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TOXICITY CRITERION FOR BETA-HEXACHLOROCYCLOHEXANE

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CONTENTS

LIST OF TABLES

ACRONYMS AND ABBREVIATIONS

ALT	alanine aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
BMD	benchmark dose
BMDL	confidence limit of the benchmark dose
CYP	cytochrome P450
DNA	deoxyribonucleic acid
EPA	U.S. Environmental Protection Agency
HCH	hexachlorocyclohexane
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
MOA	mode of action
NDEP	Nevada Division of Environmental Protection
NHL	non-Hodgkins Lymphoma
NOAEL	no-observed-adverse-effect level
OC	organochlorine
P450	cytochrome P450
PB	Phenobarbital
PD	Parkinson's Disease
POD	point of departure
RED	Reregistration Eligibility Decision
RfD	reference dose
UF	uncertainty factor
WOE	weight of evidence

EXECUTIVE SUMMARY

Integral Consulting Inc. (Integral) has developed an updated toxicity criterion for beta-hexachlorocyclohexane (beta-[HCH]). Beta-HCH has previously been regulated as a potential human carcinogen by the Nevada Division of Environmental Protection (NDEP) using toxicity criteria housed in the U.S. Environmental Protection Agency's (EPA) Integrated Risk Information System (IRIS) and last updated in1993[1]. This project was initiated by Integral on behalf of Syngenta Crop Protection and Stauffer Management Company to update the NDEP toxicity criterion for beta-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 beta-HCH that have been published since the original toxicity criterion was developed.

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

For non-cancer effects, the body of evidence suggests that the liver is the most sensitive target organ. Considering these findings and following USEPA (2000) guidance, a reference dose (RfD) was developed. The recommended RfD for beta-HCH is 0.00006 mg/kg-day. This value is based on a point of departure (POD) of 0.18 mg/kg-day for hyalinization of centrilobular cells in male rats and a total uncertainty factor (UF) of 3,000 to account for inter- and intra-species differences, use of a lowest-observed-adverse-effect level (LOAEL), use of a subchronic study, and database limitations.

For perspective, the recommended RfD is equal to the oral chronic RfD established by EPA for beta-HCH in their 2006 *Assessment of Lindane and Other Hexachlorocyclohexane Isomers* (USEPA 2006), completed as part of the Reregistration Eligibility Decision (RED) for Lindane. The chronic oral RfD proposed by EPA is based on an identical POD (i.e., a LOAEL of 0.18 mg/kg-day reported by Van Velsen et al. (1986)) and cumulative UF of 3,000 as those applied as the components of the RfD recommended here.

[1] EPA's IRIS currently classifies beta-HCH as a class C, possible human carcinogen (USEPA 2011). The current classification was last reviewed in 1993, and was based on data reported by 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.

1 INTRODUCTION

Integral Consulting Inc. (Integral) has developed an updated toxicity criterion for beta-hexachlorocyclohexane (beta-[HCH]). Beta-HCH has previously been regulated as a potential human carcinogen by the Nevada Division of Environmental Protection (NDEP) using toxicity criteria housed in the U.S. Environmental Protection Agency's (EPA) Integrated Risk Information System (IRIS) and last updated in1993. This project was initiated by Integral on behalf of Syngenta Crop Protection and Stauffer Management Company to update the NDEP toxicity criterion for beta-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 beta-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 beta-HCH. USEPA's *Guidelines for Carcinogen Risk Assessment* (2005a) provided the over-arching framework for the evaluation and assessment of potential carcinogenic effects, supplemented by recent peer-reviewed literature related to the evaluation of carcinogenic mode of action (MOA) and human relevance (Boobis et al. 2006, 2009; Butterworth 2006; Meek 2008; Meek et al. 2003). 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 to support the evaluation. Data related to the assessment of oral exposures were the focus of the review as this is a principal pathway currently for human exposures to ambient beta-HCH. EPA and Agency for Toxic Substances and Disease Registry (ATSDR) reviews of HCH toxicity (ATSDR 2005; USEPA 1987, 2001, 2006) 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 its quality and reliability using criteria developed from Klimisch et al. (1997), USEPA (2005a), and Durda and Preziosi (2000). Evaluation criteria included:

- Study uses 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 complied 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 assessment and 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 beta-HCH and identification of the most sensitive target organ/system for dose-response assessment.

2.2.1 Cancer Assessment

A weight of evidence (WOE) approach was taken to determine the carcinogenic potential of beta-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.

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[2] delivered during subchronic or chronic exposure durations because these types of exposure scenarios are most relevant for human health risk assessment (USEPA 1992). 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[3] 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 closely related to, the critical effect and then selecting the most sensitive. For threshold-based responses, 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.

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

[3]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 beta-HCH is not carcinogenic in animals or humans. Following USEPA (2005a) guidance, the following WOE cancer classification was determined for beta-HCH: "**not likely to be carcinogenic in humans**."

For non-cancer effects, the body of evidence suggests that the liver is the most sensitive target organ for toxicity. A summary of the information supporting this determination is presented 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 the carcinogenicity evaluation. Overall, the available epidemiological evidence for beta-HCH is not suggestive of carcinogenicity in humans.

The body of epidemiological evidence is limited to studies of associations between body burden and cancer incidence/risk. Relevant studies investigated associations between beta-HCH and breast cancer, non-Hodgkins lymphoma (NHL), endometrial cancer, and testicular germ cell cancer. All of these data have various methodological and statistical limitations.

