of 1,000 (10X for interspecies variability, 10X for intraspecies variability, 10X for use of LOAEL).

The process for selecting the study and endpoint for the critical effect, and for determining the POD are documented below. In addition, the basis of the UFs and/or MFs applied to the POD is provided.

### 4.1 SELECTION OF ENDPOINTS AND DATASETS

As established in Section 3.3, the immune system is the most sensitive target organ for gamma-HCH. Immunological data evaluated for the POD were based on immune response endpoints, rather than less specific non-functional endpoints (e.g., histological findings, organ weights, cell counts) that were reported in studies that evaluated immunotoxicity. Immune response endpoints directly measure function for a particular immune response and so are better potential indicators of changes in immune system function compared to the non-functional endpoints noted above (WHO 1996). Immunological data for functional endpoints were available from two studies of chronic duration (Meera et al. 1992 and Saha and Banerjee 1993). Delayed type hypersensitivity reaction, lymphocyte proliferation, and the hemolytic PFC assay were the immunological endpoints evaluated in these studies and identified as potential basis for the POD.

### 4.2 DETERMINATION OF POINT OF DEPARTURE

A traditional RfD approach was applied for the determination of the POD. The LOAEL of 0.012 mg/kg-day associated with immunotoxic effects from Meera et al. (1992) was selected as the POD for gamma-HCH. This LOAEL was associated with effects on both cell-mediated immunity (based upon results of a delayed-type hypersensitivity reaction test and a lymphocyte transformation test), and humoral immunity (based upon results of a hemolytic PFC assay). This value was the lowest of the LOAELs available for immunotoxicity. The delayed-type hypersensitivity assay and the PFC assay have been recommended by the World Health Organization (WHO) as predictive of human toxicity (WHO 1996); thus, the human relevance of these effects is supported.

A BMD modeling approach was not applied to the Meera et al. (1992) study data because they did not meet the recommended criteria established by EPA for BMD modeling. Specifically, the Meera et al. (1992) response data for the most sensitive effects were not consistently monotonic

and USEPA (2000) recommends that BMD modeling not be applied in this situation. Only one other immunotoxicity study of chronic duration was considered in the dose-response assessment (i.e., Saha and Banerjee 1993); however, the effects levels in this study were more than 100 times higher than that found in Meera et al. (1992), and therefore were not considered further in the POD development. It is additionally noted that the LOAELs for functional immune endpoints evaluated in studies of sub-chronic duration were elevated above the LOAELs from both chronic studies (Table 5).

#### 4.3 APPLICATION OF UNCERTAINTY AND MODIFYING FACTORS TO THE POINT OF DEPARTURE

UFs and MFs determined appropriate for the derivation of a toxicity criterion for gamma-HCH from the selected POD are presented below.

- **Intraspecies Extrapolation Factor** - A value of 10 was selected for this factor to account for the variation in sensitivity among the members of the human population.
- **Interspecies Extrapolation Factor** - A value of 10 was selected for this factor to account for the uncertainty involved in extrapolating from animal data to humans.
- **Subchronic-to-Chronic Duration Factor** - A value of 1 was selected for this factor. The Meera et al. (1992) study was chronic in duration<sup>5</sup>.
- **LOAEL-to-NOAEL Factor** - A value of 10 was selected for this factor. The POD selected was a LOAEL.
- **Database UF** - A value of 1 was selected for this factor. The overall database for toxicity of gamma-HCH was sufficient to support the determination of the POD.
- **Additional MF** - No additional MFs were determined necessary for the derivation of the toxicity criterion.

The total UF to be applied to the POD is 1,000.

#### 4.4 RECOMMENDED TOXICITY CRITERION FOR GAMMA-HCH

The recommended toxicity criterion for gamma-HCH is an oral RfD of 0.00001 mg/kg-day. This value is based on a POD of 0.012 mg/kg-day for immunotoxicity reported by Meera et al. (1992) and a cumulative UF of 1,000 (10 each to account for intra- and inter-species extrapolation, and 10 for extrapolation from a LOAEL to NOAEL).

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<sup>5</sup> Studies with exposure durations greater than 90 days were considered chronic (USEPA 2011).

## 5 SUMMARY

Integral has developed an updated toxicity criterion for the chemical gamma-HCH.

The cancer classification for gamma-HCH is: **“not likely to be carcinogenic in humans.”** For non-cancer effects, the body of evidence suggests that the immune system is the most sensitive target system. There is sufficient evidence to demonstrate that gamma-HCH is hepatotoxic and immunotoxic in rodents. Additionally, gamma-HCH has been shown to be neurotoxic at low doses to animals exposed during critical developmental stages. The immune system was determined to be the most sensitive target organ/system following subchronic and chronic exposure to gamma-HCH. Therefore, the recommended oral RfD for gamma-HCH was based upon a POD of 0.012 mg/kg-day for effects measured in immunoassays, including delayed-type hypersensitivity reaction, lymphocyte transformation, and haemolytic PFC assays (Meera et al. 1992). A total UF of 1,000 (10 each to account for intra- and inter-species extrapolation, and 10 for extrapolation from a LOAEL to NOAEL) was applied to the POD. The recommended oral RfD for gamma-HCH is 0.00001 mg/kg-day.

For perspective, the recommended RfD is equal to the intermediate MRL established by the ATSDR and more than two orders of magnitude less than the oral chronic RfD proposed by EPA. The intermediate oral MRL proposed by ATSDR is based on an identical POD for immunotoxicity and cumulative UF as those applied as the components of the RfD recommended here. In their 2002 *Reregistration Eligibility Decision for Lindane*, EPA established an oral RfD of 0.0047 mg/kg-day based on hepatic toxicity. IRIS additionally houses a chronic oral RfD of 0.0003 mg/kg-day; however, the criterion was last updated in 1988 and is based upon renal effects that are no longer considered relevant to humans (USEPA 1991).

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## **TABLES**

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Table 1. Epidemiological Evidence: Gamma-HCH and Cancer.

Endpoint	Study	Summary of Findings	Study Limitations
<b>Breast Cancer</b>			
456	Mathur et al. (2002)	Case-control study of women from India.  Found higher levels of gamma-HCH in blood of women (age 41-50) with breast cancer compared to controls. Relationship was not significant for other age groups.	Potential confounders including the presence of other organochlorine pesticides were not controlled for. Lipids in blood were not measured. Method for selecting control group was not discussed fully. Potential for retrospective questionnaire bias was not discussed.
459	Mills and Yang (2006)	Population-based study in a California farm worker community. Used Cancer Registry and pesticide use data.  Found no association between Lindane use and breast cancer.	Ecological study design does not allow for precise and specific exposure assessment. Isomer composition of Lindane product changed during the time period for which exposures were ascertained.
460	Muir et al. (2004)	Population level study. Mapping and statistical evaluation of breast cancer incidence rates and historical application of Lindane in two counties in England.  Found an association between breast cancer and Lindane use in rural areas of one county, but not in a second neighboring county.	Pesticide use data for areal units is not a precise or specific measure of exposure to individuals. Use data for 1991 only was assumed to represent historical use/exposure. Did not characterize isomers in Lindane applied in 1991. Breast cancer cases were evaluated at the location of diagnosis which may not represent where cases lived and were exposed to pesticides over time. Potential confounding factors were not measured or considered.
<b>Non-Hodgkin's Lymphoma (NHL)</b>			
436	Blair et al. (1998)	Pooled analysis of case-control studies in Kansas, Nebraska, Iowa, and Minnesota.  Found weak association between reported agricultural use of Lindane and risk of NHL (OR=1.5; 95%CI = 1.1-2.0). Association was diminished, and not statistically significant, when adjustments for some combinations of potential confounders were made.	The time frame over which exposures were ascertained is unclear. The makeup of Lindane, and the potential for it to have changed over the time period for which exposures were ascertained are not discussed. Use of proxy data results in potential misclassification of exposure. Differences in questionnaire and interview tools between individual studies required adjustments for pooling data and introduced potential for misclassification or loss of information.
533	Lee et al. (2004)	Pooled analysis of population-based case-control studies in Iowa, Minnesota, and Nebraska.  Increased OR (2.4, 95% CI = 1.0-5.7) for NHL for asthmatics who reported using Lindane compared to non-farmers without asthma. No association was found for non-asthmatic farmers.	Pesticide use data does not provide a specific measure of exposure. The time frame over which exposures were ascertained is unclear. The makeup of Lindane and the potential for it to have changed over this time period is not discussed. Potential for misclassification of asthma status and exposure (obtained via interview). Study had limited power for assessing interactions.
457	McDuffie et al. (2001)	Population-based case-control study in Canadian men with a diversity of occupations.  Found weak association between frequency of Lindane use and NHL (OR=2.06; 95% CI=1.01-4.22).	Incomplete information on questionnaire used, including the time frame over which exposures were ascertained, was provided. Potential for recall bias and misclassification of pesticide exposure. Low response rate. Information on study population/respondents not included.
329	Rafnsson (2006)	Nested case-control study of sheep owners in Iceland.  Increased OR for NHL (3.86; 95% CI=1.59-8.53) for individuals who dipped 100 or more sheep (used as exposure metric) compared to those who dipped less than 100 sheep.	Use of the number of sheep dipped as a surrogate for exposure is not specific or precise. Isomer content of HCH insecticide changed within the time over which exposures were ascertained; this factor is recognized but not accounted for in the analysis. Potential confounding factors including other exposures to pesticides, health status, and lifestyle factors were not ascertained or controlled for.
<b>Prostate Cancer</b>			
458	Mills and Yang (2003)	Nested case-control study in California farm workers union. Used Cancer Registry and pesticide use data.  Found association between prostate cancer risk and Lindane use (positive trends with levels of use at county level).	Evaluation did not ascertain or control for exposures prior to workers' association with the union. Ecological study design (i.e., county level pesticide use data by year) does not allow for specific or precise exposure metric. Complete data on subjects and exposure is not provided.
<b>General Cancer</b>			
543	Purdue et al. (2006)	Prospective study of pesticide applicators in the Agricultural Health Study (AHS) cohort.  Found association between intensity-weighted lifetime days exposed to Lindane and NHL risk (trends with increasing categories of exposure, $p=0.04$ ). Found no trend for increased risk with alternate exposure metric of life-time days exposed.  Study evaluated multiple cancer types. Results for lung cancer are not clear. No other cancer types were associated with Lindane use.	Use of questionnaire to estimate exposure may result in recall bias and/or misclassification. Low response rate (44% of subjects filled out the questionnaire). Short follow-up period. Isomer content of Lindane product changed over the time for which exposures were ascertained; this factor was not discussed or accounted for in the study's analysis. Large number of statistical comparisons decreases the confidence in observed chemical-specific associations.
Notes:	AHS	= Agricultural Health Study	
	CI	= confidence interval	
	HCH	= hexachlorocyclohexane	
	NHL	= non-Hodgkin's lymphoma	
	OR	= odds ratio	

Studies in which pesticides were measured, but not detected with adequate frequency for statistical analysis are not included in this table. For gamma-HCH these studies were Cocco et al. (2008), Quintana et al. (2004), Sturgeon et al. (1998), and Guttes et al. (1998).

Table 2. Gamma-HCH Animal Carcinogenicity and Related Data.

Reference	Species/Sex	Study Design	Summary of Findings	Major Study Limitations
487 Anilakumar et al. (2009)	Rat (Wistar), male	<b>Duration:</b> 48 hours <b>Sample Size:</b> 8/group <b>Route:</b> intraperitoneal <b>Dose Levels:</b> 0, 300 mg/kg	Liver ascorbic acid, malondialdehyde (TBARS), conjugated dienes, and hydroperoxides significantly increased. Catalase, SOD, glucose-6-phosphate dehydrogenase, and GST all significantly decreased; GSH peroxidase and GGT significantly increased. Femur bone marrow micronuclei significantly increased.	Only one dose level evaluated. Only males evaluated. Small sample size. Potentially irrelevant route of exposure. Mortality/general toxicity data not reported. GSH assay did not differentiate between reduced and oxidized forms.
651 Azzalis et al. (1995)	Rat (Wistar), male	<b>Duration:</b> 24 hours <b>Sample Size:</b> 2-6/group <b>Route:</b> intraperitoneal <b>Dose Levels:</b> 0, 40 mg/kg	No abnormal liver histology or increased liver weights were seen with lindane alone or lindane plus ethanol. Total P450, cytochrome b5, P450 reductase, NADPH oxidase, superoxide production, and TBARS were all significantly increased in lindane-treated rats. NADPH oxidase increase was potentiated in lindane and ethanol co-treated rats. Total GSH, GSH peroxidase, alpha-tocopherol, and ubiquinol 9 and 10 were significantly decreased in the ethanol-lindane co-treated rats relative to lindane alone. No changes in these endpoints were observed with lindane alone.	Only one dose level evaluated. Only males evaluated. Small sample size. Potentially irrelevant route of exposure.
652 Bainy et al. (1993)	Rat (Wistar), male	<b>Duration:</b> 60 and 90 days <b>Sample Size:</b> 2-21/group <b>Route:</b> dietary, ad libitum <b>Dose Levels:</b> 0, 1000 ppm	Total P450, P450 reductase, superoxide production, NADPH oxidase, GSSG reductase, TBARS, and chemiluminescence were all significantly increased after 60 and 90 days; no clear temporal trend. Total P450 was significantly increased at 90 days compared to 60 days. GSH peroxidase was significantly decreased at 90 days; catalase was significantly decreased at 60 and 90 days.	Only males evaluated. Only one dose level evaluated. Mortality and general toxicity not reported. Small sample size.
576 Barros et al. (1988)	Rat (Wistar), male	<b>Duration:</b> 24 hours <b>Sample Size:</b> 6-10/group <b>Route:</b> intraperitoneal <b>Dose Levels:</b> 0, 60 mg/kg	Hepatic reduced GSH was significantly decreased 4 hours post-dose; hepatic oxidized GSH was significantly increased 2, 4, and 24 hours post-dose. Hepatic GSH/GSSG ratio significantly changed 2, 4, and 24 hours post-dose. Biliary reduced GSH and its excretion rate were significantly decreased 4 hours post-dose; biliary oxidized GSH and its excretion rate were significantly increased 4 and 24 hours post-dose. Recovery of hepatic and biliary reduced GSH levels is seen 24 hours post-dose although GSSG is still significantly elevated at that time, suggesting up-regulation of GSH synthesis.	Small sample size. Mortality and general toxicity data not reported. Only males evaluated.
381 Barros et al. (1991)	Rat (Wistar), male	<b>Duration:</b> 15 or 30 days <b>Sample Size:</b> 6-22/group <b>Route:</b> dietary, ad libitum <b>Dose Levels:</b> 0, 20 ppm	Significant time-dependent increases in microsomal total P450 and superoxide production and TBARS formation (homogenates and microsomes). SOD activity significantly increased in treated vs. control rats at 15 and 30 days. P450 reductase, glucose-6-phosphate dehydrogenase, GSSG reductase, GSH peroxidase, and catalase not changed. No microscopic changes seen in livers from treated rats.	Small sample size. Only one dose level evaluated. Only males evaluated. Mortality not reported.
488 Cornejo et al. (2001)	Rat (SD), male	<b>Duration:</b> 24 hours <b>Sample Size:</b> 4-12/group <b>Route:</b> intraperitoneal <b>Dose Levels:</b> 0, 40 mg/kg	Cytosolic NO production significantly increased. No increase in mitochondrial superoxide production. Liver protein carbonyls significantly increased.	Only one dose level evaluated. Only males evaluated. Small sample size. Potentially irrelevant route of exposure.
489 Descampiaux et al. (1996)	Human Hep3B cell line	<b>Duration:</b> up to 72 hours <b>Sample Size:</b> 3-10 experiments <b>Route:</b> <i>in vitro</i> <b>Dose Levels:</b> 0-50 mg/L	Significant dose-dependent increases in GSSG, GST, and SOD after 24 hours exposure. Significant dose-dependent decrease in GSH after 24 hours exposure. Catalase and GSSG reductase were not significantly changed (24 hours). Significant dose- and time-dependent increases in SOD activity over 72 hours. Lindane/vitamin E co-treatment resulted in SOD decrease at low vitamin E concentrations and SOD increase at high vitamin E concentration. Potential cytostatic effect of lindane (as opposed to cytotoxic effect).	Hepatoma cells are not as physiologically relevant as primary cultures. Evaluation of MnSOD may have been confounded by pre-treatment with KCN; therefore, their evaluation of Cu,ZnSOD may be skewed.
490 Dubois et al. (1996)	Rat fetal liver cells Human Hep G2 cells	<b>Duration:</b> not reported for cytotoxicity or enzyme induction experiments. Data from Western blots were from 72 hour treatments. <b>Sample Size:</b> 3 or more measures/group <b>Route:</b> <i>in vitro</i> <b>Dose Levels:</b> 0, 50 µM or 250 µM; 0-50 µM	LDH leakage was significantly increased in 250 µM lindane-treated Hep G2 cells but not fetal rat hepatocytes. MTT conversion was significantly decreased in 250 µM lindane-treated fetal rat hepatocytes and Hep G2 cells. CYP1A enzyme activity (EROD assay) was not increased in 50 µM lindane-treated fetal rat hepatocytes or Hep G2 cells. General CYP activity (ECOD assay) was significantly increased in 50 µM lindane-treated fetal rat hepatocytes and Hep G2 cells. The increase was dose-dependent in fetal rat hepatocytes over a 0-50 µM range. CYP3A protein appeared to be increased in Hep G2 cells treated with 50 µM lindane for 72 hours.	Treatment duration not reported for most experiments. Strain and source of rat livers not reported. Only one lindane concentration evaluated in all but one experiment.
491 Fernandez et al. (2003)	Rat (SD), male	<b>Duration:</b> 24 hours <b>Sample Size:</b> 4-15/group <b>Route:</b> intraperitoneal <b>Dose Levels:</b> 0, 40 mg/kg	Total P450 content and P450 reductase activity significantly increased. Superoxide production significantly increased. SOD activity significantly decreased. CYP2E protein and activity significantly increased.	Only one dose level evaluated. Only males evaluated. Small sample size. Potentially irrelevant route of exposure.
382 Fitzhugh et al. (1950)	Rat (Wistar), male/female	<b>Duration:</b> 107 weeks <b>Sample Size:</b> 10/sex/group <b>Route:</b> dietary, ad libitum <b>Dose Levels:</b> 0, 5, 10, 50, 100, 400, 800, 1600 ppm	Significantly reduced body weight in the 1600 ppm males and females. Mean age of death decreased in the 400, 800, and 1600 ppm groups (significant at 800 and 1600). MTD exceeded at highest dose. Relative liver weight significantly increased at dose levels at or above 100 ppm (dose-dependent). Very slight to moderate gross and microscopic liver changes noted at or above 100 ppm. No gross tumors reported during macroscopic examination.	Small sample size. Minimal details on histopathology. High overall mortality in the study; evaluations were based either on severely ill or found dead animals. Data were not stratified by sex.
502 Giavarotti et al. (1998)	Rat (SD), male	<b>Duration:</b> 24 hours <b>Sample Size:</b> 4-9/group <b>Route:</b> intraperitoneal <b>Dose Levels:</b> 0, 20 mg/kg	Total P450 and P450 reductase activity were not changed 24 hours post-dose. Superoxide production and SOD activity were not changed 24 hours post-dose. Catalase, GSH peroxidase, and glucose-6-phosphate dehydrogenase were not affected. Alpha-tocopherol and beta-carotene content were not changed. Lycopene was significantly decreased 24 hours post-dose.	Only males evaluated. Only one dose level evaluated. Small sample size. Potentially irrelevant route of exposure.
641 Goel et al. (1988)	Rat (SD), female	<b>Duration:</b> up to 9 days <b>Sample Size:</b> 4-8/group <b>Route:</b> oral gavage <b>Dose Levels:</b> 0, 25, 50, 100 mg/kg	TBARS was significantly increased 12 hours post-dose, was maximal 24 hours post-dose, and decreased thereafter. GSH peroxidase was not changed. Hepatic lipid peroxidation (i.e., TBARS) increase peaked 1 day post-dose and diminished to control values 3 days post-dose. Significant dose-dependent increases in lipid peroxidation 24 hours post-dose.	Only females evaluated; small sample size. Only one dose level evaluated. Significant body weight loss in treated animals 24 hours post-dose. Functional consequences of lipid peroxidation not evaluated.
383 Goto et al. (1972)	Mouse (ICR-JCL), male	<b>Duration:</b> 26 weeks <b>Sample Size:</b> 10/group <b>Route:</b> dietary (unknown if ad libitum) <b>Dose Levels:</b> 0, 300, 600 ppm	Gross liver tumor incidence 5/10 in the 600 ppm mice; tumor incidence in control mice not reported. Microscopically, these "hepatomas" were described as atypical proliferation or hyperplastic knot. Increased relative liver weight and decreased whole body weight at 600 ppm. No metastases noted.	Small sample size. Only males tested. No statistical analysis. Inadequate characterization of histopathological changes. Mortality not reported. Inadequate translation from German did not allow for comprehensive review.
492 Guan and Ruch (1996)	Rat (WB-F344) liver epithelial cells	<b>Duration:</b> up to 4 hours <b>Sample Size:</b> 3 dishes/group <b>Route:</b> <i>in vitro</i> <b>Dose Levels:</b> 0, 50 µM	Dye coupling (i.e., intercellular communication) significantly inhibited within 10 minutes of lindane exposure. Plasma membrane connexin43 staining was decreased beginning 1 hour after treatment; cytoplasmic staining was evident at some time points. Connexin43 phosphorylation is reduced with lindane treatment. Phospho-connexin43 is endocytosed and degraded with lindane treatment.	Experiments not repeated. Only one concentration evaluated. Cell viability and treatment cytotoxicity not reported.