As shown in Table 1, nine studies evaluating the potential association between beta-HCH and breast cancer were located in the literature. Seven of these studies (Aronson et al. 2000; Demers et al. 2000; Guttes et al. 1998; Hoyer et al. 1998; Lopez-Carillo et al. 2002; Ward et al. 2000; Zheng et al. 1999) found no significant association between beta-HCH body burden and increased risk of breast cancer. Two of the nine studies (Mathur et al. 2002 and Mussalo-Rauhamaa et al. 1990) reported a positive association, but significant study limitations compromise the ability to link any response to beta-HCH exposure. Mathur et al. (2002) for example, did not measure or account for body fat levels (a parameter which is associated with breast cancer [ATSDR 2005]) in the analysis, or control for the presence of other organochlorine (OC) pesticides in the blood which could have contributed to the incidence of breast cancer. Mussalo-Rauhamaa et al. (1990) used cadavers and did not control for potential confounders including life-style factors which are known to be associated with breast cancer. Additionally, the small sample size did not allow for stable estimates of risk to be ascertained.

Four case-control studies evaluating the potential association between beta-HCH tissue concentration and NHL risk were available. Two of the four studies detected an association between beta-HCH and NHL risk in at least one model. Spinelli et al. (2007) found a weak association between plasma levels of beta-HCH and risk of NHL, although the study's low response rate and low statistical power limit its usefulness for making conclusions on any causal association between the agent and disease. Quintana et al. (2004) reported a significant association between beta-HCH in adipose tissue in the single pesticide model, but the association was not significant for the two-pesticide model that was used in order to explore the influence of potential confounding factors. Cantor et al. (2003) and Cocco et al. (2008) did not find an association between concentrations of beta-HCH and NHL risk; these studies also had methodological limitations.

The two epidemiological studies evaluating reproductive cancers found no associations between body burden of beta-HCH and cancer.

Overall, the body of epidemiological studies evaluating a potential association between body burden of beta-HCH and cancer risk do not show a consistent or strong relationship linking beta-HCH exposure to cancer. Although there are limitations associated with the collective body of evidence, the epidemiological data do not indicate that beta-HCH is carcinogenic in humans.

3.1.2 Animal Bioassays

Overall, the animal bioassay data do not indicate that beta-HCH is carcinogenic in animals. Table 2 presents summaries of animal bioassays reviewed for evaluation of beta-HCH carcinogenic potential.

A total of nine studies were reviewed. The studies utilized one rat strain and three mouse strains. Three of the reviewed studies (Fitzhugh et al. 1950; Goto et al. 1972; Thorpe and Walker 1973) were ultimately not included in the determination of carcinogenicity due to limitations of the studies, as described in Table 2. Of particular note, the Thorpe and Walker (1973) study which provided the basis for EPA's original 1993 classification of beta-HCH as a "possible human carcinogen" suffers multiple limitations which make it unreliable for determining the compound's carcinogenicity. High mortality was noted in the mice. Additionally, an increased incidence of spontaneous tumors was reported in control animals. Moreover, in USEPA's (2001) *Cancer Assessment Document, Evaluation of the Carcinogenic Potential of Lindane*, they dismissed the Thorpe and Walker study as unreliable for classifying carcinogenicity.

Five of the remaining studies evaluated effects following chronic or lifetime dietary exposure to beta-HCH. No tumors were observed in four studies - two in mice (Ito et al. 1973a,b) and two in rats (Ito et al. 1975; Van Velsen et al. 1986) – although hepatotoxicty and/or other toxicity was

observed. Hanada et al. (1973) reported mammary tumors in 2/8 mid-dose female mice but none in the higher dose, and no liver or other tumors at any dose tested.

Beta-HCH is not a tumor initiator: no hepatic foci were observed in partially hepatectomized rats given a single dose of beta-HCH followed by 15 weeks of dietary Phenobarbital (PB) (Schroter et al. 1987).

3.1.3 Mutagenicity and Genotoxicity Assays

Overall, the available evidence for beta-HCH suggests that it is not mutagenic but could cause deoxyribonucleic (DNA) fragmentation. Table 3 summarizes the short-term mutagenicity and genotoxicity assays for beta-HCH. Tanooka (1977) reported negative results for an *in vitro* gene mutation assay. Sagelsdoff et al. (1983) found that beta-HCH did not bind to DNA following *in vivo* exposures in mice. In a genotoxicity assay, Kalantzi et al. (2004) reported positive results for a comet assay measuring DNA fragmentation performed with high-doses of beta-HCH in human MC-7 breast and PC-3 prostate carcinoma cells but not at lower doses (data not shown by authors).

3.1.4 Summary of Carcinogenicity and Uncertainties for the Weight of Evidence

The collective WOE indicates that beta-HCH is not carcinogenic in humans or animals. The collective database suffers from some limitations due to study design, analysis, and/or reporting which are sources of uncertainty in the carcinogenicity evaluation.

3.2 NON-CANCER ENDPOINTS

Human and animal data were reviewed for non-cancer effects. Overall, the quality of the human epidemiological data is very limited and cannot be used to assess potential non-cancer effects in humans. In animals, non-cancer effects observed following subchronic and chronic exposures to beta-HCH include hepatic, renal, immunological (including hematopoietic), neurological, reproductive, and developmental effects (ATSDR 2005). Table 4 presents a summary of literature reviewed for non-cancer effects. Tables 5 through 8 present summaries of the study designs and findings for hepatic, immunological, neurological, and reproductive effects. Hematological effects evaluated were limited to red blood cell and neutrophil concentrations and were, therefore, encompassed in the category of immunological effects. Renal effects were determined to be of limited utility for the sensitivity evaluation, due to observed effects in controls.

Overall, hepatic, reproductive and immunological endpoints were associated with the lowest LOAELs across the endpoints evaluated. Of these, liver is the most sensitive target organ for beta-HCH toxicity. The data supporting this conclusion are presented below.

3.2.1 Human Data

Available epidemiological studies that evaluated the relationship between body burden of beta-HCH and various adverse effects were reviewed. Epidemiological studies were reviewed for neurological, immunological, and reproductive endpoints. The following conclusions were reached:

- Epidemiological studies that examined a potential relationship between immunological effects (NHL) and exposure to beta-HCH were inconclusive. Two studies suggested a weak association between beta-HCH exposure and NHL (Quintana et al. 2004; Spinelli et al. 2007) while two suggested no association (Cantor et al 2003; Cocco et al. 2008). All four studies had serious limitations.
- There were insufficient epidemiological data to assess neurological effects. Only one study was reviewed that indicated neurological effects in humans. This study found detectable levels of beta-HCH in a greater number of patients with Parkinson's Disease (PD) than in controls; however, this study had several limitations, including small sample size. In addition, a substantial number of the patients with PD had no detectable levels of beta-HCH (Richardson et al. 2009).
- Epidemiological studies that focused on reproductive effects were inconclusive. Epidemiological evidence for relationships between body burdens of HCH and breast cancer is inconclusive, with some studies suggesting a positive correlation, but most others failing to demonstrate a relationship (Calle et al. 2002; Zou and Matsumura 2003). Other studies that looked at body burden of beta-HCH in females and effects on reproductive outcomes did not identify significant adverse effects associated with beta-HCH. Studies that looked at body burden of beta-HCH in males and developmental effects either were inconclusive or found no significant associations between beta-HCH and adverse testicular effects (Pierek et al. 2007; Hosie et al. 2000; McGlynn et al. 2008).

Due to limitations in study design, results, and reporting, data from epidemiological studies are not sufficient to inform either the types of toxicity or the most sensitive endpoint following beta-HCH exposures.

3.2.2 Animal Bioassays

Only four animal studies were identified that met the criteria adopted for data quality, exposure duration, and low dose exposure. These four studies were reviewed to determine the most sensitive toxic endpoint for beta-HCH. Toxic responses observed in these studies are documented by endpoint in Tables 5 through 8.

The most comprehensive study evaluated responses associated with all four target endpoints of interest (Van Velsen et al. 1986). The critical review of the data determined that hepatic effects were the most sensitive in this study. In the two highest dose groups, distinct hyalinization of centrilobular cells was observed in males and increased number of mitoses was detected in females. In the two lowest dose groups, slight hyalinization of centrilobular cells was observed in males only. The lowest LOAEL for hepatic effects in this study was 0.18 mg/kg-day for hyalinization of centrilobular cells in males.

Two additional studies found LOAELs of 0.03 mg/kg-day (Schroter et al. 1987) and 0.79 mg/kg-day (Fitzugh et al. 1950) for hepatic effects in rats. The LOAEL of 0.03 mg/kg-day, although significant, was not part of a dose-dependent trend (dose-dependent effects were observed to begin at 3 mg/kg-day) (Schroter et al. 1987).

LOAELs reported by Van Velsen et al. (1986) for other effects (0.13 mg/kg-day for both immunological and renal effects in females) are of questionable significance. Specifically, the LOAEL of 0.13 mg/kg-day reported for immunological effects was not dose-dependent and was not consistently observed in both sexes. Additionally, the LOAEL of 0.13 mg/kg-day reported for renal effects was associated with increased relative kidney weights in females, but not males, and there were adverse effects also observed in the kidneys of the control females. Because of these limitations, the Van Velsen et al. (1986) results for these endpoints were not used to identify the most sensitive endpoint for dose-response modeling.

3.3 MOST SENSITIVE TARGET ORGAN

Overall, the available data indicate that the liver is the most sensitive target organ following subchronic or chronic exposure to beta-HCH. The animal bioassay which evaluated the greatest number of endpoints identified liver as the most sensitive target organ (Van Velsen et al. 1986), and this was supported by the results of two other studies (Schroter et al. 1987; Fitzugh et al. 1950) with hepatic effects at similar dose levels.

4 TOXICITY CRITERION

A final oral RfD of 0.00006 mg/kg-day was established for beta-HCH. The toxicity criterion is based on the LOAEL of 0.18 mg/kg-day from Van Velsen et al. (1986) for hyalinization of centrilobular cells in the liver and the combined UF of 3,000.

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

The available evidence supports the conclusion that beta-HCH does not cause cancer in humans or laboratory animals. The liver was determined to be the most sensitive target organ for beta-HCH; measured adverse effects in the liver, therefore, provide the appropriate endpoint for the derivation of a toxicity criterion for the compound.

Data evaluated for the derivation of a toxicity criterion were limited to three studies of hepatic effects of beta-HCH (see Table 5).

Table 9 presents a comprehensive listing of the hepatic endpoints available for toxicity criterion development for beta-HCH. It provides a summary of which of the endpoints that were considered appropriate for the POD determination. It additionally shows which data were amenable to BMD analysis. The specific reasoning for data excluded from the BMD analysis was provided.