Table 2. (continued)

Reference	Species/Sex	Study Design	Summary of Findings	Major Study Limitations
439	Guan et al. (1995) Rat (WB-F344) liver epithelial cells	<b>Duration:</b> up to 14 days <b>Sample Size:</b> 3 cultures <b>Route:</b> <i>in vitro</i> <b>Dose Levels:</b> 0-100 µM	Dye coupling (i.e., intercellular communication) was significantly reduced after 10 minutes of lindane treatment. Dye coupling was also significantly reduced with increasing lindane concentration. The number of gap junctions per cell also decreased as a function of time and concentration. Decreased phospho-connexin43 was observed beginning 3 hours after treatment and with increasing concentration. Connexin43 message decreased over time, beginning 4 hours after treatment.	Cell viability and treatment cytotoxicity were not reported. Evidence for reversibility of the changes in phospho-connexin43 protein was very limited. Repetition of the experiments was not reported.
385	Hanada et al. (1973) Mouse (DD), male/female	<b>Duration:</b> 32 weeks plus 5-6 weeks recovery <b>Sample Size:</b> 3-10/sex/group (treated); 14-18/sex (control) <b>Route:</b> dietary, ad libitum <b>Dose Levels:</b> 0, 100, 300, 600 ppm	Increased mortality in the 600 ppm group. Dose-dependent increase in mortality after 32 weeks plus recovery. 2/2 600 ppm males had liver tumors at the week 26 laparotomy. Atypical proliferation (i.e., foci of enlarged cells) seen in 5/9 300 ppm males, 1/7 300 ppm females, 4/4 600 ppm males and 3/3 600 ppm females; hepatoma seen in 600 ppm males (3/4) and females (1/3) after exposure plus recovery. No metastases or peritoneal invasion noted.	Small sample size. No statistical analysis. No evaluation done at the end of the 32 week exposure period; regression of changes could not be evaluated. Greater than 50% mortality in the high dose group after 26 weeks; MTD exceeded.
442	Herbst et al. (1975) Mouse (NMRI), male/female	<b>Duration:</b> 80 weeks <b>Sample Size:</b> 50/sex (treated); 100/sex (control) <b>Route:</b> dietary (unknown if ad libitum) <b>Dose Levels:</b> 0, 12.5, 25, 50 ppm	No treatment-dependent increase in liver tumors was observed. Cirrhosis and cell hypertrophy not observed. No apparent increase in mortality or decrease in body weight in treated animals.	Body weights/food consumption not evaluated for all animals. Unclear mortality data. Only liver evaluated. The doses evaluated were too low to elicit a toxic effect.
363	Ito et al. (1973a) Mouse (DDY), male	<b>Duration:</b> 24 weeks <b>Sample Size:</b> 20/group <b>Route:</b> dietary, ad libitum <b>Dose Levels:</b> 0, 100, 250, 500 ppm	Slight increase in relative liver weight at 500 ppm only. Body weight not affected. Equivocal evidence of liver hypertrophy at 100 and 250 ppm; slight hypertrophy noted at 500 ppm. No liver tumors observed. Slight proliferation of endoplasmic reticulum noted.	Only males evaluated. No statistical analysis. Only examined liver histologically. Mortality not reported.
364	Ito et al. (1973b) Mouse (DDY), male	<b>Duration:</b> 24 weeks <b>Sample Size:</b> 20-28/group <b>Route:</b> dietary (unknown if ad libitum) <b>Dose Levels:</b> 0, 50, 100, 250 ppm	No increase in relative liver weight. Equivocal evidence of hypertrophy (liver) only at 250 ppm. No liver tumors observed. Body weight not affected.	No statistical evaluation. Only males evaluated. Unclear if extra-hepatic tumors/metastases were evaluated microscopically. Mortality not reported. The doses evaluated were too low to elicit a toxic effect.
386	Ito et al. (1975) Rat (Wistar), male	<b>Duration:</b> 24 or 48 weeks <b>Sample Size:</b> 6-8/group <b>Route:</b> dietary, ad libitum <b>Dose Levels:</b> 0, 500 ppm	Equivocal evidence of liver cell hypertrophy at 48 weeks only. No liver tumors observed. Potential effect of treatment on liver weight could not be evaluated because the control and treated animals were sacrificed at different times/ages. The dose evaluated may not have been adequate to produce a toxic effect.	Mortality not reported. Unclear if metastases were evaluated grossly or microscopically. Only males evaluated. Small sample size. No statistical evaluation.
286	Johri et al. (2007) Rat (Wistar), male and female adults; male pups	<b>Duration:</b> daily maternal doses on GD 5-21 <b>Sample Size:</b> 3 or 6 male pups/group/time point <b>Route:</b> oral (dams); gestational and lactational (pups) <b>Dose Levels:</b> 0, 0.0625, 0.125, 0.25 mg/kg-day (maternal administered dose)	Hepatic P450 isoform activity increased postnatally in male pups in a dose- and time-dependent manner, with significant increases in CYP1A and CYP2B activities (EROD and PROD assays) in the two highest dose groups compared to controls; the greatest enzyme activity values were at 6 weeks of age. CYP2E activity was significantly increased in male pups in the two highest dose groups at 3 and 6 weeks of age and in the highest dose group at 9 weeks of age compared to controls. For CYP2E, the increases were dose-dependent but did not further increase with pup age. Similar dose-dependent increases in CYP1A, CYP2B, and CYP2E protein in male pups were evident; but maximal protein values were observed at 3 weeks and declined thereafter. Dose- and time-dependent CYP message increases were also observed.	Only male pups evaluated. Protein and message methods were not quantitative. No toxicity or mortality data presented.
287	Johri et al. (2008a) Rat (Wistar), male and female adults; male pups	<b>Duration:</b> daily maternal doses on GD 5-21 <b>Sample Size:</b> 3 or 6 male pups/group/time point <b>Route:</b> oral (dams); gestational and lactational (pups) <b>Dose Levels:</b> 0, 0.0625, 0.125, 0.25 mg/kg-day (maternal administered dose)	Dose-dependent increases in hepatic CYP1A1, CYP1A2, CYP2B1, CYP2B2, CYP2E1 message and enzyme activity (EROD, PROD, and NDMA-d) were observed in male pups, which were significant in the two highest dose groups and, in some cases, all treated groups. For most isozymes, P450 message and activity reached a maximal level or plateaued and declined thereafter over time.	Only male pups evaluated. Message method was not quantitative. No toxicity or mortality data presented.
288	Johri et al. (2008b) Rat (Wistar), male and female adults; male pups	<b>Duration:</b> daily maternal doses on GD 5-21 <b>Sample Size:</b> 3 or 6 pups/group/time point <b>Route:</b> oral (dams); gestational and lactational (pups) <b>Dose Levels:</b> 0, 0.25 mg/kg-day (maternal administered dose)	Hepatic CYP1A and CYP2B enzyme activity were significantly increased in male pups of exposed dams. Hepatic CYP2B protein was significantly increased in male pups; CYP1A protein was not significantly increased. CYP1A2, CYP2B1 and CYP2B2 message were not increased in male pups; CYP1A1 message was significantly increased.	Only male pups evaluated. Message method was not quantitative. No toxicity or mortality data presented.
493	Junge et al. (2001) Rat (SD), male	<b>Duration:</b> 24 hours <b>Sample Size:</b> 3-8/group <b>Route:</b> intraperitoneal <b>Dose Levels:</b> 0, 40 mg/kg	Hepatic MPO activity, biliary GSSG efflux, liver protein carbonyls significantly increased.	Only one dose level evaluated. Only males evaluated. Small sample size. Potentially irrelevant route of exposure.
354	Junqueira et al. (1986) Rat (Wistar), male	<b>Duration:</b> 24 hours <b>Sample Size:</b> 6-18/group <b>Route:</b> intraperitoneal <b>Dose Levels:</b> 0, 20, 40, 60, 80 mg/kg	Progressive microscopic lipid accumulation was seen at all dose levels. Significant dose-dependent increases in total P450, superoxide production, and TBARS. SOD and catalase activities significantly decreased at dose levels at or above 40 mg/kg.	Difficult to evaluate dose-response for their microscopy data. Only males evaluated. Small sample size. Potentially irrelevant route of exposure.
355	Junqueira et al. (1988) Rat (Wistar), male	<b>Duration:</b> up to 24 hours <b>Sample Size:</b> 5-30/group <b>Route:</b> oral gavage <b>Dose Levels:</b> 0, 60 mg/kg	Total P450 and superoxide production were significantly increased 24 hours post-dose. TBARS production was significantly increased 4, 6, and 24 hours post-dose (time-dependent). Time-dependent increases in chemiluminescence observed. SOD and catalase activities significantly decreased 6 and 24 hours post-dose (time-dependent). Periportal necrosis and fatty changes seen 24 hours post-dose.	Non-specific nature of chemiluminescence assay; potential confounding by iron present in the reaction mixture. Only one dose level evaluated. Only males evaluated. Small sample size.
580	Junqueira et al. (1993) Rat (Wistar), male	<b>Duration:</b> up to 24 hours <b>Sample Size:</b> 5-14/time point <b>Route:</b> intraperitoneal <b>Dose Levels:</b> 0, 60 mg/kg	Total GSH was significantly decreased 4 hours post-dose; recovery to control levels observed 24 hours post-dose. GSH peroxidase, GST, and GGT not affected up to 24 hours post-dose. Turnover of radiolabeled reduced GSH was increased in lindane-treated rats over 3 hours, but total GSH was the same in control and treated rats.	Non-specific GSH assay for the time course. Only one dose level evaluated. Small sample size. GSH synthesis not evaluated. Potentially irrelevant route of exposure.
581	Junqueira et al. (1994) Rat (Wistar), male	<b>Duration:</b> 3 daily doses <b>Sample Size:</b> 4-11/group <b>Route:</b> intraperitoneal <b>Dose Levels:</b> 0, 20 mg/kg-day	Significant increases in hepatic total P450, P450 reductase, NADPH oxidase, microsomal superoxide production, TBARS formation (microsomes and homogenates), and chemiluminescence were observed. No hepatic "morphological alterations" were observed. Hepatic total GSH, GSH peroxidase, and GSSG reductase were increased and catalase was decreased - these changes were not statistically significant. SOD was not changed.	Only males evaluated. Small sample size. Only one dose-level evaluated. Non-specific GSH assay. Potentially irrelevant route of exposure.

Table 2. (continued)

Reference	Species/Sex	Study Design	Summary of Findings	Major Study Limitations
289 Junqueira et al. (1997)	Rat (Wistar), male	<b>Duration:</b> up to 7 days after a single dose <b>Sample Size:</b> 8/group <b>Route:</b> intraperitoneal <b>Dose Levels:</b> 0, 60 mg/kg	Catalase was significantly decreased 1 and 2 days after the lindane dose. SOD activity was significantly decreased 1 day after dosing. TBARS and total P450 were significantly increased 1, 2, and 3 days post-dose. Superoxide production was significantly increased 1 and 2 days post-dose. Periportal necrosis and fatty changes were observed 1 day after dosing. Mitochondrial abnormalities were seen 1 day after dosing (electron microscopy). All of these changes had returned to control values or had regressed by the end of the 7 day experimental period.	Only one dose level evaluated. Only males evaluated. Small sample size. Potentially irrelevant route of exposure. Treatment toxicity was not reported.
494 Klaunig et al. (1990)	Mouse (B6C3F1), male hepatocytes	<b>Duration:</b> up to 48 hours <b>Sample Size:</b> 3 dishes/ group <b>Route:</b> <i>in vitro</i> <b>Dose Levels:</b> 0-125 µM	Significant dose-dependent inhibition of cell dye coupling (i.e., intercellular communication) was observed. Maximal inhibition was achieved after 1 hour of treatment. Dye coupling returned to control values 2 hours after cessation of exposure. Co-treatment with a cAMP analogue prevented lindane-mediated cell communication inhibition. Co-treatment with a CYP inhibitor did not.	Efficacy of the P450 inhibitor was not evaluated. Cytotoxicity data was not shown; the authors claim the treatments were "sublethal." The maximal degree of inhibition was different for each experiment.
389 Kraus et al. (1981)	Rat (Wistar), male	<b>Duration:</b> 7 days (5 daily doses followed by a 2 day recovery) <b>Sample Size:</b> 5/group <b>Route:</b> intraperitoneal <b>Dose Levels:</b> 0, 10 mg/kg-day	GST activity was significantly increased when the substrate was a specific HCH metabolite but not when a general GST substrate was used.	Only males evaluated. Only one dose level evaluated. Small sample size. Potentially irrelevant route of exposure (intraperitoneal).
495 Kroll et al. (1999)	Rat (Wistar), male whole animal and Kupffer cell cultures	<b>Duration:</b> 2, 5, or 56 days ( <i>in vivo</i> ), 1-24 hours ( <i>in vitro</i> ) <b>Sample Size:</b> 3/group ( <i>in vivo</i> ); 3 experiments ( <i>in vitro</i> ) <b>Route:</b> dietary, ad libitum; <i>in vitro</i> <b>Dose Levels:</b> 0, 350 mg/kg ( <i>in vivo</i> ); 0, 10 µM ( <i>in vitro</i> )	Release of prostaglandins D2, E2, and F2alpha was increased after a 1 hour incubation of lindane with Kupffer cells. COX2 protein was increased after 1, 8, and 24 hours of lindane treatment. COX2 message, protein, and activity were increased in rats treated with lindane for 2, 5, or 56 days <i>in vivo</i> .	No statistical evaluation. Kupffer cell viability and treatment toxicity were not reported. Treatment toxicity during the <i>in vivo</i> experiments also not reported. No loading control on their Western blots. PCR conditions/primers not reported. Small sample size. Only males evaluated. Only one dose level/concentration evaluated. Small sample size.
644 Kumar and Dwivedi (1988)	Rat (Wistar), male	<b>Duration:</b> 4 days <b>Sample Size:</b> number of animals per group not reported; experiments were conducted in triplicate with 6 iterations per group for the EROD assay. <b>Route:</b> intraperitoneal <b>Dose Levels:</b> 0, 25 mg/kg bw/day	Cytochrome P450 b/e (i.e., CYP2B) was increased after 4 daily doses of lindane. Cytochrome P450 c/d (i.e., CYP1A) was not increased. Positive controls were included and showed an appropriate response. EROD (i.e., CYP1A) activity was increased ~4 fold in lindane-treated animals relative to control.	Microsomes were not used for the P450 enzyme activity assay. Only males evaluated. Loading control not included on Western blot. No statistical evaluation.
454 Leibold and Schwarz (1993)	Rat (Wistar), male hepatocytes	<b>Duration:</b> 5 hours <b>Sample Size:</b> experiments conducted two or three times <b>Route:</b> <i>in vitro</i> <b>Dose Levels:</b> 0-50 µM	Significant dose-dependent decrease in cell dye coupling (i.e., intercellular communication). Dye coupling returned to control value 2 hours after cessation of exposure. Co-treatment with vitamin E reduced lindane-mediated loss of dye coupling. Co-treatment with SOD, catalase, or aspirin did not attenuate lindane-mediated loss of dye coupling.	For some endpoints the number of iterations/cultures per experiment was not reported. Treatment cytotoxicity was not reported.
294 Loch-Carusio et al. (2004)	WB-F344 rat liver epithelial cell cultures (neo resistant line)	<b>Duration:</b> up to 4 hours <b>Sample Size:</b> 9-12 dishes/group <b>Route:</b> <i>in vitro</i> <b>Dose Levels:</b> 0, 50 µM (100 µM used in one experiment)	Intercellular dye transfer was significantly decreased 0.5 and 4 hours after treatment. Connexin43 protein (non-phospho and phospho forms) appears not to be affected by up to 4 hour lindane treatment, nor was it affected by a higher dose of lindane (100 µM). Increased punctate localization of S368-phospho-connexin43 after 0.5 hour lindane treatment; this was not seen after 4 hours of treatment. Suggests transient effect.	Unclear solvent concentration in their incubations. Questionable relevance of neo-resistant cell type. Questionable "quantitation" of Western blots (summing various bands).
300 Matsuura et al. (2005)	Rat (SPF) male and female	<b>Duration:</b> total exposure duration not reported. Approximate exposures were: 12 weeks (F0 males); approximately 19 weeks (F0 females); approximately 19 weeks (F1 males); approximately 26 weeks (F1 females) <b>Sample Size:</b> 10-24/sex/group (F0 adults); 10-22/sex/group (F1 adults); 4 males/group (P450 and UGT analysis) <b>Route:</b> dietary (F0 generation); gestation, lactation, and dietary (F1 generation) <b>Dose Levels:</b> 0, 10, 60, 300 ppm	Dose-dependent increases in F0 male and female absolute and relative liver weights were observed; many increases were significant. Absolute and relative liver weight were also increased in F1 adult males and females, with some significant values. Dose-dependent increases in macroscopic and microscopic (i.e., hypertrophy) liver abnormalities were observed in F0 and F1 animals, particularly in the higher dose groups. Significant increases in hepatic P450 content, MROD, EROD, BROD, testosterone hydroxylase, and UGT activity were observed, largely in the highest dose F0 and F1 males; some significant increases were noted in the mid-dose F1 males. P450 and UGT increases were dose-dependent.	Small sample size and only males evaluated for P450 assays. Exact duration of exposure not reported.
405 NTP (1977)	Mouse (B6C3F1), male/female; Rat (Osborne-Mendel), male/female	<b>Duration:</b> 90-91 weeks (mice); 108-110 weeks (rats); design was exposure plus recovery. <b>Sample Size:</b> 50/sex/group (treated); 10/sex/group (control) <b>Route:</b> dietary, ad libitum <b>Dose Levels:</b> 0, 80 or 160 ppm (mice); 236 or 472 ppm (male rats); 135 or 270 ppm (female rats)	Treatment-related liver or extra-hepatic tumors were not observed at a significant incidence in rats; liver tumor incidence was significantly increased in low-dose male mice but not in high-dose males or females. No body weight loss was observed in treated rats or mice, although increased clinical signs of toxicity were seen in treated animals toward the end of the study.	Small size of control group. Organ weights not reported. The effect of true lifetime exposure was not evaluated; rats and mice both had a recovery period following lindane treatment.
583 Oesch et al. (1982)	Mouse (CF1), male/female; Mouse (B6C3F1), male/female; Rat (Osborne-Mendel), male/female	<b>Duration:</b> 3 days or 3 months <b>Sample Size:</b> 3/group <b>Route:</b> dietary, ad libitum <b>Dose Levels:</b> 0, approx. 50, 125, and 300 ppm	Relative liver weight was significantly increased in the high-dose CF1 mice after 3 days (females only) and 3 months (males and females) and in O-M males and females after 3 months; relative liver weight also significantly increased in mid-dose O-M females after 3 months. Some significantly decreased liver weight values were seen but no trend was evident. B6C3F1 mice were highly susceptible to lindane-induced mortality.  Dose-dependent increases in ECOD activity were observed after 3 days and 3 months in all rats and mice. The magnitude of the effect was very small for the rats. Significantly increased EH activity in mid-dose CF1 males and high-dose males and females after 3 days and in high-dose females after 3 months; in low-dose B6C3F1 females after 3 days and 3 months and mid-dose males and females after 3 months; and in low-dose O-M male rats and high-dose males and females after 3 days and in low-dose females and mid-and high-dose male and female rats after 3 months.  Dose-dependent GST activity increases seen with some significant values, particularly in CF1 mice and rats. Increases in UGT activity seen in all animals; inconsistent dose-response pattern. No clear temporal trends for the enzyme activities.	Small sample size. Increased mortality, particularly in B6C3F1 mice.
498 Parmar et al. (2003)	Rat (Wistar), male	<b>Duration:</b> up to 21 days <b>Sample Size:</b> 10/group <b>Route:</b> oral gavage <b>Dose Levels:</b> 0, 2.5, 5, 10, 15 mg/kg bw/day	Significant dose- and time-dependent increases in total P450, CYP1A activity (EROD), CYP2B activity (PROD), and CYP2E activity (NMDA) seen after 5 daily doses of 0-15 mg/kg bw/day lindane and after 15 and 21 daily doses of 2.5 mg/kg bw/day lindane. Progressive increases in CYP2B and CYP2E proteins over time. Attenuation of lindane-mediated increase in CYP2B, CYP1A, and CYP2E activities observed with specific antibody and global P450 inhibitor.	Only males evaluated. Small sample size. Did not evaluate cellular or tissue-level sequelae of P450 increases.

Table 2. (continued)

Reference	Species/Sex	Study Design	Summary of Findings	Major Study Limitations
647 Pereira et al. (1982)	Rat (SD), male/female	<b>Duration:</b> 45 days plus 7 days recovery <b>Sample Size:</b> 8-19/group <b>Route:</b> dietary, ad libitum <b>Dose Levels:</b> 0, 76 ppm	Lindane-only treatment appears to result in low foci density. Foci density is increased in lindane-treated, partially hepatectomized or DEN-initiated females compared to treated PH or DEN-initiated males and compared to lindane-only. Foci density was highest in PH, DEN-initiated, lindane-treated females and, to a lesser degree, males. Background foci density was higher in females than in males.	Mortality and body weight data not reported. Only one dose level and one endpoint evaluated. Unclear statistical comparisons. Small sample size. No evaluation was conducted at the end of the 45 day exposure period; the effect of the 7 day recovery could not be evaluated.
544 Radosavljevic et al. (2008)	Rat (Wistar), male	<b>Duration:</b> 30 minutes <b>Sample Size:</b> 8-18/group <b>Route:</b> intraperitoneal <b>Dose Levels:</b> 0, 8 mg/kg	Liver enzyme increases (e.g., ALT, AST, ALP, LDH) observed.	Only one dose level evaluated. Only males evaluated. Small sample size. Potentially irrelevant route of exposure.
584 Ravinder et al. (1989)	Mouse (Swiss), male	<b>Duration:</b> 2 weeks <b>Sample Size:</b> 10-12/group <b>Route:</b> dietary (unknown if ad libitum) <b>Dose Levels:</b> 0, 200, 400 ppm	Significant increase in relative liver weight in treated mice. Significant dose-dependent increase in serum AST and ALT but not ALP. Significant dose-dependent increases in hepatic ALP, acid phosphatase, acid cathepsin; glucose 6 phosphate dehydrogenase, glucose-6-phosphatase, and aldolase significantly increased at 400 ppm; hepatic AST and LDH were significantly decreased (dose-dependent). Intestinal acid phosphatase and amylase were significantly increased; intestinal ALP significantly increased only at 400 ppm. Intestinal sucrase, lactase, and dipeptidase were significantly decreased.	Functional consequences of changes in enzyme activities not evaluated. Only males evaluated. Small sample size.
500 Ruch and Klaunig (1986)	Mouse (B6C3F1), male hepatocytes	<b>Duration:</b> 8 hours <b>Sample Size:</b> 3-6 cultures/concentration <b>Route:</b> <i>in vitro</i> <b>Dose Levels:</b> 0-5 µg/mL; 0-35 µ/mL for cytotoxicity	Transfer of tritiated uridine from donor to recipient hepatocytes was significantly reduced in the presence of lindane (dose-dependent). Treatments above 10 µg/mL were significantly cytotoxic (LDH release). RNA synthesis was not affected by lindane. Co-treatment with SOD, vitamin E, or DPPD (an antioxidant) attenuated the lindane-mediated decrease in intercellular radiolabel transfer.	Subjectivity and potential non-specificity of the intercellular communication assay.
465 Ruch et al. (1987)	Mouse (B6C3F1), male hepatocytes	<b>Duration:</b> up to 24 hours <b>Sample Size:</b> 3-4 replicates/time point <b>Route:</b> <i>in vitro</i> <b>Dose Levels:</b> 0, 0.1, 0.5, 1.0, 5.0 µg/mL	Hepatocytes were labeled with tritiated uridine. Transfer of radiolabel from donor to recipient hepatocytes was significantly reduced in the presence of lindane (dose-dependent). Lindane treatment was not toxic (LDH release).	Experiments not repeated. Subjectivity and potential non-specificity of the intercellular communication assay.
390 Schroter et al. (1987)	Rat (Wistar), female	<b>Duration:</b> 17 weeks (initiation); 15-20 weeks following initiation by NNM (promotion) <b>Sample Size:</b> 3-8/group (initiation) <b>Route:</b> oral gavage (initiation); dietary, ad libitum (promotion) <b>Dose Levels:</b> 0, 30 mg/kg (initiation); 0-30 mg/kg bw/day (promotion)	<b>Initiation Study:</b> No increase in GGT-positive hepatic foci in partially hepatectomized rats given a single oral dose of lindane followed by dietary phenobarbital. <b>Promotion Study:</b> Dose- and time-dependent increase in hepatic foci density and area after NNM-initiation followed by 15 or 20 weeks of lindane exposure. Foci area was significantly increased relative to control at mid- to high-doses. Liver mass and liver DNA were significantly increased in the 30 mg/kg-day group after 15 or 20 weeks; no temporal trend. Slight dose-dependent P450 induction was seen after 4, 15, and 20 weeks at 30 mg/kg-day; no temporal trend. Relative liver weight was not strongly correlated with foci area.	Small sample size. Only females evaluated. Not all data were statistically evaluated. Mortality not reported. Sample size for some endpoints not reported. Only liver evaluated. The effect of gamma-HCH only, without initiation, on foci formation not evaluated.
319 Sumida et al. (2007)	Rat (Fischer), male	<b>Duration:</b> up to 28 days <b>Sample Size:</b> 4-20/group/time point <b>Route:</b> oral gavage <b>Dose Levels:</b> 0, 1, 10 mg/kg-day	After 28 days, 10 mg/kg/day animals weighed significantly less than the control. No consistent increases in relative liver weight were observed. No microscopic liver abnormalities observed. Changes in gene expression were generally moderate (less than 2-fold) with some instances of 3 or 4 fold changes. Potentially meaningful changes may have occurred in the areas of fatty acid metabolism, retinoid X receptor, early growth response, cell growth, transport, and proteolysis.	Potential changes in expression were not confirmed with quantitative PCR, so their meaning is uncertain. Treatment mortality not reported. Only males evaluated. Small sample size.
395 Thorpe and Walker (1973)	Mouse (CF1), male/female	<b>Duration:</b> 2 years <b>Sample Size:</b> 30/sex (treated); 45/sex (control) <b>Route:</b> dietary (unknown if ad libitum) <b>Dose Levels:</b> 0, 400 ppm	Increased mortality overall during the study. Liver enlargement noted after 50 weeks in treated males and females. Livers had nodules and necrotic areas. High incidence of hepatic and extra-hepatic tumors in treated mice dying early; extra-hepatic tumor incidence was reduced compared to control mice. Higher incidence of liver tumors and lung metastases in males vs. females.	Only one dose level evaluated. Increased mortality. High incidence of spontaneous lung, liver, and lymphoid tumors in untreated control animals.
656 USEPA (1983)	Rat (Wistar), male/female	<b>Duration:</b> 12 weeks or 12 weeks plus 6 weeks recovery <b>Sample Size:</b> 20/sex/group <b>Route:</b> dietary, ad libitum <b>Dose Levels:</b> 0, 0.2, 0.8, 4, 20, 100 ppm	Significant dose-dependent increase of total P450 levels was seen in the females but not in the males. P450 increases regressed after the recovery period. Slight increases in relative liver and kidney weight were seen at 20 and 100 ppm (males and females). Microscopic changes in the livers (hypertrophy; Kupffer cell proliferation) of treated males and females were seen. Hypertrophy incidence was dose-dependent. No microscopic liver changes were seen after the recovery period.	Moderate to significant weight loss in treated males with recovery period. P450 levels evaluated in liver homogenates instead of microsomes.
653 USEPA (1989)	Rat (CrI:(WI)BR), male/female	<b>Duration:</b> 6 weeks, 13 weeks, or 13 weeks plus 6 weeks recovery <b>Sample Size:</b> 13/sex/group (6 week and recovery); 23/sex/group (13 week) <b>Route:</b> dermal <b>Dose Levels:</b> 0, 10, 60, 400 mg/kg-day	Relative liver weights were significantly increased in the 60 mg/kg and 400 mg/kg males and 400 mg/kg females after 6 and 13 weeks and in the 60 mg/kg females after 13 weeks. These increases in relative liver weight regressed after the 6 week recovery period, although absolute liver weight was still increased in the 400 mg/kg males. Dose- and time-dependent increase in incidence of centrilobular hypertrophy (10/18 and 8/17 60 mg/kg males and females; 20/20 and 13/13 400 mg/kg males and females at termination). No rats had hypertrophy after the recovery period. Focal necrosis was observed in male rats after the recovery, but not during treatment.	Increased mortality among high-dose females but not males. Small sample size.
654 USEPA (1992b)	Rat (Wistar), male/female	<b>Duration:</b> 104 weeks with interim sacrifices and recovery <b>Sample Size:</b> up to 55/sex/group <b>Route:</b> dietary, ad libitum <b>Dose Levels:</b> 0, 1, 10, 100, 400 ppm	Significant dose-dependent increase in male and female relative liver weight at 30 days, 26 weeks, 52 weeks, and 104 weeks (400 ppm); significantly increased in the 100 ppm males and females at 104 weeks. No clear temporal trend. No increases in liver weight were seen in the recovery animals. Periportal hypertrophy observed in males and females with dose-dependent increase in incidence; no clear temporal trend. No hypertrophy observed in the recovery animals. One liver tumor seen in a high-dose female.	Cumulative percent mortality approximately 15-20% in the 400 ppm males and females and the 100 ppm males; significant for 400 ppm females. No significant change in body weight.
274 USEPA (2001)	Mouse (CD-1), male/female	<b>Duration:</b> 78 weeks <b>Sample Size:</b> 50/sex/dose <b>Route:</b> dietary (unknown if ad libitum) <b>Dose Levels:</b> 0, 10, 40, 160 ppm	Liver adenomas and carcinomas were observed in control and treated male mice at similar, non-dose-dependent incidence. One high-dose female had a liver adenoma; no carcinomas were observed in the female mice. Dose-dependent increase in incidence of hepatocellular hypertrophy and foci were seen in males only; significant at 160 ppm. No microscopic liver changes seen in female mice.  Dose-dependent increases in lung adenomas were seen in treated females; significant at 160 ppm. Carcinoma incidence was not significantly increased. Adenoma incidence was high in control males and was not further increased with treatment.  Mortality in control and treated animals was acceptable (less than ~15%).	These data were evaluated in summary form only. Body weight, organ weight, and other general toxicity data not provided.