Responses including early microscopic changes to the liver (e.g., foci formation, hyalinization of centrilobular cells, mitoses, focal cell necrosis, periportal fat accumulation) were brought forward for the POD evaluation. These endpoints do not constitute adverse effects; but they are potential precursors of adverse effects in rodent hepatotoxicity (Klaassen 2008). Measures indicative of liver injury including, alanine aminotransferase (ALT) and hepatic glycogen were also considered. Finally, toxic endpoints, including gross macroscopic changes to the liver were brought forward for the POD determination. Cytochrome P450 (CYP450) concentrations and activity were not included as endpoints for the POD evaluation because such responses are not tightly linked to toxic endpoints and; thus, do not represent a critical effect that is both consistently predictive of adverse toxicity and biologically significant.

4.2 DETERMINATION OF POINT OF DEPARTURE

Two approaches for deriving the POD were explored: a traditional RfD approach and BMD modeling. Although BMD modeling has recognized advantages over the traditional RfD approach (USEPA 2000; Castorina and Woodruff 2003), all data sets are not amenable to BMD modeling[4]. Exploring results via both approaches allowed for a comprehensive evaluation of the available data.

For the traditional RfD approach, the lowest effect level of the endpoints considered was reported by Van Velsen et al. (1986). This study reported a LOAEL of 0.18 mg/kg-day for hyalinization of centrilobular cells in male rats exposed to beta-HCH via the diet. The statistical significance of this effect was not evaluated by the authors. This effect was observed at the lowest tested dose, and therefore a NOAEL for the effect was not established.

The results of the BMD modeling are provided in Table 10. Of the low-dose studies/endpoints identified for the POD evaluation, only a subset of the data from a single study (Van Velsen et al. 1986) modeled successfully. The most sensitive modeled effect was an increase in mitoses in the liver of female Wistar rats exposed to beta-HCH through the diet for 13 weeks. The confidence limit of the benchmark dose (BMDL) associated with this effect was 0.90 mg/kg-day. Data for hyalinization of centrilobular cells (reported as the most sensitive effect above for the traditional RfD approach, above) could not be modeled because the statistical significance of the effect was not recorded in the study.

The POD was conservatively selected using a traditional RfD approach because the lowest measured hepatic LOAEL was lower than the lowest BMDL. The POD determined for beta-HCH was 0.18 mg/kg-day based on the LOAEL for hyalinization of centrilobular cells reported by Van Velsen et al. (1986). This effect was observed at the lowest tested dose, and therefore a NOAEL for the effect was not established. This response was selected as the POD because it was the most sensitive hepatic effect observed in the most comprehensive low dose animal bioassay.

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 beta-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.

[4] All BMD modeling was completed using EPA's Benchmark Dose Software version 2.1, and following EPA guidance on benchmark dose modeling (USEPA 2000).

- **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 3 was selected for this factor. The Van Velsen et al. (1986) study was subchronic in duration.
- **LOAEL-to-NOAEL Factor** - A value of 10 was selected for this factor. The POD selected was a LOAEL.
- **Database UF** - A value of 3 was selected for this factor to account for data gaps in the investigation of non-hepatic effects. However, significant data gaps that would affect the determination of the critical effect and the POD for that critical effect for hepatic effects were not identified.
- **Additional MF** - No additional MFs were determined necessary for the derivation of the toxicity criterion.

Although the mathematical combination of all these factors would equal a total UF of 9,000; the maximum UF to be applied to any POD is 3,000, per USEPA (2002) guidance. Therefore, the total UF to be applied to the POD in this case is 3,000.

4.4 RECOMMENDED TOXICITY CRITERION FOR BETA-HCH

The recommended toxicity criterion for beta-HCH is an oral RfD of 0.00006 mg/kg-day. This value is based on a POD of 0.18 mg/kg-day for hyalinization of centrilobular cells reported by Van Velsen et al. (1986) and an UF of 3,000.

5 SUMMARY

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

The cancer classification for beta-HCH is "**not likely to be carcinogenic in humans**." For non-cancer effects, the body of evidence indicates that the liver is the most sensitive target organ following chronic exposure to beta-HCH.

The recommended toxicity criterion for beta-HCH is an oral RfD of 0.00006 mg/kg-day. The criterion is derived using a POD of 0.18 mg/kg-day for hyalinization of centrilobular cells in male rats reported by Van Velsen et al. (1986). The use of this response as the POD is conservative, because this response is a precursor event in the biological continuum of rodent hepatotoxicity. The RfD includes a total UF of 3,000 to account for inter- and intra-species differences, use of a LOAEL, use of a subchronic study, and database limitations.

For perspective, the recommended RfD is equal to the oral chronic RfD established by EPA for beta-HCH in their 2006 *Assessment of Lindane and Other Hexachlorocyclohexane Isomers* (USEPA 2006), completed as part of the Reregistration Eligibility Decision (RED) for Lindane. The chronic oral RfD proposed by EPA is based on an identical POD (i.e., a LOAEL of 0.18 mg/kg-day reported by Van Velsen et al. (1986)) and cumulative UF of 3,000 as those applied as the components of the RfD recommended here.