Table 2. (continued)

Reference	Species/Sex	Study Design	Summary of Findings	Major Study Limitations
503 Valencia et al. (2004)	Rat (SD), female	<b>Duration:</b> 18 hours <b>Sample Size:</b> 4-14 <b>Route:</b> intraperitoneal <b>Dose Levels:</b> 0, 50 mg/kg	Liver NFkB DNA binding, liver protein carbonyls, sinusoidal LDH efflux, and serum TNFalpha level significantly increased; liver total glutathione significantly decreased.	Only one dose level evaluated. Only females evaluated. Small sample size. Potentially irrelevant route of exposure. The GSH assay did not differentiate between reduced and oxidized forms.
505 Videla et al. (1991)	Rat (Wistar), male	<b>Duration:</b> up to 24 hours <b>Sample Size:</b> 37 total rats; 3-6 evaluations per time point <b>Route:</b> intraperitoneal <b>Dose Levels:</b> 0, 25, 40, 60 mg/kg	Hepatic LDH efflux and oxygen consumption increased (dose-dependent) 24 hours post-dose. Hepatic oxygen consumption increased over time, which could be partially mitigated by co-treatment with an antioxidant. Total GSH content decreased and then recovered over 24 hours. Total glutathione efflux decreased and then recovered over 24 hours. LDH efflux increased 6 hours post-dose, with further increase 24 hours post-dose. These changes were generally statistically significant.	Non-specific GSH assay. Small sample size. Only males evaluated. Functional consequences of the GSH/oxygen changes were not evaluated. Potentially irrelevant route of exposure.
504 Videla et al. (1997)	Rat (SD), male	<b>Duration:</b> 24 hours <b>Sample Size:</b> 4-6/group <b>Route:</b> intraperitoneal <b>Dose Levels:</b> 0, 5, 10, 20, 40, 60 mg/kg	Significant dose-dependent increases in hepatic oxygen consumption were seen. Hepatic carbon intake and carbon-induced oxygen consumption were significantly increased up to 20 mg/kg lindane. These increases were significantly attenuated by gadolinium chloride pre-treatment (inhibitor of Kupffer cells). Lindane- and carbon-mediated increases in sinusoidal LDH efflux were somewhat attenuated by gadolinium chloride pre-treatment.	Biochemical/functional consequences of lindane-activated Kupffer cells not evaluated. Small sample size; only males evaluated. Potentially irrelevant route of exposure.
400 Videla et al. (2000)	Rat (Wistar), male	<b>Duration:</b> 4 hours <b>Sample Size:</b> 6/group <b>Route:</b> intraperitoneal <b>Dose Levels:</b> 0, 60 mg/kg	Phenobarbital pretreatment significantly reduces tissue lindane concentration. Liver TBARS formation and chemiluminescence significantly increased; liver GSH content, biliary GSH efflux significantly decreased. These decreases attenuated in phenobarbital-pretreated animals; TBARS and chemiluminescence remained elevated. Total P450, P450 reductase, and superoxide not increased by lindane. SOD, catalase, GSH peroxidase, GSSG reductase, glucose-6-phosphate dehydrogenase, and GST not changed by lindane. Lindane caused periportal necrosis; attenuated in phenobarbital-pretreated animals.	Only one dose level evaluated. Only males evaluated. Small sample size. Potentially irrelevant route of exposure.
473 Videla et al. (2004)	Rat (SD), male	<b>Duration:</b> up to 22 hours <b>Sample Size:</b> 3-11/group <b>Route:</b> intraperitoneal <b>Dose Levels:</b> 0, 50 mg/kg	NFkB DNA binding significantly increased over time; liver oxygen consumption, LDH efflux, and protein oxidation significantly increased; liver total GSH significantly decreased. Liver TNFalpha and IL-1alpha expression and serum levels significantly increased. Each of these changes significantly attenuated by alpha-tocopherol or gadolinium chloride pre-treatment.	Only one dose level evaluated. Only males evaluated. Small sample size. Potentially irrelevant route of exposure.
471 Weisse and Herbst (1977)	Mouse (NMRI), male/female	<b>Duration:</b> 80 weeks <b>Sample Size:</b> 50/sex (treated); 100/sex (control) <b>Route:</b> dietary (unknown if ad libitum) <b>Dose Levels:</b> 0, 12.5, 25, 50 ppm	**These are follow-up tumor and electron microscopy data from Ref#442. Control and treated mice had leukemia and lung and liver tumors. For each of these tumor types, the incidence in treated mice was similar to or less than the incidence in control mice. No treatment-dependent changes in livers examined by electron microscopy were noted.	Body weights/food consumption not evaluated for all animals. Unclear mortality data. The doses evaluated were too low to elicit a toxic effect.
498 Wolff et al. (1987)	Mouse (black, yellow, pseudoagouti), female	<b>Duration:</b> 6, 12, 18, or 24 months <b>Sample Size:</b> 13-96/group <b>Route:</b> dietary, ad libitum <b>Dose Levels:</b> 0, 160 ppm	Liver weights of treated yellow and pseudoagouti mice were significantly greater than untreated controls at all time points; slight temporal trend. Liver weights of treated black mice were significantly increased relative to untreated black mice at 24 months; slight temporal trend. Liver adenoma incidence was increased in the 18 and 24 month treated yellow mice and in the 24 month pseudoagouti mice but not in treated black mice (all relative to untreated control of the same phenotype). Liver carcinoma incidence was slightly increased in the 24 month treated yellow mice vs. untreated yellow mice. Temporal trend evident for liver tumor formation in yellow mice. Clara cell hyperplasia was increased in treated yellow, pseudoagouti, and black mice at all time points relative to the appropriate control. No clear temporal trend. Lung tumors were increased in 18 and 24 month treated yellow mice, in 24 month treated pseudoagouti mice, and in 18 month treated black mice. Temporal trend evident for yellow and pseudoagouti mice. Hepatocellular adenoma and carcinoma and lung tumor incidence decreased in yellow mice after cessation of exposure. Clara cell hyperplasia incidence decreased in treated yellow and black mice after cessation of exposure.	Only females evaluated. Only one dose level evaluated. Uncertain relevance of the transgenic mouse model.
662 Yang and DiSilvestro (1992)	Rat (Sprague Dawley) male	<b>Duration:</b> up to 24 hours <b>Sample Size:</b> 3 or 5/group <b>Route:</b> intraperitoneal <b>Dose Levels:</b> 0, 60 mg/kg	Hepatic TBARS were significantly increased 16 and 24 hours post-dose. Cu,ZnSOD activity was not different from control over 24 hours. This pattern was independent of dietary copper levels.	Only male rats evaluated. Only one concentration evaluated. No cytotoxicity data presented.
275 Zucchini-Pascal et al. (2009)	Rat (SD), male hepatocytes	<b>Duration:</b> up to 72 hours <b>Sample Size:</b> 3 cultures/group <b>Route:</b> in vitro <b>Dose Levels:</b> 0, 10, 25, 50, 75 µM	Lindane induced formation of acidic vacuoles (i.e., lysosomes). Markers of autophagy (LC3 and Beclin 1 proteins) were significantly increased following lindane treatment. This represents delayed/inhibited maturation of the autophagy process. Lindane induces anti-apoptotic Bcl xL protein and inhibits cytochrome c release from the mitochondria in a dose- and time-dependent manner. Caspase 9 and 3 activities are decreased in a dose- and time-dependent manner. Lindane induces necrosis rather than apoptosis.	No major limitations noted.

Table 2. (continued)

Notes:	ALP	= alkaline phosphatase	MPO	= myeloperoxidase
	ALT	= alanine aminotransferase	MROD	= 7-Methoxyresorufin O-demethylation assay
	AST	= aspartate aminotransferase	MTD	= maximum tolerated dose
	BROD	= benzyloxyresorufin O-dealkylation assay	MTT	= 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
	bw/day	= body weight per day	NADPH	= nicotinamide adenine dinucleotide phosphate
	cAMP	= cyclic adenosine monophosphate	NFKB	= nuclear factor kappa-light-chain-enhancer of activated B cells
	COX	= cyclooxygenase	NMDA	= N-methyl D-aspartate
	CYP	= cytochrome P450	NNM	= N-nitrosomorpholine
	DEN	= diethylnitrosamine	NO	= nitric oxide
	DNA	= deoxyribonucleic acid	O-M	= Osborne-Mendel
	DPPD	= N,N'-diphenyl-1,4-phenylenediamine	P450	= cytochrome P450
	ECOD	= ethoxycoumarin-O-deethylase	PCR	= polymerase chain reaction
	EH	= epoxide hydrolase	PH	= partial hepatectomy
	EROD	= ethoxyresorufin-O-deethylase	ppm	= part per million
	GGT	= gamma-glutamyl transpeptidase	PROD	= pentoxyresorufin-O-dealkylase
	GSH	= glutathione	RNA	= ribonucleic acid
	GSSG	= glutathione disulfide	ROS	= reactive oxygen species
	GST	= glutathione-S-transferase	SD	= Sprague Dawley
	HCH	= hexachlorocyclohexane (gamma isomer; also called lindane)	SOD	= superoxide dismutase (Mn - manganese; Cu,Zn - copper, zinc)
	IL	= interleukin	TBARS	= thiobarbituric acid reactive substance
	KCN	= potassium cyanide	TNF	= tumor necrosis factor
	LDH	= lactate dehydrogenase	UGT	= UDP-glucuronosyl transferase
	mg/kg	= milligram per kilogram	µg/ml	= microgram per milliliter
	mg/kg-day	= milligram per kilogram per day	µM	= micromolar
	mg/L	= milligram per liter		

Table 3. Summary of Mutagenicity and Genotoxicity Assays for Gamma-HCH.

Reference	Test System		Assay/Test	Endpoint	Treatment	Result	Comments
	In Vitro / In Vivo	Species/Strain/ Cell Type					
<b>Mutation</b>							
768	Gopaldaswamy and Nair (1992)	<i>In vitro</i>	<i>Salmonella typhimurium</i> TA98	Ames assay	Mutation	50 and 100 µg/plate w/ and w/out activation	Positive
499	Pool-Zobel et al. (1993)	<i>In vitro</i>	CHO cells	HPRT assay	Point mutation	0.063-300 µg/ml w/ and w/out activation	Negative
<b>DNA Binding</b>							
768	Gopaldaswamy and Nair (1992)	<i>In vivo</i>	Wistar rats liver	--	DNA binding	25 mg/kg w/ and w/out activation	Weakly positive Lindanes metabolite hexachlorobenzene showed lower binding.
		<i>In vitro</i>	Calf thymus DNA	--	DNA binding	NA w/ and w/out activation	Weakly positive Lindanes metabolite hexachlorobenzene showed lower binding.
422	Iverson et al. (1984)	<i>In vitro</i>	Calf thymus DNA	--	DNA binding	1 µm	Weakly positive Low levels of DNA binding only.
		<i>In vivo</i>	Mouse liver	--	DNA binding	25 mg/kg	Weakly positive Low levels of DNA binding only.
408	Sagelsdorff et al. (1983)	<i>In vivo</i>	NMRI, CF1, and C6B3F1 mouse liver	HPLC analysis of nucleosides	DNA binding	8.7-23 mg/kg	Weakly positive Authors characterize results as "minute DNA binding", stating that the level of binding is more than three orders of magnitude lower than would be expected if the mechanism of tumor induction was genotoxicity mediated by DNA binding.
<b>DNA Damage, Fragmentation, and Repair</b>							
789	Ahmed et al. (1977)	<i>In vitro</i>	Human VA-4 cells	UDS by BUdR	Unscheduled DNA Synthesis (indicative of excision repair)	1 and 1,000 µM w/ and w/out activation	Negative
790	Anguiano et al. (2007)	<i>In vivo</i>	hemocytes from <i>Crassostrea gigas</i> (Pacific Oysters)	Comet assay	DNA fragmentation	0-5mg/L	Positive
770	Jenssen and Ramel (1980)	<i>In vivo</i>	CBA male mice	Micronucleus assay	Micronuclei induction	75 mg/kg	Negative
290	Kalantzi et al. (2004)	<i>In vitro</i>	Human MCF-7 breast carcinoma cells	Micronucleus assay	Micronuclei induction	10 <sup>-12</sup> -10 <sup>-10</sup> M	Positive
			Human PC-3 prostate carcinoma cells	Micronucleus assay	Micronuclei induction	10 <sup>-12</sup> -10 <sup>-10</sup> M	Positive
			Human MCF-7 breast carcinoma cells	Comet assay	DNA fragmentation	10 <sup>-4</sup> M	Positive Authors note that at lower concentrations no comet-forming effects were observed, however the specific treatment dose or data results are not provided.
			Human PC-3 prostate carcinoma cells	Comet assay	DNA fragmentation	10 <sup>-4</sup> M	Positive Authors note that at lower concentrations no comet-forming effects were observed, however the specific treatment dose or data results are not provided.
792	Martin et al. (1999)	<i>In vitro</i>	Human MCL-5 cells	Comet assay	DNA fragmentation	0.16-1.56 mM w/ and w/out inhibitors of DNA repair	Positive In cells treated without DNA repair inhibitors only the highest tested dose showed a significant increase in DNA fragmentation. Effect was enhanced with co-exposure to inhibitors of DNA repair.
499	Pool-Zobel et al. (1993)	<i>In vitro</i>	Primary rat hepatocytes	--	DNA damage (single strand breaks)	0.0625-1.0 µmol/tube	Negative
			Primary cells of the gastric mucosa from Sprague-Dawley rats	Comet assay	DNA fragmentation	0.0625-1.0 µmol/tube	Positive
			Primary cells of the nasal mucosa from Sprague-Dawley rats	Comet assay	DNA fragmentation	0.125-1.0 µmol/ml	Positive

Table 3. (continued)

Reference	Test System		Assay/Test	Endpoint	Treatment	Result	Comments
	<i>In Vitro</i> / <i>In Vivo</i>	Species/Strain/ Cell Type					
		Human peripheral lymphocytes	Comet assay	DNA fragmentation	0.125-1.0 µmol/ml	Positive	
	<i>In vivo</i>	Sprague-Dawley rat liver cells	--	DNA damage (single strand breaks)	30 and 60 mg/kg	Negative	
		Gastric mucosa cells from Sprague-Dawley rats	Comet assay	DNA fragmentation	60 mg/kg	Positive	
		Nasal mucosa cells from Sprague-Dawley rats	Comet assay	DNA fragmentation	100 µg/kg	Positive	
		Bone marrow cells from NMRI mice	Micronucleus assay	Micronuclei induction	35-70 mg/kg	Negative	
		Bone marrow cells from Chinese hamsters	Micronucleus assay	Micronuclei induction	60-120 mg/kg	Negative	
		Bone marrow cells from Sprague-Dawley rats	Micronucleus assay	Micronuclei induction	15-60 mg/kg	Negative	
781	Rocchi et al. (1980)	<i>In vitro</i>	Human lymphocytes	UDS	Unscheduled DNA Synthesis (indicative of excision repair)	500 µg/ml	Negative
426	Sasaki et al. (1997)	<i>In vivo</i>	CD-1 mice (liver, kidney, lung, spleen, bone marrow)	Comet assay	DNA fragmentation	80 mg/kg	Negative
<b>Chromosomal Alterations</b>							
499	Pool-Zobel et al. (1993)	<i>In vitro</i>	CHO cells	SCE	Exchanges of DNA between two sister chromatids of a duplicating chromosome	0.063-300 µg/ml with and without activation	Negative
		<i>In vivo</i>	Chinese hamster bone marrow cells	SCE	Exchanges of DNA between two sister chromatids of a duplicating chromosome	120 mg/kg	Negative

Notes: BUdR = photolysis of 5-bromo-2'-deoxyuridine, technique used to provide a rapid and quantitative estimate of DNA repair.  
 CHO = Chinese hamster ovary  
 DNA = deoxyribonucleic acid  
 HCH = hexachlorocyclohexane  
 HPLC = high performance liquid chromatography  
 M = molar mass  
 HPRT = hypoxanthine-guanine phosphoribosyltransferase  
 mg/kg = milligram per kilogram  
 mg/L = milligram per liter  
 mM = millimole  
 NA = not available, dose not specified or unclear  
 SCE = sister chromatid exchange  
 UDS = unscheduled DNA synthesis  
 µg/kg = microgram per kilogram  
 µg/ml = microgram per milliliter  
 µg/plate = microgram per plate  
 µM = micromole  
 µmol/ml = micromole per milliliter  
 µmol/tube = micromole per tube  
 -- = specific test name not provided. Only endpoint is provided.

Table 4. Inclusion of Studies Evaluating Gamma-HCH Toxicity, *Non-Cancer Endpoints and Sensitive Subpopulations*, by Endpoint.

Reference <sup>a</sup>	Included in Endpoint Sensitivity Evaluation	Reason for Exclusion <sup>b</sup>
<b>Cardiovascular Endpoints</b>		
735 Anand et al. (1995)	Yes	
279 Ananya et al. (2005)	Yes	NA
<b>Hematological Endpoints</b>		
657 Grabarczyk et al. (1990)	No	Endpoint not evaluated
581 Junqueira et al. (1994)	No	Endpoint not evaluated
653 USEPA (1989)	No	Endpoint not evaluated
<b>Hepatic Endpoints</b>		
487 Anilakumar et al. (2009)	No	* MOA endpoint/ <i>in vitro</i>
651 Azzalis et al. (1995)	No	* Acute exposure/High dose
652 Bainy et al. (1993)	No	* MOA endpoint/ <i>in vitro</i>
576 Barros et al. (1988)	No	* Acute exposure/High dose
381 Barros et al. (1991)	Yes	* NA
635 Busser and Lutz (1987)	No	* Reliability Rank
488 Cornejo et al. (2001)	No	* Acute exposure/High dose
489 Descampiaux et al. (1996)	No	* MOA endpoint/ <i>in vitro</i>
490 Dubois et al. (1996)	No	* MOA endpoint/ <i>in vitro</i>
491 Fernandez et al. (2003)	No	* Acute exposure/High dose
382 Fitzhugh et al. (1950)	Yes	* NA
639 Fitzloff et al. (1982)	No	* MOA endpoint/ <i>in vitro</i>
502 Giavarotti et al. (1998)	No	* Acute exposure/High dose
641 Goel et al. (1988)	No	* Acute exposure/High dose
383 Goto et al. (1972)	No	* Acute exposure/High dose
657 Grabarczyk et al. (1990)	Yes	* NA
439 Guan et al. (1995)	No	* MOA endpoint/ <i>in vitro</i>
492 Guan and Ruch (1996)	No	* MOA endpoint/ <i>in vitro</i>
385 Hanada et al. (1973)	No	* Acute exposure/High dose
442 Herbst et al. (1975)	Yes	* NA
363 Ito et al. (1973a)	No	* Acute exposure/High dose
364 Ito et al. (1973b)	No	* Acute exposure/High dose
386 Ito et al. (1975)	No	* Acute exposure/High dose
286 Johri et al. (2007)	Yes	* NA
287 Johri et al. (2008a)	No	* MOA endpoint/ <i>in vitro</i>
288 Johri et al. (2008b)	No	* MOA endpoint/ <i>in vitro</i>
493 Junge et al. (2001)	No	* Acute exposure/High dose & MOA endpoint/ <i>in vitro</i>
354 Junqueira et al. (1986)	No	* Acute exposure/High dose & MOA endpoint/ <i>in vitro</i>
355 Junqueira et al. (1988)	No	* Acute exposure/High dose & MOA endpoint/ <i>in vitro</i>
580 Junqueira et al. (1993)	No	* Acute exposure/High dose & MOA endpoint/ <i>in vitro</i>
581 Junqueira et al. (1994)	No	* Acute exposure/High dose & MOA endpoint/ <i>in vitro</i>
289 Junqueira et al. (1997)	No	* Acute exposure/High dose & MOA endpoint/ <i>in vitro</i>
494 Klaunig et al. (1990)	No	* Acute exposure/High dose & MOA endpoint/ <i>in vitro</i>
389 Kraus et al. (1981)	No	* Acute exposure/High dose & MOA endpoint/ <i>in vitro</i>
495 Kroll et al. (1999)	No	* MOA endpoint/ <i>in vitro</i>
644 Kumar and Dwivedi (1998)	No	* Acute exposure/High dose & MOA endpoint/ <i>in vitro</i>
454 Leibold and Schwarz (1993)	No	* Acute exposure/High dose & MOA endpoint/ <i>in vitro</i>
294 Loch-Caruso et al. (2004)	No	* Acute exposure/High dose & MOA endpoint/ <i>in vitro</i>
300 Matsuura et al. (2005)	Yes	* NA
405 NTP (1977)	No	* Acute exposure/High dose
583 Oesch et al. (1982)	No	* Acute exposure/High dose
498 Parmar et al. (2003)	Yes	* NA
647 Pereira et al. (1982)	Yes	* NA
544 Radosavljevic et al. (2008)	No	* Acute exposure/High dose & MOA endpoint/ <i>in vitro</i>
584 Ravinder et al. (1989)	No	* Acute exposure/High dose
500 Ruch and Klaunig (1986)	No	* Acute exposure/High dose & MOA endpoint/ <i>in vitro</i>
465 Ruch et al. (1987)	No	* Acute exposure/High dose & MOA endpoint/ <i>in vitro</i>
390 Schroter et al. (1987)	Yes	* NA
319 Sumida et al. (2007)	Yes	* NA
395 Thorpe and Walker (1973)	Yes	* NA
656 USEPA (1983)	Yes	* NA
653 USEPA (1989)	Yes	* NA
654 USEPA (1992b)	Yes	* NA
274 USEPA (2001)	Yes	* NA
570 Valencia et al. (2004)	No	* Acute exposure/High dose & MOA endpoint/ <i>in vitro</i>
505 Videla et al. (1991)	No	* Acute exposure/High dose & MOA endpoint/ <i>in vitro</i>
504 Videla et al. (1997)	No	* Acute exposure/High dose & MOA endpoint/ <i>in vitro</i>
503 Videla et al. (2000)	No	* Acute exposure/High dose & MOA endpoint/ <i>in vitro</i>
400 Videla et al. (2004)	No	* Acute exposure/High dose & MOA endpoint/ <i>in vitro</i>
471 Weisse and Herbst (1997)	Yes	* NA

Table 4. (continued)

	Reference <sup>a</sup>	Included in Endpoint Sensitivity Evaluation	Reason for Exclusion <sup>b</sup>	
473	Wolff et al. (1987)	No	*	Acute exposure/High dose
662	Yang and DiSilvestro (1992)	No	*	Acute exposure/High dose
275	Zucchini-Pascal et al. (2009)	No	*	Acute exposure/High dose & MOA endpoint/ <i>in vitro</i>
<b>Immunological Endpoints</b>				
510	Banerjee et al. (1996)	Yes		NA
436	Blair et al. (1998)	Yes	*	NA
522	Daniel et al. (2001)	No		Reliability Rank
626	Das et al. (1990)	No		Reliability Rank
637	Desi et al. (1978)	No		Reliability Rank
282	Devos et al. (2004)	No		Acute exposure/High dose & MOA endpoint/ <i>in vitro</i>
638	Dewan et al. (1980)	Yes		NA
640	Gerhard et al. (1991)	No		Reliability Rank
529	Karmaus et al. (2005)	No		Reliability Rank
508	Kato et al. (2004)	No		Reliability Rank
627	Koner et al. (1998)	Yes		NA
533	Lee et al. (2004)	Yes	*	NA
300	Matsuura et al. (2005)	Yes		NA
457	McDuffie et al. (2001)	Yes	*	NA
536	Mediratta et al. (2008)	Yes		NA
366	Meera et al. (1992)	Yes		NA
628	Meera et al. (1993)	Yes		NA
303	Olgun and Misra (2006)	No		Acute exposure/High dose & MOA endpoint/ <i>in vitro</i>
543	Purdue et al. (2006)	Yes	*	NA
329	Rafnsson (2006)	No	*	Reliability Rank
357	Saha and Banerjee (1993)	Yes		NA
660	Sweet et al. (2006)	No		MOA endpoint/ <i>in vitro</i>
654	USEPA (1992b)	Yes		NA
323	Wang et al. (2006)	No		Multiple isomer treatment
<b>Mutagenicity/Genotoxicity Endpoints</b>				
789	Ahmed et al. (1977)	No	*	MOA endpoint/ <i>in vitro</i>
790	Anguiano et al. (2007)	No	*	MOA endpoint/ <i>in vitro</i>
768	Gopalaswamy and Nair (1992)	No	*	MOA endpoint/ <i>in vitro</i>
422	Iverson et al. (1984)	No	*	MOA endpoint/ <i>in vitro</i>
770	Jenssen and Ramel (1980)	No	*	MOA endpoint/ <i>in vitro</i>
290	Kalantzi et al. (2004)	No	*	MOA endpoint/ <i>in vitro</i>
792	Martin et al. (1999)	No	*	MOA endpoint/ <i>in vitro</i>
499	Pool-Zobel et al. (1993)	No	*	MOA endpoint/ <i>in vitro</i>
781	Rocchi et al. (1980)	No	*	MOA endpoint/ <i>in vitro</i>
408	Sagelsdorff et al. (1983)	No	*	MOA endpoint/ <i>in vitro</i>
426	Sasaki et al. (1997)	No	*	MOA endpoint/ <i>in vitro</i>
<b>Neurological Endpoints</b>				
277	Agrawal et al. (1995)	Yes		NA
634	Anand et al. (1998)	Yes		NA
351	Aoki et al. (2008)	No		MOA endpoint/ <i>in vitro</i>
573	Arisi et al. (1994)	No		Acute exposure/High dose
516	Bist and Bhatt (2009)	No		Acute exposure/High dose & MOA endpoint/ <i>in vitro</i>
664	Corrigan et al. (2000)	Yes		NA
523	Firestone et al. (2005)	Yes		NA
666	Fleming et al. (1994)	Yes		NA
524	Gupta et al. (1999)	Yes		NA
525	Hancock et al. (2008)	Yes		NA
286	Johri et al. (2007)	Yes		NA
287	Johri et al. (2008a)	No		MOA endpoint/ <i>in vitro</i>
288	Johri et al. (2008b)	No		MOA endpoint/ <i>in vitro</i>
300	Matsuura et al. (2005)	Yes		NA
539	Nyitrai et al. (2002)	No		Acute exposure/High dose
498	Parmar et al. (2003)	Yes		NA
545	Richardson et al. (2009)	Yes		NA
547	Rivera et al. (1991)	No		Acute exposure/High dose & MOA endpoint/ <i>in vitro</i>
629	Rivera et al. (1998)	Yes		NA
312	Sahaya et al. (2007)	No		Acute exposure/High dose
630	Sahoo et al. (2000)	No		MOA endpoint/ <i>in vitro</i>
482	Samanic et al. (2008)	Yes		NA
358	Srivastava and Shivanadappa (2005)	No		Acute exposure/High dose & MOA endpoint/ <i>in vitro</i>
649	Tilson et al. (1987)	No		Acute exposure/High dose
321	Toscano et al. (2008)	No		Reliability Rank
<b>Renal Endpoints</b>				
382	Fitzhugh et al. (1950)	Yes		NA
657	Grabarczyk et al. (1990)	Yes		NA
300	Matsuura et al. (2005)	Yes		NA
653	USEPA (1989)	Yes		NA

Table 4. (continued)

	Reference <sup>a</sup>	Included in Endpoint Sensitivity Evaluation	Reason for Exclusion <sup>b</sup>
654	USEPA (1992b)	Yes	NA
<b>Reproductive/Developmental Endpoints</b>			
514	Beard and Rawlings (1998)	Yes	NA
511	Beard and Rawlings (1999)	Yes	NA
512	Beard et al. (1999a)	Yes	NA
513	Beard et al. (1999b)	Yes	NA
625	Cooper et al. (1989)	Yes	NA
519	Dalsenter et al. (1996)	Yes	NA
520	Dalsenter et al. (1997a)	No	Acute exposure/High dose
521	Dalsenter et al. (1997b)	No	Acute exposure/High dose
369	Di Consiglio et al. (2009)	No	Acute exposure/High dose
640	Gerhard et al. (1991)	No	Reliability rank
285	Hassoun and Stohs (1996)	No	Acute exposure/High dose
642	Hosie et al. (2000)	Yes	NA
531	La Sala et al. (2009)	No	Acute exposure/High dose
298	Maranghi et al. (2007)	No	Acute exposure/High dose
456	Mathur et al. (2002)	No	* Reliability rank
300	Matsuura et al. (2005)	Yes	NA
458	Mills and Yang (2003)	Yes	* NA
459	Mills and Yang (2006)	Yes	* NA
302	Mograbi et al. (2003)	No	Acute exposure/High dose & MOA endpoint/ <i>in vitro</i>
460	Muir et al. (2004)	No	* Reliability rank
646	Palmer et al. (1978)	Yes	NA
542	Pathak et al. (2009)	Yes	NA
305	Pflieger-Bruss et al. (2006)	Yes	NA
587	Samanta and Chainy (2002)	No	Reliability rank
314	Saradha et al. (2008a)	Yes	NA
313	Saradha et al. (2008b)	Yes	NA
551	Shivanandappa and Krishnakumari (1983)	No	Multiple isomer treatment
565	Siddiqui et al. (2003)	Yes	NA
597	Silvestroni and Palleschi (1999)	Yes	NA
596	Silvestroni et al. (1997)	Yes	NA
566	Sircar and Lahiri (1989)	Yes	NA
650	Sujatha et al. (2001)	Yes	NA
568	Traina et al. (2003)	No	Acute exposure/High dose
<b>Respiratory Endpoints</b>			
274	USEPA (2001)	No	Endpoint not evaluated

Notes: HCH = hexachlorocyclohexane

MOA = mode of action

NA = not applicable

\* = study determined useful for other aspects of the evaluation (carcinogenicity and/or MOA evaluation).

<sup>a</sup> Table includes only primary literature, or studies for which a comprehensive review of the study was available. All studies shown are included in the database of literature for the evaluation.

<sup>b</sup> Studies were not selected for presentation, for a variety of reasons, as presented below:

**Reliability rank** - animal bioassay was determined to be unreliable for the toxicity evaluation. Due to limited human data, some epidemiological studies for which the reliability was classified as unreliable were presented in the review. In these cases the reliability rank is noted.

**Acute exposure/High dose** - study was conducted at acute exposure duration and/or at high doses, which were determined not to inform the sensitivity evaluation. For the sensitivity evaluation, studies with a treatment dose of less than 10 mg/kg-day and an exposure duration greater than 2 weeks were included. In a few cases, a low dose study of gestation or early development was also included, even though the exposure duration was less than 2 weeks.

**Endpoint not evaluated** - endpoint showed no evidence of being a sensitive endpoint based upon data reported in the ATSDR (2005) Toxicological Profile.

**MOA endpoint /*In vitro*** - study may be useful for determining MOA however does not support dose-response for toxic effects. *In vitro* dose-response data is not comparable to *in vivo* studies.

**Multiple isomer treatment** - study evaluated treatment with technical HCH or technical Lindane that reportedly contained substantial amounts of multiple isomers.

Table 5. Hazard Identification for Gamma-HCH: Summary of Animal Bioassay Studies at Low Doses, Immunological Effects.