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TABLES

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

Endpoint	Study	Summary of Findings	Study Limitations
Breast Cancer			
684	Aronson et al. (2000)	Hospital-based case-control study in Ontario, Canada. Found no association between beta-HCH levels in breast adipose tissue and breast cancer risk. (Significant associations were found for other pesticides).	Use of hospital controls only. Differences existed in cases and controls for which tissue was obtained, compared to others identified for study inclusion and resulted in more narrowly defined study population. In the case that the disease process may have modulated pesticide concentrations the close temporal relationship between collection of samples and time of diagnosis may have influenced the results.
685	Demers et al. (2000)	Case-control study in Quebec City, Canada. Used hospital and general-population controls. Found no relationship between levels of beta-HCH in lipid adjusted serum and the relative risk of breast cancer. Found an association between beta-HCH levels in lipid adjusted serum and large tumors with lymph-node invasion in women diagnosed with breast cancer (OR=2.25; 95% CI=1.12-4.51). Results suggest that exposure to beta-HCH may influence growth or aggressiveness of breast cancer, rather than initiate breast cancer.	In the case that the disease process may have modulated pesticide concentrations the close temporal relationship between collection of samples and time of diagnosis may have influenced the results.
595	Guttes et al. (1998)	Analysis of surgically removed breast tissue from patients in Germany with either benign or malignant breast disease. Found no difference between concentrations of beta-HCH in breast tissue of patients with malignant breast disease compared to benign breast disease.	Small sample size (N=45 cases, 20 controls). Did not measure or control for potential confounding factors/risk factors for breast disease. Use of patient-controls with breast disease only; if an association between beta-HCH and benign breast disease exists the use of benign breast disease patients as controls could lead to an underestimation of true relative risk for carcinoma.
686	Hoyer et al. (1998)	Prospective study in Danish women participating in the Copenhagen City Heart Study. Found no significant association between lipid adjusted serum concentrations of beta-HCH and breast cancer (slight increasing trend was not significant).	Limited statistical power.
535	Lopez-Carillo et al. (2002)	Hospital-based case-control study of women from Mexico City. Found no association between beta-HCH in lipid adjusted serum and breast cancer.	No discussion of follow-up for breast-cancer diagnosis in controls. In the case that the disease process may have modulated pesticide concentrations the close temporal relationship between collection of samples and time of diagnosis may have influenced the results.
456	Mathur et al. (2002)	Hospital-based case-control study in women from India. Found higher levels of beta-HCH in blood in women (age 31-50) with breast cancer compared to controls.	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.
537	Mussalo-Rauhamaa et al. (1990)	Case-control study of women in Finland. Controls were from cadavers of accident fatalities. Concentrations of beta-HCH in adipose breast tissue of breast cancer patients was greater than in controls (p =0.026).	Small sample size (N=44 cases, 33 controls). The disease and treatment status of cases was not fully described. Controls were obtained from post-mortem examinations that did not allow for collection of information on potential confounders.
571	Ward et al. (2000)	Prospective case-control study using samples collected from Norwegian serum bank. Found no association between concentrations of beta-HCH in lipid adjusted serum and breast cancer risk.	No data available on some potential confounders including menopausal status and BMI. Slightly negative associations between OC levels and disease suggest potential for systmatic bias in the selection of cases and controls.
474	Zheng et al. (1999)	Case-control study using surgically removed breast tissue from patients in Connecticut with either benign or malignant breast disease. Found no association between concentrations of beta-HCH in adipose breast tissue and risk of breast cancer carcinoma.	Relatively low levels of beta-HCH were measured and do not allow for a full dose response relationship to be explored. If an association between beta-HCH and benign breast disease exists the use of benign breast disease patients as controls could lead to an underestimation of true relative risk for carcinoma.
Non-Hodgkins Lymphoma (NHL)			
480	Cantor et al. (2003)	Prospective case-control study based on data from cancer registry in Washington County, Maryland. Found no association between beta-HCH in lipid adjusted serum and risk of NHL.	Did not measure or control for all potential confounders. Analytical sample results had large variance which would have the potential to obscure associations of relatively small magnitude.
517	Cocco et al. (2008)	Case-control study of individuals from France, Germany, and Spain participating in a European multicenter study of environmental exposures. Found no association between beta-HCH in lipid adjusted plasma and risk of NHL.	Limited discussion of ascertainment of disease and selection of controls. In the case that disease process and/or chemotherapy alter pesticide levels the timing of sample collection may have influenced results. Potential for measurment bias due to time lag between blood withdrawal and analysis.
509	Quintana et al. (2004)	Nested case-control study using samples collected from cadavers and surgical patients as part of the EPA National Human Adipose Tissue Survey. Found an association between concentrations of beta-HCH in adipose tissue and NHL (quartile trend of ORs, p <0.05) in the single pesticide model. No association was present in the two-pesticide model applied to explore potential confounding.	Due to post-mortem collection, there is a lack of detailed information about potential confouders including lifestyle factors and other disease conditions. OC analyses were completed in different laboratories over time. NHL cases were limited to those with poor prognosis or fatal effects.

Table 1. (continued)

Endpoint	Study	Summary of Findings	Study Limitations
317	Spinelli et al. (2007)	Case-control study of individuals in Canada enrolled in the British Columbia Cancer Registry. Found a weak association between plasma levels of beta-HCH and NHL (quartile trend of ORs, p <0.05).	Low response rate. Study had limited power to detect interactions among variables. Incomplete information on type and length of exposure.
Endometrial Cancer			
567	Sturgeon et al. (1998)	Case-control study of patients in 5 geographic areas of the United States. Found no association between lipid adjusted serum concentrations of beta-HCH and endometrial cancer incidence. (Significant associations were found for other pesticides).	Time frame between diagnosis and blood collection was not clear. In the case that disease process and/or therapy alter pesticide levels the timing of sample collection may have influenced results. Incomplete information on follow-up of controls.
Testicular Germ Cell Cancer			
301	McGlynn et al. (2008)	Prospective case-control study of military servicemen. Found no association between serum levels of beta-HCH and the risk of testicular germ cell tumors. (Positive associations were found for other OC pesticides).	Potential for recall bias from questionnaire (cases were asked to answer questions in reference to a historical date prior to diagnosis). Some parameters including body weight were self-reported rather than measured. Analysis included multiple comparisons which may influence reliability of results. Study did not ascertain when or how exposure occured, and therefore the critical window of exposure could not be analyzed.

Notes:
BMI = body mass index
CI = confidence interval
EPA = U.S. Environmental Protection Agency
HCH = hexachlorocyclohexane
NHL = non-Hodgkin's lymphoma
OC = organochlorine
OR = odds ratio

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

	Reference	Species, Sex	Study Design	Summary of Findings	Major Study Limitations
382	Fitzhugh et al. (1950)	Rat (Wistar), male/female	**Duration:** Approximately 107 weeks **Sample Size:** 10/sex/group **Route:** dietary, ad libitum **Dose Levels:** 0, 10, 100, 800 ppm	Dose-dependent decrease in survival, which was significant at highest dose tested (800 ppm). Significant dose-dependent increase in relative liver weight. Dose-dependent increase in gross and microscopic liver changes. No gross tumors reported.	Small sample size. Minimal details on histopathology. High overall mortality in the study; evaluations were based either on moribund or found dead animals. Inadequate discussion of mortality/general toxicity. Data were not stratified by sex.
383	Goto et al. (1972)	Mouse (ICR-JCL), male	**Duration**: 26 weeks **Sample Size:** 10/group evaluated **Route:** dietary (unknown if ad libitum) **Dose Levels:** 0, 600 ppm	Increased relative liver weight. Microscopically, "hepatomas" were observed and described as atypical proliferation or hyperplastic knot. Hepatoma incidence in control animals not reported.	Only one dose tested. 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.
385	Hanada et al. (1973)	Mouse (dd), male/female	**Duration:** 32 weeks plus 5-6 weeks recovery **Sample Size:** 10-11/sex/treatment group; 21 males and 20 females for the control group at start of experiment **Route:** dietary, ad libitum **Dose Levels:** 0, 100, 300, 600 ppm	No liver tumors seen during week 26 laparotomy. No liver tumors seen after exposure plus recovery. Atypical hepatocellular proliferation seen in 300 and 600 ppm males (4/8 and 8/8) and females (2/8 and 3/4) after exposure plus recovery. No atypical cellular changes or tumors in control animals. No peritoneal invasion or extra-hepatic metastases seen microscopically. 2/8 300 ppm females had mammary carcinoma.	Potentially increased mortality, particularly in 600 ppm females. Small sample size. No statistical analysis. General toxicity data were not reported. No evaluation done at the end of the 32 week exposure period; regression of changes could not be evaluated.
363	Ito et al. (1973a)	Mouse (dd), male	**Duration:** 24 weeks **Sample Size:** 20/group **Route:** dietary, ad libitum **Dose Levels:** 0, 100, 250, 500 ppm	No tumors. Relative liver weight slightly increased (dose-dependent). Liver cell hypertrophy seen at 500 and 250 ppm; more pronounced at 500 ppm. No nodules or HCC in treated or control animals. Proliferation of smooth endoplasmic reticulum seen at 500 ppm.	Only males tested. No statistical analysis. Only examined liver histologically. Mortality not reported.
364	Ito et al. (1973b)	Mouse (dd), male	**Duration:** 24 weeks **Sample Size:** 20-28/group **Route:** dietary (unknown if ad libitum) **Dose Levels:** 0, 50, 100, 250 ppm	No tumors. Relative liver weight slightly increased; similar across doses. Centrilobular hypertrophy seen at 250 ppm. No nodules or HCC in treated or control animals. No cirrhosis or metastases. Body weight not affected.	No statistical evaluation. Only males evaluated. Unclear if extra-hepatic tumors/metastases were evaluated microscopically. Mortality not reported.
386	Ito et al. (1975)	Rat (Wistar), male	**Duration:** 72 weeks; interim sacrifices **Sample Size:** 5-8/group **Route:** dietary, ad libitum **Dose Levels:** 0, 500, 1000 ppm	No tumors. Increased relative liver weight; not dose-dependent. No benign nodules or HCC at 24 or 48 weeks. Hepatocellular hypertrophy was observed in the 500 ppm 48 week group and in the 1000 ppm 24 week group but not in the 500 ppm 24 week group. No metastases.	Control animals sacrificed at different time than treated animals. Mortality not reported. Unclear if metastases were evaluated grossly or microscopically. Insufficient description of general toxicity. Only males evaluated. Small sample size. No statistical evaluation.
390	Schroter et al. (1987)	Rat (Wistar), female	**Duration:** 17 weeks (initiation); 15-20 weeks following initiation by NNM (promotion) **Sample Size:** 3-7/group (initiation) **Route:** oral gavage (initiation); dietary, ad libitum (promotion) **Dose Levels:** 0, 100 mg/kg (initiation); 0, 0.03 0.2, 1, 3, 10 mg/kg (promotion)	**Initiation Study:** No increase in GGT-positive foci in rats subject to partial hepatectomy, then a single oral dose of HCH (100 mg/kg), then dietary phenobarbital for 15 weeks. **Promotion Study:** Dose-dependent increase in GGT-positive foci number and area after 15 or 20 weeks of beta-HCH exposure in NNM-initiated rats. Foci number and area were increased at 20 weeks relative to 15 weeks, particularly at high doses. Foci area was significantly increased relative to control at mid- to high-doses. No other temporal trends were evident. Dose-dependent increases in liver DNA and liver mass after 15 or 20 weeks, with some significant findings. Slight dose-dependent increases in P450 activity after 15 and 20 weeks relative to NNM-only rats. Correlation analysis suggested that foci growth is not strongly correlated with P450 induction.	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 HCH alone, without initiation, was not evaluated in the promotion study.
395	Thorpe and Walker (1973)	Mouse (CF1), male/female	**Duration:** 2 years **Sample Size:** 30/sex/group (treated); 45/sex/group (control) **Route:** dietary (unknown if ad libitum) **Dose Levels:** 0, 200 ppm	Decreased survival in treated animals vs. controls. Liver enlargement seen after 50-60 weeks. Some treated mice exhibited ataxia before death. Mice dying early had hepatic and extra-hepatic tumors; males were more susceptible to hepatic tumors than females. Lung metastases noted in males but not females.	Only one dose level evaluated. Increased mortality. High incidence of spontaneous lung, liver, and lymphoid tumors in untreated control animals.
399	Van Velsen et al. (1986)	Rat (Wistar), male/female	**Duration:** 13 weeks **Sample Size:** 10/sex/group **Route:** dietary, ad libitum **Dose Levels:** 0, 2, 10, 50, 250 mg/kg	No tumors. Total P450 significantly increased in the 50 and 250 mg/kg males; P450 activity increases seen starting at 2 mg/kg (males) and 250 mg/kg (females). Significant dose-dependent increases in relative liver weights (males and females).	Considerable mortality (>50%), adverse clinical signs (ataxia, comatose), and reduced body weight gain seen in 250 mg/kg group. Many gross and microscopic changes in multiple organs at 250 mg/kg.