Reference <sup>a</sup>	Species, Sex	Study Design	Dose (exposure)			Sample Size	Test Employed/Effects Tested	Response			
			Dose Range (mg/kg-day)	Exposure Duration				Observed Response <sup>b</sup>	LOAEL (s)	NOAEL(s)	Major Study Limitations
510	Banerjee et al. (1996)	Mouse, male	Multiple dose dietary bioassay	0, 2.4, 7.3, 12 (0, 10, 30, 50 ppm) <sup>c</sup>	12 weeks	10-14/group	Relative liver weight	Significantly increased at 7.3 mg/kg-day (30 ppm) at 12 weeks and at 12 mg/kg-day (50 ppm) at 6, 8, and 12 weeks.	7.3	2.4	Food consumption rate not reported. Insufficient reporting of histological results.
						Relative spleen weight	No significant differences from control.			12	
						Relative thymus weight	No significant differences from control.			12	
						Serum antibody titre to sheep red blood cells	Significantly decreased primary antibody response observed at 12 mg/kg-day (50 ppm) at 12 weeks. Significantly decreased secondary antibody response observed at 12 mg/kg-day (50 ppm) at 3 weeks. Significantly decreased secondary antibody response observed at 7.3 mg/kg-day (30 ppm) at 12 weeks. Response is dose- and time-dependent.	7.3		2.4	
						Splenic-PFC response	Significantly decreased PFC response at 7.3 mg/kg-day and 12 mg/kg-day starting at 6 weeks. Response is both dose and time dependent.	7.3		2.4	
638	Dewan et al. (1980)	Rat, male/female	Multiple dose, oral intubation bioassay	0, 6.25, 25	5 weeks	5/sex/group	Serum antibody titre to <i>Salmonella typhi</i> and <i>S. paratyphi</i>	Significantly decreased antibody response observed at 6.25 and 25 mg/kg-day at 21 days.	6		Isomer purity not reported. Statistical results not clearly reported. Insufficient description of assay methods.
657	Grabarczyk et al. (1990)	Rabbit, male	Single dose oral intubation bioassay	0, 7	4 weeks	5/group <sup>d</sup>	Phagocytic activity of peripheral blood neutrophils	Significant decrease in the phagocytosis index of the neutrophils at 7 mg/kg-day at 1 week. Increase in the number of non-phagocytizing neutrophils at 7 mg/kg-day at 1 week. The most significant decrease in phagocytosis noted at 3 weeks.	7		Insufficient reporting of sample size, dosing regimen, and results. Treatment toxicity and mortality not reported
						Nucleolar activity in lymphocytes	A significant increase in the percentage of lymphocytes with non-active nucleoli was observed at 7 mg/kg-day at 4 weeks.	7			
627	Koner et al. (1998)	Wistar Rat, male	Multiple dose dietary bioassay	0, 3.7, 7.4 (0, 40, 80 ppm) <sup>e</sup>	8 weeks	8-10/group	Serum TBARS level	Significantly increased TBARS level observed at 40 and 80 ppm.	3.7		Isomer purity and mortality data not reported.
						SOD activity in red blood cells	Significantly increased SOD activity in red blood cells observed at 40 and 80 ppm.	3.7			
						Serum antibody titre to sheep red blood cells	Significantly decreased antibody titre observed at 40 and 80 ppm.	3.7			
300	Matsuura et al. (2005)	Crl:CD (SD)IGS Rat, male/female	Two generation reproductive dietary bioassay	0, 0.56-1.5, 3.4-8.9, 17-45 (0, 10, 60, and 300 ppm) <sup>f</sup>	F0: 10 weeks before mating, through mating until terminal necropsy (males); and through mating, gestation, lactation until F1 weaning at post partum Day 21 (females); F1: treated same manner as F0 animals after weaning at post partum Day 21	4-24/group	Offspring necropsy/histological findings	No significant differences from control in thymus and spleen of F1 and F2 offspring.		28 (dam)	None identified.
							Offspring organ weight	Significantly increased relative spleen weight in F1 females at 10 and 60 ppm, but not at 300 ppm.	0.88 (dam)		
536	Mediratta et al. (2008)	Wistar Rat, male	Single dose oral intubation bioassay	0, 10	21 days	8/group	Serum MDA level	Significant increase in MDA level observed at 10 mg/kg-day.	10		Isomer purity not reported. Test conditions not sufficiently reported. Histological effects not examined.
						Reduced glutathione content in blood	Significant decrease in reduced glutathione content observed at 10 mg/kg-day.	10			
						SRBC antibody titre	Significant decrease observed in anti-SBRC antibody titre at 10 mg/kg-day.	10			
						Delayed type hypersensitivity reaction (footpad)	Significant decrease in percent change in footpad thickness observed at 10 mg/kg-day.	10			
366	Meera et al. (1992)	Mouse, female	Multiple dose dietary bioassay with interval tests/sacrifice every 4 weeks	0, 0.012, 0.12, 1.2	24 weeks	6/group	Delayed type hypersensitivity reaction (footpad)	Increased diameter of induration on footpad up to 12 weeks, decreased diameter of induration on footpad at 16-24 weeks.	0.012		No discussion of statistical tests used to assess statistical significance. Insufficient reporting of histological results by dose. Treatment purity of only 97%.
						Lymphocyte transformation	Lymphocyte proliferative response to ConA increased up to 8 weeks, then decreased after 12 weeks, but not all increases/decreases were significant.	0.012			
						Mixed lymphocyte reaction	No significant differences from control.			1.2	
						Haemolytic PFC assay	Significant increase in PFC number up to 8 weeks followed by significant suppression up to 24 weeks.	0.012			
						Macrophage Phagocytic activity	No significant differences from control.			1.2	
						Histology of thymus	Increase in size of medulla and decrease in cellular population of cortex at 4 weeks, further loss of distinction between the cortex and medulla at 24 weeks. Not all data reported, but effects shown for 1.2 mg/kg-day.	1.2			
						Histology of lymph node	Increased activity in the lymphoid follicles at 4 weeks, no difference from control at 12 weeks, loss of demarcation between cortex and paracortex at 24 weeks. Not all data reported, but effects shown for 1.2 mg/kg-day.	1.2			
						Histology of spleen	No significant differences at 4 weeks, reduction in overall cellularity of red pulp and white pulp areas at 24 weeks. Not all data reported, but effects shown for 1.2 mg/kg-day.	1.2			

Table 5. (continued)

Reference <sup>a</sup>	Species, Sex	Study Design	Dose (exposure)			Sample Size	Response		LOAEL (s)	NOAEL(s)	Major Study Limitations
			Dose Range (mg/kg-day)	Exposure Duration	Test Employed/Effects Tested		Observed Response <sup>b</sup>				
628 Meera et al. (1993)	Mouse, female	Multiple dose dietary bioassay with interval tests at 4 and 12 weeks	0, 0.012, 0.12, 1.2	24 weeks	6/group	Calcium uptake in lymphocytes	Significant increase in calcium influx observed in all dose groups at 4 weeks, followed by a significant decrease in all dose groups at 12 and 24 weeks. Effects were both dose and time dependent.	0.012		Not clear if these are the same animals discussed in Meera et al. (1992), or a different population. No discussion of statistical tests used to assess statistical significance. Treatment purity of only 97%.	
						Lymphocyte proliferation in presence of verapamil (calcium channel blocker)	Decreased lymphoproliferative response at all doses. Effects were dose dependent, but not necessarily time dependent.	0.012			
						Lymphocyte proliferation in presence of trifluoperazine (calmodulin inhibitor)	Decreased lymphoproliferative response at all doses. Effects were dose dependent, but not time dependent.	0.012			
357 Saha and Banerjee (1993)	Rat, male	Multiple dose dietary bioassay with interval sacrifice at 8, 12, 18, and 22 weeks	0, 0.4, 1.5, 2.2 (0, 5, 20, 30 ppm) <sup>9</sup>	22 weeks	10-12/group	Relative spleen weight	No significant differences from control.			2.2	Treatment purity of only 97% pure lindane and isomer purity not reported. Food consumption not reported. Insufficient reporting of histological results.
						Relative thymus weight	No significant differences from control.			2.2	
						Albumin/Globulin ratio	Significantly decreased globulin level in stimulated rats at 30 ppm (2.2 mg/kg-day) at 18 weeks and 20 ppm (1.5 mg/kg-day) and 30 ppm (2.2 mg/kg-day) at 22 weeks.	1.5		0.4	
						Serum antibody titre to tetanus toxoid	Significant decrease in serum antibody titre to tetanus toxoid at 20 ppm (1.5 mg/kg-day) and 30 ppm (2.2 mg/kg-day) at 12, 18, and 22 weeks.	1.5		0.4	
						Serum immunoglobulin levels (IgM and IgG)	Significantly lower increases of IgG and IgM levels after tetanus toxoid at 20 ppm (1.5 mg/kg-day) at 22 weeks and at 30 ppm (2.2 mg/kg-day) at 18 and 22 weeks.	1.5		0.4	
654 USEPA (1992b)	Rat, male/female	Multiple dose, dietary bioassay	0, 0.05, 0.47, 4.81, and 19.66 for males; 0, 0.06, 0.59, 6.00 and 24.34 for females	104 weeks with interval blood collection and sacrifice	15/sex/group	Leukocyte count (WBC)	No significant differences from control reported.			19.66/24.34 (males/females)	Lack of histopathology data for adrenal gland. Kidney effects noted, but not relevant for human health risk assessment.
						Platelet count	Significant increases in platelet counts seen only at 12 and 24 weeks at the 100 and 400 ppm doses (4.81 and 19.66 mg/kg-day) in males and at 24 weeks at 100 and 400 ppm (6 and 24.34 mg/kg-day) in females. No significant differences seen at later intervals.	4.81/6.00 (males/females)		0.47/0.59 (males/females)	
						Erythrocyte count	Significant decrease in erythrocyte count in both males and females at 104 weeks at 400 ppm.	19.66/24.34 (males/females)		4.81/6.00 (males/females)	
						Packed cell volume	Significantly decreased at 104 weeks at 400 ppm.	19.66/24.34 (males/females)		4.81/6.00 (males/females)	
						Spleen weight (absolute and relative)	Absolute and relative spleen weights significantly increased at 52 weeks at 400 ppm, but not at 104 weeks. Relative spleen weight significantly increased at 104 weeks at 100 ppm, but not at 400 ppm. Not dose-dependent.	4.81/6.00 (males/females)		0.47/0.59 (males/females)	
						Spleen histology	No pathological findings in spleen.			19.66/24.34 (males/females)	
						Bone marrow histology	No significant difference from control in mean myeloid to erythroid ratio; cellularity; composition; incidence of fatty marrow.			19.66/24.34 (males/females)	
						Albumin/Globulin ratio	Significantly decreased at 400 ppm.	19.66/24.34 (males/females)		4.81/6.00 (males/females)	

Source: Default dose conversion values obtained from EPA (1988).

Notes: HCH = hexachlorocyclohexane  
 kg = kilogram  
 kg/day = kilogram per day  
 LOAEL = lowest-observed-adverse-effect level  
 MDA = malondialdehyde  
 mg/kg-day = milligram per kilogram per day  
 NOAEL = no-observed-adverse-effect level  
 PFC = plaque forming cell  
 ppm = part per million  
 SOD = superoxide dismutase  
 SRBC = sheep red blood cell  
 TBARS = thiobarbituric acid reactive substance  
 WBC = white blood cell

<sup>a</sup> Studies selected for inclusion in this table were limited to those with at least one treatment dose of 10 mg/kg-day or less; and those with subchronic/chronic exposure durations or exposure during early development.

<sup>b</sup> Responses were considered significant only for effects reported to be statistically significant at  $p < 0.05$ .

<sup>c</sup> Dietary concentrations in ppm converted to dose in mg/kg/day using estimated food consumption rate of 0.0051 kg/day and average body weight of 0.021 kg.

<sup>d</sup> Number of animals/group was inferred from a single graphic depicted in the literature and was not specifically reported for each experimental regimen.

<sup>e</sup> Dietary concentrations in ppm converted to dose in mg/kg/day using estimated food consumption rate of 0.02 kg/day and average body weight of 0.217 kg.

<sup>f</sup> Range of dietary doses provided for each dietary concentration per "Table 1 Daily intake of lindane" as reported in the literature. Time weighted average of maternal intake over all dosing periods shown as LOAEL/NOAEL.

<sup>9</sup> Dietary concentrations in ppm converted to dose in mg/kg/day using estimated food consumption rate of 0.034 kg/day and average body weight of 0.462 kg.

Table 6. Hazard Identification for Gamma-HCH: Summary of Animal Bioassay Studies at Low Doses, Liver Effects.

Reference <sup>a</sup>	Species, Sex	Study Design	Dose (exposure)			Response			Major Study Limitations	
			Dose Range	Exposure Duration	Sample Size	Observed Response <sup>b</sup>	LOAEL (s) (mg/kg-day)	NOAEL (s) (mg/kg-day)		
510	Banerjee et al. (1996)	Mouse, male	Multiple dose dietary bioassay	0, 2.4, 7.3, 12 mg/kg-day (0, 10, 30, 50 ppm) <sup>c</sup>	12 weeks	10-14/ group	Significantly increased relative liver weight at 7.3 mg/kg-day (30 ppm) at 12 weeks and at 12 mg/kg-day (50 ppm) at 6, 8, and 12 weeks.	7.3	2.4	Food consumption rate not reported. Insufficient reporting of histological results.
381	Barros et al. (1991)	Rat (Wistar), male	Single dose dietary bioassay	0, 20 ppm (0, 2 mg/kg-day) <sup>d</sup>	15 or 30 days	6-22/group	Significant increase in P450 content after both 15 and 30 days. Significant increase in TBARS production after both 15 and 30 days. Significant increase in superoxide anion production after both 15 and 30 days. Significant increase in SOD activity after both 15 and 30 days.	2 2 2 2		Only 1 dose tested. Insufficient reporting of methods, treatment purity.
382	Fitzhugh et al. (1950)	Rat (Wistar), male/female	Multiple dose dietary bioassay	0, 5, 10, 50, 100, 400, 800, 1600 ppm (0, 0.4, 0.8, 3.9, 7.9, 31.6, 63.2, 126.3 mg/kg-day) <sup>e</sup>	Approximately 107 weeks	10/sex/group; 20/sex/group controls	Significant increase in relative liver weight at 100, 800, and 1600 ppm. Slight microscopic liver changes seen at 100 ppm. Gross histological liver changes and microscopic changes seen at 400, 800, and 1600 ppm.	7.9 7.9 31	3.9 3.9 7.9	Substantial mortality in both control and all treatment groups.
657	Grabarczyk et al. (1990)	Rabbit, male	Single dose oral intubation bioassay	0, 7 mg/kg-day	4 weeks	5/group <sup>f</sup>	Noticeable hepatocellular damage and frequent aggregation of erythrocytes in hepatic lobules observed after 4 weeks at 7 mg/kg-day.	7		Insufficient reporting of sample size, dosing regimen, and results. Treatment toxicity and mortality not reported.
442	Herbst et al. (1975)	Mouse (Chbi:NMRI (SPF)), male/female	Multiple dose dietary bioassay	0, 12.5, 25, 50 ppm (0, 2.1, 4.1, 8.2 mg/kg-day males; 0, 2, 3.9, 7.8 mg/kg-day females) <sup>g</sup>	80 weeks	50/sex/treatment group 100/sex/control group	Tumor production not related to treatment but found to occur spontaneously in this strain.		7.8 (females) 8.2 (males)	Insufficient reporting of mortality data and other results. High background incidence of tumors.
286	Johri et al. (2007)	Rat, male/female	Multiple dose gestation (day 5-21) oral bioassay with interval sacrifice of pups at 3, 6, or 9 weeks	0, 0.0625, 0.125, and 0.25 mg/kg-day	17 days	6/group	Significant increase in activity of CYP monooxygenases in liver for doses of 0.125 and 0.25 seen consistently at 3, 6 and 9 weeks postnatal.	0.125 (dam)	0.0625 (dam)	Treatment was "Lindane - technical grade" and isomer content/purity was not reported. Not clear how many animals were evaluated for each treatment at each dose.
300	Matsuura et al. (2005)	Crj:CD (SD)IGS Rat, male/female	Two generation reproductive dietary bioassay	0, 0.56-1.5, 3.4-8.9, 17-45 mg/kg-day (0, 10, 60, and 300 ppm) <sup>h</sup>	F0: 10 weeks before mating, through mating until terminal necropsy (males); and through mating, gestation, lactation until F1 weaning at post partum Day 21 (females): F1: treated same manner as F0 animals after weaning at post partum Day 21	4-24/ group	Relative liver weight significantly increased for F0 males at 10 ppm and greater and for F0 females at 60 and 300 ppm. Relative liver weight significantly increased for F1 males at 300 ppm and for F1 females at 60 and 300 ppm. Centrilobular hypertrophy of hepatocytes in F0 males and females at 60 and 300 ppm. Centrilobular hypertrophy of hepatocytes in F1 males at all doses and in F1 females at 60 and 300 ppm. Significantly increased activity of drug-metabolizing enzymes in F1 offspring at 17 weeks at both 60 and 300 ppm. Enlargement of liver was observed at 60 and 300 ppm in both F0 and F1 females, but not in males. The significance of this effect was not reported.	0.56 (males) 5.2 (females) 23 (males) 5.6 (females) 3.4 (males) 5.2 (females) 0.74 (males) 5.6 (females) 5.2 (dam)	0.88 (females) 0.94 (females) 0.56 (males) 0.88 (females) 0.94 (females) 0.88 (dam)	None identified.
583	Oesch et al. (1982)	CF1 Mouse, male/female	Multiple dose dietary bioassay	0, 56, 111, 360 ppm (0, 11, 22 and 72 mg/kg-day males; 0, 11, 23, and 76 mg/kg-day females) <sup>i</sup>	3 months	3/group	Significant increase in absolute liver weight for both males and females at 360 ppm. Significant increase in activity of 7-ethoxycoumarin-O-dealkylase at 56 ppm in males and 111 ppm in females. Significant increase in activity of EH in liver microsomes at 111 ppm in males and 360 ppm in females. Significant increase in activity of GST in liver supernatant at 56 ppm in females and 111 ppm in males. Significant increase in activity of UGT in liver microsomes in males at 360 ppm.	72 (males) 76 (females) 11 (males) 23 (females) 22 (males) 76 (females) 22 (males) 11 (females) 72 (males)	22 (males) 23 (females) 11 (females) 23 (females) 11 (males) 22 (males) 76 (females)	Mortality at highest dose for CF1 mice. Very small group size. Treatment was Lindane, isomer content not reported.
		B6C3F1 Mouse, male/female	Multiple dose dietary bioassay	0, 56, 170, 270 ppm (0, 10, 31 and 49 mg/kg-day males; 0, 11, 33, and 53 mg/kg-day females) <sup>i</sup>	3 months	3/group	No significant differences observed in absolute liver weight for both males and females at lower doses. No results for highest dose level due to mortality. Significant increase in activity of 7-ethoxycoumarin-O-dealkylase at 170 ppm in males and females. Significant increase in activity of EH in liver microsomes at 170 ppm in males and 56 ppm in females.		31 (males) 33 (females) 31 (males) 11 (females)	

Table 6. (continued)