Notes:
DNA = deoxyribonucliec acid
GGT = gamma-glutamyl transpeptidase
GST = glutathione-S-transferase
HCC = hepatocellular carcinoma
HCH = hexachlorocyclohexane (beta isomer)
mg/kg = milligram per kilogram
NNM = N-nitrosomorpholine
P450 = cytochrome P450
ppm = part per million

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

Reference	Test System: *In Vitro / In Vivo*	Test System: Species/Strain/ Cell Type	Assay/Test	Endpoint	Treatment	Result	Comments
Mutation							
433 Tanooka (1977)	*In vitro*	*Bacillus subtilis* TKJ5211	Spot test	Gene mutation	5,000 μg/plate	Negative	
DNA Binding							
408 Sagelsdorff et al. (1983)	*In vivo*	NMRI mice	HPLC analysis of nucleosides	DNA binding	7.3-7.7 mg/kg	Negative	
DNA Damage, Fragmentation, and Repair							
290 Kalantzi et al. (2004)	*In vitro*	Human MCF-7 breast carcinoma cells	Comet assay	DNA fragmentation	10^{-4} 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^{-4} 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.

Notes:
DNA = deoxyribonucleic acid
HCH = hexachlorocyclohexane
HPLC = high performance liquid chromatography
M = molar mass
mg/kg = milligram per kilogram
μg/plate = microgram per plate

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

Reference [a]		Included in Endpoint Sensitivity Evaluation		Reason for Exclusion [b]
Hematological Endpoints				
399	Van Velsen et al. (1986)	Yes		NA
Hepatic Endpoints				
382	Fitzhugh et al. (1950)	Yes	*	NA
383	Goto et al. (1972)	No	*	Acute exposure/High dose
385	Hanada et al. (1973)	No	*	Acute exposure/High dose
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
389	Kraus et al. (1981)	No	*	Acute exposure/High dose & MOA endpoint/*in vitro*
390	Schroter et al. (1987)	Yes	*	NA
395	Thorpe and Walker (1973)	Yes	*	NA
399	Van Velsen et al. (1986)	Yes	*	NA
Immunological Endpoints				
480	Cantor et al. (2003)	Yes	*	NA
517	Cocco et al. (2008)	Yes	*	NA
518	Cornacoff et al. (1988)	Yes		NA
522	Daniel et al. (2001)	No		Reliability Rank
626	Das et al. (1990)	No		Reliability Rank
633	Nigam et al. (1993)	No		Multiple isomer treatment
509	Quintana et al. (2004)	Yes	*	NA
317	Spinelli et al. (2007)	Yes	*	NA
660	Sweet et al. (2006)	No		MOA endpoint/*in vitro*
399	Van Velsen et al. (1986)	Yes		NA
323	Wang et al. (2006)	No		Multiple isomer treatment
Neurological Endpoints				
518	Cornacoff et al. (1988)	Yes		NA
534	Lopez-Espinosa et al. (2009)	Yes		NA
633	Nigam et al. (1993)	No		Multiple isomer treatment
545	Richardson et al. (2009)	Yes		NA
358	Srivastava and Shivanandappa (2005)	No		Acute exposure/High dose & MOA endpoint/*in vitro*
399	Van Velsen et al. (1986)	Yes		NA
Renal Endpoints				
382	Fitzhugh et al. (1950)	Yes		NA
399	Van Velsen et al. (1986)	Yes		NA
Reproductive/Developmental Endpoints				
278	Alvarez-Pedrerol et al. (2008)	Yes		NA
684	Aronson et al. (2000)	Yes		NA
518	Cornacoff et al. (1988)	Yes		NA
685	Demers et al. (2000)	Yes		NA
595	Guttes et al. (1998)	Yes		NA
353	Hatakeyama et al. (2002)	Yes		NA
642	Hosie et al. (2000)	Yes		NA
686	Hoyer et al. (1998)	Yes		NA
527	Itoh et al. (2009)	Yes		NA
291	Khanjani and Sim (2006)	Yes		NA
535	Lopez-Carrillo et al. (2002)	Yes		NA
456	Mathur et al. (2002)	Yes		NA
301	McGlynn et al. (2008)	Yes	*	NA
537	Mussalo-Rauhamaa et al. (1990)	Yes		NA
542	Pathak et al. (2009)	Yes		NA
306	Pierik et al. (2007)	Yes		NA
551	Shivanandappa and Krishnuakumari (1983)	No		Multiple isomer treatment
565	Siddiqui et al. (2003)	Yes		NA
466	Steinmetz et al. (1996)	Yes		NA
567	Sturgeon et al. (1998)	Yes	*	NA