Reference <sup>a</sup>	Species, Sex	Study Design	Dose (exposure)			Response			Major Study Limitations	
			Dose Range	Exposure Duration	Sample Size	Observed Response <sup>b</sup>	LOAEL (s) (mg/kg-day)	NOAEL (s) (mg/kg-day)		
						Significant increase in activity of GST in liver supernatant at 170 ppm in females. No significant effects observed for males at lower doses. No results for highest dose level due to mortality.	33 (females)	31 (males) 11 (females)		
						Significant increase in activity of UGT in liver microsomes in females at 170 ppm. No significant differences observed for males at lower doses. No results for highest dose level due to mortality.	33 (females)	31 (males) 11 (females)		
	Osborne-Mendel Rat, male/female	Multiple dose dietary bioassay	0, 56, 111, 360 ppm (0, 4.9, 9.7, and 31 mg/kg-day males; 0, 5.3, 10, and 34 mg/kg-day females) <sup>k</sup>	3 months	3/group	Significant increase in absolute liver weight for males at 360 ppm and for females at 111 ppm.	31 (males) 10 (females)	9.7 (males) 5.3 (females)		
						Significant increase in activity of 7-ethoxycoumarin-O-dealkylase at 56 ppm in both males and females.	4.9 (males) 5.3 (females)			
						Significant increase in activity of EH in liver microsomes at 111 ppm in males and 56 ppm in females.	9.7 (males) 5.3 (females)	4.9 (males)		
						Significant increase in activity of GST in liver supernatant at 111 ppm in males and 56 ppm in females.	9.7 (males) 5.3 (females)	4.9 (males)		
						Significant increase in activity of UGT in liver microsomes in males and females at 360 ppm.	31 (males) 34 (females)	9.7 (males) 10 (females)		
646	Palmer et al. (1978)	CD Rat, male/female	Three generation reproductive dietary bioassay	0, 7, 14, 28 mg/kg-day (0, 25, 50, 100 ppm) <sup>l</sup>	60 days prior to mating, through mating and gestation and 21 days postpartum	10 males/group; 10-20 females/group	Significantly increased relative liver weight in males at 100 ppm and females at all doses. No other significant effects for organ weights.	7 (females) 28 (males)	14 (males)	Treatment was lindane; isomer content and purity not reported. Inconsistent reporting of statistical significance.
498	Parmar et al. (2003)	Rat, male	Single dose oral bioassay	0, 2.5, 5, 10, 15 mg/kg-day	5-21 days	10/group	Significant increase in activity of CYP monooxygenases in liver at 5-15 mg/kg-day for 5 day exposure. Significant increase in activity of CYP monooxygenases in liver at 2.5 mg/kg-day for 15 and 21 day exposure. Dose- and time-dependent.	2.5		Treatment was "Lindane - technical grade" and isomer content/purity was not reported. Dose-response information was only available for an exposure duration of 5 days. Only a single dose evaluated for 21 days.
							Significant increase in absolute liver weight at 2.5 mg/kg-day for 21-day exposure. No significant change in relative liver weight at the same dose/time.	2.5		
647	Pereira et al. (1982)	Rat (SD), male/female	Single dose dietary bioassay	0, 76 ppm (0, 5.2 mg/kg-day males; 0, 6.1 mg/kg-day females) <sup>m</sup>	45 days after DENA	5-19/group	Increased incidence of DENA-initiated GGase-positive foci in liver, indicating lindane is a promoter of hepatocarcinogenesis, although not an initiator.	5.2 (males) 6.1 (females)		Only 1 dose tested. Insufficient reporting of results. Inadequate statistical evaluation.
390	Schroter et al. (1987)	Rat (Wistar), female	Single dose initiation and multiple dose dietary promotion study	0, 30 mg/kg-day (initiation); 0, 0.1, 0.5, 2.5, 10, 30 mg/kg-day (promotion)	Single dose (initiation); 15 or 20 weeks (promotion)	3-8/group (initiation)	Significant increase in liver DNA after 15 and 20 weeks at 30 mg/kg. Significant increase in liver mass after 15 and 20 weeks at 30 mg/kg. Dose-dependent increase in monooxygenase activity all doses (not significant). Significant increase in foci area after 20 weeks at 2.5 mg/kg; after 15 and 20 weeks at 10 mg/kg; and after 4, 15, and 20 weeks at 30 mg/kg.	30 30 2.5	10 10 0.5	Only females were tested. Promotion measured following initiation with known carcinogen.
319	Sumida et al. (2007)	Rat (F344), male	Multiple dose oral bioassay	0, 1, 10 mg/kg-day	28 days; interim sacrifices at 1, 3, 7, 14, and 28 days	4/group	Significant increase in relative liver weight at 7 days with 10 mg/kg-day. Significant decrease in ALT levels at 28 days at 10 mg/kg-day.	10 10	1 1	Only males were tested.
656	USEPA (1983)	Rat (Wistar), male/female	Multiple dose dietary bioassay	0, 0.2, 0.8, 4, 20, 100 ppm (0, 0.02, 0.06, 0.29, 1.6, 7.3 mg/kg-day males; 0, 0.02, 0.06, 0.33, 1.7, 7.9 mg/kg-day females) <sup>n</sup>	12 weeks or 12 weeks plus 6 weeks recovery	20/sex/group	Dose-dependent, significant increase of total P450 levels was seen in the 0.8, 4, 20, 100 ppm females; an increase in P450 levels also seen in the 100 ppm males, but this finding was not significant. P450 increases regressed after the recovery period. Slight increase in relative liver weight seen at 20 and 100 ppm (males and females). Statistical methods for all noted effects were described, but significance was not indicated for specific effects. It was assumed that all reported treatment differences were significant. Microscopic changes in the livers included hypertrophy in 4 ppm males and greater and in 20 ppm females and greater (dose-dependent); Kupffer cell proliferation in 0.8 ppm males and greater and in 0.2 ppm females and greater (no dose-dependent trend). Reported dose dependent treatment effects were assumed to be significant. No microscopic liver changes were seen after the recovery period.	0.06 (females) 1.6 (males) 1.7 (females) 0.29 (males) 1.7 (females)	0.02 (females) 0.29 (males) 0.33 (females) 0.06 (males) 0.33 (females)	P450 measured in liver homogenates instead of microsomes.
653	USEPA (1989)	Rat (CrI:(WI)BR), male/female	Dermal	0, 10, 60, 400 mg/kg-day	6 weeks, 13 weeks, or 13 weeks plus 6 weeks recovery	13/sex/group (6 week and recovery); 23/sex/group (13 week)	Absolute liver weights were significantly increased in the 60 mg/kg and 400 mg/kg males and 400 mg/kg females after 6 and 13 weeks and in the 60 mg/kg females after 13 weeks. These increases regressed after the 6 week recovery period, except for males in the 400 mg/kg group where liver weights remained statistically significantly elevated. Dose-dependent increase in incidence of centrilobular hypertrophy in males and females. No rats had hypertrophy after the recovery period. Focal necrosis was observed in male rats after the recovery, but not during treatment. Statistical significance was not reported for any of these effects.	60	10	Increased mortality at the high dose.

Table 6. (continued)

Reference <sup>a</sup>	Dose (exposure)					Response			Major Study Limitations
	Species, Sex	Study Design	Dose Range	Exposure Duration	Sample Size	Observed Response <sup>b</sup>	LOAEL (s) (mg/kg-day)	NOAEL (s) (mg/kg-day)	
654 USEPA (1992b)	Rat (Wistar), male/female	Multiple dose dietary bioassay	0, 0.05, 0.47, 4.81, 19.66 (males); 0, 0.06, 0.59, 6, 24.34 (females)	104 weeks	15-50/sex/group	Significant dose-dependent increase in male and female relative liver weight at 30 days, 26 weeks, 52 weeks, and 104 weeks (19.66 mg/kg-day males and 24.34 mg/kg-day females); significantly increased in the 4.81 mg/kg-day males and 6 mg/kg-day females at 104 weeks. No increases in liver weight were seen in the recovery animals.  Periacinar hypertrophy observed in males and females with dose- and time-dependent increase in incidence (significant at 4.81 mg/kg-day in males and at 6 mg/kg-day in females). No hypertrophy observed in the recovery animals.  One liver tumor seen in a high-dose female (dose and significance not reported).	4.81 (males) 6 (females)	0.47 (males) 0.59 (females)	Increased mortality at the highest dose.
274 USEPA (2001)	Mouse (CD-1) male/female	Multiple dose dietary bioassay	0, 1.3, 5.2, and 21 mg/kg- day males; 1, 1.8, 7.1 and 27 mg/kg-day females (0, 10, 40, and 160 ppm)	78 weeks	50/sex/group	No significant differences from control observed in number of liver adenomas or carcinomas in males and females at all doses.  Significant increase in incidence of centrilobular hepatocyte hypertrophy observed in males at 160 ppm; no significant differences in females at all doses.  Significant increase in incidence of eosinophilic foci of hepatocellular alteration observed in males at 160 ppm; no significant differences in females at all doses.	20.5 (males) 26.8 (females)	5.2 (males) 26.8 (females)	Unable to review primary study; only reviewed synopsis provided by EPA. Unable to assess data quality.
471 Weisse and Herbst (1977)	Mouse (Chbi:NMRI (SPF)), male/female	Multiple dose dietary bioassay	0, 12.5, 25, 50 ppm (0, 2.1, 4.1, 8.2 mg/kg-day males; 0, 2, 3.9, 7.8 mg/kg-day females) <sup>g</sup>	80 weeks	50/sex/treatment group  100/sex/control group	Tumor production not related to treatment but found to occur spontaneously in this strain.		7.8 (females) 8.2 (males)	Insufficient reporting of mortality data and other results. High background incidence of tumors.

Source: Default dose conversion values obtained from EPA (1988).

- Notes:
- ALT = alanine aminotransferase
  - CYP = cytochrome P450
  - DENA = N-nitrosodiethylamine
  - DNA = deoxyribonucleic acid
  - EH = epoxide hydrolase
  - EPA = U.S. Environmental Protection Agency
  - GGT = gamma-glutamyl transpeptidase
  - GST = glutathione-S-transferase
  - HCH = hexachlorocyclohexane
  - kg = kilogram
  - kg = kilogram per day
  - LOAEL = lowest-observed-adverse-effect level
  - mg/kg = milligram per kilogram
  - mg/kg-day = milligram per kilogram per day
  - NOAEL = no-observed-adverse-effect level
  - ppm = part per million
  - P450 = cytochrome P450
  - SOD = superoxide dismutase
  - TBARS = thiobarbituric acid reactive substance
  - UGT = UDP-glucuronosyl transferase

<sup>a</sup> Studies selected for inclusion in this table were limited to those with at least one treatment dose of 10 mg/kg-day or less; and those with subchronic/chronic exposure durations or exposure during early development.

<sup>b</sup> Responses were considered significant only for effects reported to be statistically significant at  $p < 0.05$ .

<sup>c</sup> Dietary concentrations in ppm converted to dose in mg/kg/day using estimated food consumption rate of 0.0051 kg/day and average body weight of 0.021 kg.

<sup>d</sup> Dietary concentrations in ppm converted to dose in mg/kg-day using estimated food consumption rate of 0.02 and average body weight of 0.2 taken from study.

<sup>e</sup> Dietary concentrations in ppm converted to dose in mg/kg-day using an estimated average food consumption rate for males and females of 0.03 kg/day and an average body weight for males and females of 0.38 kg.

<sup>f</sup> Number of animals/group was inferred from a single graphic depicted in the literature and was not specifically reported for each experimental regimen.

<sup>g</sup> Dose conversion provided by study authors.

<sup>h</sup> Range of dietary doses provided for each dietary concentration per "Table 1 Daily intake of lindane" as reported in the literature. Time weighted average doses calculated for F0 and F1 females across pre-mating, gestation and lactation periods. NOAEL and LOAEL for effects in offspring are time weighted maternal dose averages

<sup>i</sup> Dietary concentrations in ppm converted to dose in mg/kg/day using estimated food consumption rate of 0.0045 kg/day for males and 0.0043 kg/day for females and body weight of 0.0223 kg for males and 0.0204 kg for females.

<sup>j</sup> Dietary concentrations in ppm converted to dose in mg/kg/day using estimated food consumption rate of 0.0057 kg/day for males and 0.0048 kg/day for females and body weight of 0.0316 kg for males and 0.0246 kg for females.

<sup>k</sup> Dietary concentrations in ppm converted to dose in mg/kg/day using estimated food consumption rate of 0.023 kg/day for males and 0.019 kg/day for females and body weight of 0.263 kg for males and 0.201 kg for females.

<sup>l</sup> Doses estimated based upon average reported initial body weight of 75 g and mean food consumption rates reported for F1B and F2B generations in Table II of the literature (0.021 g/day).

<sup>m</sup> Dietary concentrations in ppm converted to dose in mg/kg-day using estimated food consumption rate of 0.036 (males) and 0.027 (females) kg/day and average body weight of 0.523 (males) and 0.338 (females) kg.

<sup>n</sup> Doses calculated by study authors.

Table 7. Hazard Identification for Gamma-HCH: Summary of Animal Bioassay Studies at Low Doses, Neurological Effects.

Reference <sup>a</sup>	Species, Sex	Study Design	Dose (exposure)			Sample Size	Test Employed/Effects Tested	Response			
			Dose Range (mg/kg-day)	Exposure Duration				Observed Response <sup>b</sup>	LOAEL (s)	NOAEL(s)	Major Study Limitations
277	Agrawal et al. (1995)	Rat, female	Single dose oral bioassay	0, 5 mg/kg-day	3-6 months	5/group	Phosphoinositide levels in rat erythrocyte membranes	Significant decrease seen at 6 months, but not at 3 months.	5		Treatment was "technical grade HCH" and isomer content/purity was not reported. Only a single dose was tested, for 5 days per week over the exposure duration.
						Phosphoinositide levels in rat forebrain (cerebrum)	Significant decrease at 5 mg/kg-day at both 3 and 6 months.	5			
634	Anand et al. (1998)	Rat, male	Multiple dose intraperitoneal bioassay	0, 2, 3, and 5 mg/kg-day	90 days	24/group	Tremors	No significant effects with lindane alone. Significant effects at 3 and 5 mg/kg-day of lindane when challenged with leptazol.	3	2	Treatment was gamma-HCH, but isomer purity not reported. Statistical methods not identified. Unclear how many animals were tested for each specific endpoint.
						Negative geotaxis	Significant increase in negative geotaxis at all doses when challenged with leptazol (GABA antagonist).	2			
						Negative geotaxis	Significant blockage in negative geotaxis at 3 and 5 mg/kg-day when administered with GABA agonist.	3	2		
						Spontaneous locomotor activity	Significant decrease at all doses in presence of GABA agonist, significant increase in presence of GABA antagonist.	2			
						GABA levels in cerebellar region	At 1-24 hours, significant increase at 3 and 5 mg/kg. No significant effect at 2 mg/kg. At 0 hours, all doses showed significant decrease.	3	2		
						3H-flunitrazepam binding	Significant increase at 5 mg/kg only.	5	3		
741	Hughes (1999, as cited by ATSDR 2005)	Rat, male/female	Multiple dose dietary bioassay	0, 1.4, 7.1, and 28.1 mg/kg-day for males; 0, 1.6, 7.9, and 30.2 mg/kg-day for females	13 weeks		FOB	Increased rearing, walking on tiptoes, hypersensitivity to touch, hunched posture.	28.1 (males) 7.9 (females)	7.1 (males) 1.6 (females)	Unpublished study. Unable to evaluate.
						Motor activity	No significant effects reported in ATSDR.				
						Histopathology	Decreased body weight gain and food consumption, staining of urogenital region.	28.1 (males) 7.9 (females)	7.1 (males) 1.6 (females)		
286	Johri et al. (2007)	Rat, male/female	Multiple dose gestation (day 5-21) oral bioassay with interval sacrifice of pups at 3, 6, or 9 weeks	0, 0.0625, 0.125, and 0.25 mg/kg-day	17 days	6/group	Activity of CYP monooxygenases in brain	Significant increase in activity at 3 weeks postnatal for doses of 0.125 and 0.25, with decrease in effect at 6 weeks, and no significant increase seen at 9 weeks postnatal.	0.125 (dam)	0.0625 (dam)	Treatment was "Lindane - technical grade" and isomer content/purity was not reported. Not clear how many animals were evaluated for each treatment at each dose.
						Activity of CYP monooxygenases in liver	Significant increase in activity for doses of 0.125 and 0.25 seen consistently at 3, 6 and 9 weeks postnatal.	0.125 (dam)	0.0625 (dam)		
						Spontaneous locomotor activity of offspring	Significant increase in one of six measures of spontaneous locomotor activity at 0.125 mg/kg/day at 3 weeks. Significant increase in all six measures of activity at 0.25 mg/kg-day at 3 weeks. Significant increase at 0.25 mg/kg-day only for four of six measures at 6 weeks. Significant increase in activity at 0.25 mg/kg-day in only one of six measures of locomotor activity at 9 weeks postnatal.	0.125 (dam)	0.0625 (dam)		
300	Matsuura et al. (2005)	Crj:CD (SD)IGS Rat, male/female	Two generation reproductive dietary bioassay	0, 0.56-1.5, 3.4-8.9, 17-45 mg/kg-day (0, 10, 60, and 300 ppm) <sup>c</sup>	F0: 10 weeks before mating, through mating until terminal necropsy (males); and through mating, gestation, lactation until F1 weaning at post partum Day 21 (females); F1: treated same manner as F0 animals after weaning at post partum Day 21	4-24/group	Clinical signs	No significant differences from control in males. In females, mortality, convulsions, irritability and suppressed weight gain were observed at the highest dose (300 ppm). Convulsions were observed in two F1 dams, on Days 18 and 20 of gestation at 300 ppm; however significance of this effect was not reported.		23 (males) 5.6 (females)	None identified.
						Behavioral function (latency time, ambulation, rearing, grooming, defecation, urination, motor coordination, learning/memory)	No significant differences from control in F1 males and females when tested at 4 to 6 weeks of age.		23 (males) 25 (females)		
736	Myers (1999, as cited by ATSDR 2005)	Rat	Multiple dose gestation/ lactation study	0, 0.8-0.9, 4.2-4.6, or 8.0-10.5 mg/kg-day (gestation); 0, 1.2-1.7, 5.6-8.3, or 13.7-19.1 mg/kg-day (lactation)	104 weeks with interval blood collection and sacrifice	6/group	Motor activity	Increased motor activity at two highest dose levels (both sexes).	5.6 - 19.1	0.9 - 1.7	Unpublished study. Unable to evaluate.
						Auditory startle response	Reduced auditory startle response habituation in both sexes at 13.7 mg/kg dose.	13.7			
						Learning and memory	Decreased habituation of motor activity at 2 highest doses (females).				
						Brain histology	Effects not reported in ATSDR.				
498	Parmar et al. (2003)	Rat, male	Single dose oral bioassay	0, 2.5, 5, 10, 15 mg/kg-day	5-21 days	10/group	Activity of CYP monooxygenases in brain	Significant increase in activity at 10 mg/kg-day and 15 mg/kg-day for 5 days exposure. Significant increase in activity at 2.5 mg/kg-day for 15 and 21 day exposure. Dose- and time-dependent.	2.5		Treatment was "Lindane - technical grade" and isomer content/purity was not reported. Dose-response information was only available for an exposure duration of 5 days. Only a single dose evaluated for 21 days.
						Activity of CYP monooxygenases in liver	Significant increase in activity at 5-15 mg/kg-day for 5 day exposure. Significant increase in activity at 2.5 mg/kg-day for 15 and 21 day exposure. Dose- and time-dependent.	2.5			

Table 7. (continued)

Reference <sup>a</sup>	Species, Sex	Study Design	Dose (exposure)			Sample Size	Test Employed/Effects Tested	Response			Major Study Limitations
			Dose Range (mg/kg-day)	Exposure Duration				Observed Response <sup>b</sup>	LOAEL (s)	NOAEL(s)	
629 Rivera et al. (1998)	Wistar Rat, male/ female	Single dose oral intubation bioassay	0, 10 mg/kg-day	7 days (postnatal day 8-14)	8-10/ group	Passive avoidance acquisition	Significant improvement observed in passive avoidance acquisition with reduced number of entries. This is not considered a toxic effect.	10		Purity of isomer not reported. Animal body weight not reported. Insufficient reporting of mortality data.	
						Motor activity	Significant increase observed in motor activity.	10			
						Neurotransmitter ratios of 5-HIAA/Serotonin and DOPAC/Dopamine	Significant decrease observed in 5-HIAA/Serotonin ratio in pons medulla, colliculi and frontal cortex at 10 mg/kg-day. Significant decrease in DOPAC/Dopamine ratio observed in mesencephalon and significant increase in DOPAC/Dopamine ratio observed in striatum at 10 mg/kg-day.	10			

Notes: ATSDR = Agency for Toxic Substances and Disease Registry  
 CYP = cytochrome P450  
 FOB = functional observational battery  
 GABA = gamma amino butyric acid  
 HCH = hexachlorocyclohexane  
 LOAEL = lowest-observed-adverse-effect level  
 mg/kg = milligram per kilogram  
 mg/kg-day = milligram per kilogram per day  
 NOAEL = no-observed-adverse-effect level  
 ppm = part per million

<sup>a</sup> Studies selected for inclusion in this table were limited to those with at least one treatment dose of 10 mg/kg-day or less; and those with subchronic/chronic exposure durations or exposure during early development.

<sup>b</sup> Responses were considered significant only for effects reported to be statistically significant at  $p < 0.05$ .

<sup>c</sup> Range of dietary doses provided for each dietary concentration per "Table 1 Daily intake of lindane" as reported in the literature. For behavioral effects in F1 females, the estimated dose over the pre-mating period was used.

Table 8. Hazard Identification for Gamma-HCH: Summary of Animal Bioassay Studies at Low Doses, Reproductive/Developmental Effects.

Reference <sup>a</sup>	Species, Sex	Study Design	Dose (exposure)			Sample Size	Test Employed/Effects Tested	Response		
			Dose Range (mg/kg-day)	Exposure Duration				Observed Response <sup>b</sup>	LOAEL (s)	NOAEL(s)
514 Beard and Rawlings (1998)	Mink, male/female	Three generation reproductive dietary bioassay	0, 1	11 weeks (F1), 42 weeks (F2 males), 30 weeks (F3 females); F2 and F3 were exposed in utero and through mating/ lactation.	8-10/ group	Mating behavior	No significant difference in proportion of F2 mink that accepted the first mating.	1	1	Purity and isomer content of lindane treatment not reported. Single dose design does not support dose response assessment. Insufficient reporting of histological results.
						Fertility/whelping	The proportion of mated mink that subsequently whelped was reduced compared to controls, but significant only at $p < 0.1$ .			
						Fertility/ litter size	Significantly reduced litter size compared to control.			
						Fertility/ weaning	No significant difference in proportion of F3 kits weaned.			
						Serum thyrosine	Significant decrease in secretion of thyrosine in F2 and F3 males, and in F3 females.			
						Serum oestradiol	No significant difference observed in F2 or F3 mink.			
						Serum corisol	No significant difference observed in F2 females (no other groups measured).			
						Serum testosterone	No significant difference observed in F2 or F3 males.			
						Testicular growth	No significant difference observed in F2 males. Relative testis length significantly smaller in F3 males compared to controls.			
						Thyroid mass	No significant differences observed in F2 and F3 mink.			
Adrenal mass	No significant differences observed in F2 and F3 females.									
625 Cooper et al. (1989)	F344 Rat, female	Multiple dose oral gavage bioassay	0, 5, 10, 20, 40	130 days	6-12/ group	Vaginal opening	Significant delay in vaginal opening at 10 and 40 mg/kg, but not at 20 mg/kg. Not dose dependent.	10	5	Treatment was lindane; isomer content and purity not reported.
						Vaginal cycle	Significantly fewer animals with regular 5-day estrous cycles at all doses. However, effects were no longer significantly different from controls by day 111. Effects both dose and time dependent.	5		
						Body weight	Body weight significantly increased at 20 and 40 mg/kg-day.	20	10	
						Organ weights	Relative weight of pituitary, uterus and ovaries significantly decreased in dose dependent manner at 10 mg/kg-day (for pituitary) and 20 mg/kg-day for uterus and ovaries.	10	5	
						Reproductive hormone concentrations	Pituitary LH and serum LH significantly lower at 20 and 40 mg/kg-day. Pituitary FSH significantly greater at 20 and 40 mg/kg-day. Pituitary prolactin significantly lower at 40 mg/kg-day. No significant differences in serum FSH or serum progesterone.	20	10	
519 Dalsenter et al. (1996)	Wistar Rat, male	Acute dietary bioassay	0, 6 (repeated doses over 5 days), 30 (single dose)	5 days	5-10/ group	Spermatids and sperm number	Significant decrease in number of spermatids at 6 mg/kg-day, but no significant difference in number of sperms.	6	Low dose given for 5 days and high dose given as single dose. Does not support dose response.	
						Histological findings	No significant differences in sperm morphology.	6		
						Organ weights	No significant differences in relative weights of reproductive organs.	6		
742 King (1991, as cited by ATSDR 2005)	Charles River Rat, male/female	Two generation reproductive dietary bioassay	0, 0.09, 1.7, 13.1	Mating period only	NA	Clinical signs	No signs of toxicity, effects on body weight or food consumption in F0 or F1 males or females during pre-mating. Suppressed weight gain in F0 dams at 13.1 mg/kg-day during gestation.	13.1	1.7	Unpublished study. Unable to obtain for review.
						Mating	No significant differences from control.	13.1		
						Fertility	No significant differences from control.	13.1		
						Gestation survival	No significant differences from control.	13.1		
						Liveborn index	No significant differences from control.	13.1		
						Mean litter size	No significant differences from control.	13.1		
						Offspring viability	Reduced body weight and decreased viability of pups of both generations at 13.1 mg/kg-day.	13.1	1.7	
						Offspring development	Delays in onset and completion of tooth eruption and hair growth in F2 pups at 13.1 mg/kg-day.	13.1	1.7	

Table 8. (continued)

Reference <sup>a</sup>	Species, Sex	Study Design	Dose (exposure)			Response				
			Dose Range (mg/kg-day)	Exposure Duration	Sample Size	Test Employed/Effects Tested	Observed Response <sup>b</sup>	LOAEL (s)	NOAEL(s)	Major Study Limitations
300 Matsuura et al. (2005)	Crj:CD (SD)IGS Rat, male/female	Two generation reproductive dietary bioassay	0, 0.56-1.5, 3.4-8.9, 17-45 (0, 10, 60, and 300 ppm) <sup>c</sup>	F0: 10 weeks before mating, through mating until terminal necropsy (males); and through mating, gestation, lactation until F1 weaning at post partum Day 21 (females); F1:treated same manner as F0 animals after weaning at post partum Day 21	4-24/group	Histological findings	No significant differences from controls for number of primordial and secondary follicles of the ovaries and total number of ovarian follicles.		26 (F0 females) 28 (F1 females)	None identified.
						Blood hormone analysis	Significant decrease in thyroid hormones (T3, T4) in both sexes at 300 ppm. No significant differences in any of the sex hormones.	26 (females) 17 (males)	5.6 (females) 4.5 (males)	
						Estrous cycle	No significant treatment-related changes in estrous cycling of F0 or F1 females.		26 (F0 females) 28 (F1 females)	
						Sperm analysis	No significant differences in percentage of motile sperm, or sperm count.		17 (F0 males) 23 (F1 males)	
						Mating/fertility	No treatment-related effects in mating or fertility.		17 (F0 males) 23 (F1 males) 26 (F0 females) 28 (F1 females)	
						Parturition and nursing	No treatment-related effects on gestation length, number of implantations, birth index or gestation index. No treatment-related effects in nursing in F0 dams. In F1 dams, total litter loss was associated with maternal behavior at 300 ppm.	28 (F1 females)	26 (F0 females) 5.6 (F1 females)	
						Offspring viability	No effects on number of offspring, sex ratio, or viability in F1 offspring. In F2 offspring, there were significant decrease in viability at postnatal day 4 at 300 ppm, likely associated with abnormalities in nursing behavior of dams.	28 (dam)	5.6 (dam)	
						Offspring body weight	Significantly low birth weights and suppressed postnatal body weight gain in both sexes of F1 and F2 offspring at 300 ppm.	26 (dam)	5.6 (dam)	
						Offspring anogenital distance/nipple development	No significant treatment-related differences from control.		28 (dam)	
						Offspring physical development and reflex/sensory functions	No significant treatment-related differences from control.		28 (dam)	
						Sexual maturation	Significant delay in preputial separation in males and vaginal opening in females at 300 ppm in F1 offspring.	26 (dam)	5.2 (dam)	
						Offspring organ weight	Significantly increased relative spleen weight in F1 females at 10 and 60 ppm, but not at 300 ppm.	5.2 (dam)		
						Offspring necroscopy/histological findings	Changes observed in kidney and liver of F1 and F2 offspring, but not dose dependent. No significant differences from control in thymus and spleen of F1 and F2 offspring, and uterus of F1 offspring.		28 (dam)	
Hepatic drug-metabolizing enzyme activities	Significantly increased activity of drug-metabolizing enzymes in F1 offspring at 17 weeks at both 60 and 300 ppm.	5.2 (dam)	0.88 (dam)							
646 Palmer et al. (1978)	CD Rat, male/female	Three generation reproductive dietary bioassay	0, 7, 14, 28 (0, 25, 50, 100 ppm) <sup>d</sup>	60 days prior to mating, through mating and gestation and 21 days postpartum	10 males/group; 10-20 females/group	Body weight/weight gain	No significant differences from controls.		28	Treatment was lindane; isomer content and purity not reported. Inconsistent reporting of statistical significance.
						Mating performance, pregnancy rate, duration of gestation	No significant differences from controls.		28	
						Parental necroscopy	No significant differences from controls.		28	
						Total litter loss	Not dose dependent.		28	
						Offspring viability	No significant adverse effects on litter size, offspring weight, offspring viability.		28	
						Offspring malformations	One rat in the F1B generation at 50 ppm with non-patent vagina. One rat in the F3B generation showed cerebellar hypoplasia. Effects not treatment-related. Increased number of males with 14th rib at 100 ppm, but significance of effect not evaluated.	28 (dam)	14 (dam)	
						Organ weights	Significantly increased relative liver weight in males at 100 ppm and females at all doses. No other significant effects for organ weights.	7 (dam)		
Histological findings	No significant differences from control.		28 (dam)							
566 Sircar and Lahiri (1989)	Swiss Mouse, female	Multiple dose oral bioassay during pregnancy	0, 3.8, 6.3, 7.1, 10.8 <sup>e</sup>	Up to 19 days (gestation)	6/group	Number of implantation sites	Significant decrease in implantation of fetus at 10.8 mg/kg-day in early pregnancy. No significant differences associated with treatment of 6.3 mg/kg-day in mid-pregnancy.	10.8	7.1	Experimental design does not support dose response assessment (varying dose depending upon time of treatment). Significance of effects inconsistently reported.
						Number of fetuses	Significant decrease in number of fetuses associated with treatment in early pregnancy.	10.8	7.1	
						Ovary weight	Significantly decreased weight of ovary with treatment in both early and mid-pregnancy.	10.8	7.1	
						Fetus weight	Significant decrease associated with treatment of 6.3 mg/kg-day in mid-pregnancy.	6.3	3.8	
						Percent resorption	Significantly increased percent of resorption associated with treatment of 6.3 mg/kg-day in mid-pregnancy.	6.3	3.8	
						Number of offspring	No significant differences at 3.8 mg/kg-day, but potential decrease at 7.1 mg/kg-day (significance not reported).	7.1	6.3	
						Weight of offspring	Significant decrease at 3.8 and 7.1 mg/kg-day in late pregnancy.	3.8		
						Offspring viability	Decreased at both 3.8 and 7.1 mg/kg-day in late pregnancy (significance not reported).	3.8		
Histological findings	No significant differences from controls for number of primordial and secondary follicles of the ovaries and total number of ovarian follicles.		26 (F0 females) 28 (F1 females)							

Table 8. (continued)

Reference <sup>a</sup>	Species, Sex	Study Design	Dose (exposure)			Sample Size	Response			
			Dose Range (mg/kg-day)	Exposure Duration	Test Employed/Effects Tested		Observed Response <sup>b</sup>	LOAEL (s)	NOAEL(s)	Major Study Limitations
650 Sujatha et al. (2001)	Wistar Rat, male	Single dose subchronic oral bioassay	0, 5	30 days	6/group	Body weight	Body weight significantly decreased at 5 mg/kg-day.	5	Single dose study, does not support dose response.	
						Organ weights	Relative organ weights of testis, epididymis, seminal vesicles and ventral prostate significantly decreased at 5 mg/kg-day.	5		
						Antioxidant enzyme activities in testes	Significantly decreased activities of superoxide dismutase, catalase and glutathione reductase at 5 mg/kg-day.	5		
						Hydrogen peroxide generation assay	Significantly increased generation of hydrogen peroxide at 5 mg/kg-day.	5		
						Steroidogenic enzyme activities	Significantly decreased activity of 3beta-hydroxysteroid dehydrogenase and 17b-hydroxysteroid dehydrogenase at 5 mg/kg-day.	5		
Testicular DNA, RNA and protein content	Significant decrease in DNA, RNA and protein level at 5 mg/kg-day.	5								

Source: Default dose conversion values obtained from EPA (1988).

- Notes:
- DNA = deoxyribonucleic acid
  - FSH = follicle stimulating hormone
  - g = gram
  - g/day = gram per day
  - HCH = hexachlorocyclohexane
  - kg = kilogram
  - LH = luteinizing hormone
  - LOAEL = lowest-observed-adverse-effect level
  - mg/day = milligram per day
  - mg/kg = milligram per kilogram
  - mg/kg-day = milligram per kilogram per day
  - NA = not available
  - NOAEL = no-observed-adverse-effect level
  - ppm = part per million
  - RNA = ribonucleic acid

<sup>a</sup> Studies selected for inclusion in this table were limited to those with at least one treatment dose of 10 mg/kg-day or less; and those with subchronic/chronic exposure durations or exposure during early development.

<sup>b</sup> Responses were considered significant only for effects reported to be statistically significant at  $p < 0.05$ .

<sup>c</sup> Range of dietary doses provided for each dietary concentration per "Table 1 Daily intake of lindane" as reported in the literature. Time weighted averages across dosing periods were used for F0 and F1 maternal females. For offspring, the time weighted average for the most sensitive (F0 or F1) dam was shown as the LOAEL; the time-weighted average for the less sensitive (F0 or F1) dam was shown as the NOAEL. Please refer to the literature for dosing details.

<sup>d</sup> Doses estimated based upon average reported initial body weight of 75 g and mean food consumption rates reported for F1B and F2B generations in Table II of the literature (0.021 g/day). For offspring, the LOEAL/NOAEL value is the maternal dose.

<sup>e</sup> Doses estimated based upon average reported body weight of 0.024 kg and reported dose of mg/animal daily at 0.09, 0.15, 0.17, and 0.26.

## **ATTACHMENT A**

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LITERATURE REVIEW OF ALPHA-,  
BETA-, AND GAMMA-  
HEXACHLOROCYCLOHEXANE  
[ON ENCLOSED CD]