Table 4. (continued)

	Reference [a]	Included in Endpoint Sensitivity Evaluation	Reason for Exclusion [b]
399	Van Velsen et al. (1986)	Yes	NA
571	Ward et al. (2000)	Yes	NA
326	Wong and Matsumura (2007)	No	MOA endpoint/*in vitro* /Reliability rank
474	Zheng et al. (1999)	Yes	NA
361	Zho and Matsumura (2003)	Yes	NA

Notes: HCH = hexachlorocyclohexane
MOA = mode of action
NA = not applicable
* = study determined useful for other aspects of the evaluation (carcinogenicity and/or MOA evaluation).

[a] 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.

[b] Studies were not selected for the sensitivity evaluation, 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 Beta-HCH: Summary of Animal Bioassay Studies at Low Doses, Liver Effects.

Reference [a]		Dose (exposure)					Response			
		Species, Sex	Study Design	Dose Range	Exposure Duration	Sample Size	Observed Response [b]	LOAEL (s) (mg/kg-day)	NOAEL (s) (mg/kg-day)	Major Study Limitations
382	Fitzhugh et al. (1950)	Rat (Wistar), male/ female	Multiple dose dietary bioassay	0, 10, 100, 800 ppm (0, 0.8, 7.9, 63.2 mg/kg-day)[c]	Approximately 107 weeks	10/sex/group; 20/sex/group controls	Significant increase in relative liver weight at 10, 100, and 800 ppm.	0.79		Substantial mortality in both control and all treatment groups.
							Slight microscopic liver changes seen at 10 ppm.	0.79		
							Gross histological liver changes and microscopic changes seen at 100 and 800 ppm.	7.9	0.79	
390	Schroter et al. (1987)	Rat (Wistar), female	Single dose initiation and multiple dietary dose promotion study	0, 100 mg/kg-day (initiation); 0, 0.03, 0.2, 1, 3, 10 mg/kg-day (promotion)	Single dose (initiation); 15 or 20 weeks (promotion)	3-7/group (initiation)	Significant increase in liver DNA after 20 weeks at 3 and 10 mg/kg.	3	1	Only females tested. Promotion measured after initiation with known carcinogen.
							Significant increase in liver mass after 15 or 20 weeks at 10 mg/kg. Dose-dependent increase in monooxygenase activity after 15 or 20 weeks all doses (not significant).	10	3	
							Significant increase in foci area after 20 weeks at 0.03, 3, and 10 mg/kg; and after 15 weeks at 10 mg/kg. Dose-dependent increase started at 3 mg/kg-day.	0.03		
399	Van Velsen et al. (1986)	Rat (Wistar) male/ female	Multiple dose dietary bioassay	0, 2, 10, 50, 250 ppm (0, 0.18, 4.5, 22 mg/kg-day males; 0, 0.13, 0.66, 3.3, 16 mg/kg-day females)[d]	13 weeks	10/sex/group	Significant increase in hepatic glycogen concentration at 250 ppm in males.	22 (males)	4.5 (males)	Food consumption rate not reported. Highest dose may exceed maximum tolerated dose.
							Significantly higher microsomal enzyme (AH and APDM) and P450 concentrations in 50 and 250 ppm males.	4.5 (males)	0.89 (males)	
							P450 activity increases seen starting at 2 ppm males (significant at 50 ppm) and 250 ppm females (not significant).	4.5 (males)	0.89 (males)	
							Significant dose-dependent increase in absolute liver weight at 10, 50, and 250 ppm groups (males and females).	0.89 (males) 0.66 (females)	0.18 (males) 0.13 (females)	
							Significant dose-dependent increase in relative liver weight at 10, 50, and 250 ppm females and 50 and 250 ppm males.	4.5 (males) 0.66 (females)	0.89 (males) 0.13 (females)	
							Hyalinization of centrilobular cells (beginning at 10 ppm) and focal cell necrosis, increased mitoses, and Kupffer cell activity beginning at 50 ppm were reported, but statistical significance was not evaluated for these effects.	0.18 (males)		

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

Notes:
AH = aniline hydroxylase
APDM = aminopyrin-N-demethylase
DNA = deoxyribonucleic acid
HCH = hexachlorocyclohexane
kg = kilogram
kg/day = 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

[a] 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.

[b] Responses were considered significant only for effects reported to be statistically significant at $p < 0.05$.

[c] 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.

[d] Dietary concentrations in ppm converted to dose in mg/kg-day using estimated food consumption rates of 0.034 kg/day (males) and 0.025 kg/day (females) and default average body weight for both sexes of 0.38 kg. in the study.

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

		Dose (exposure)					Response				
	Reference [a]	Species, Sex	Study Design	Dose Range (mg/kg-day)	Exposure Duration	Sample Size	Test Employed/Effects Tested	Observed Response [b]	LOAEL (s)	NOAEL (s)	Major Study Limitations
518	Cornacoff et al. (1988)	Mouse, female	Multiple dose dietary bioassay	0, 19, 58, 192 (0, 100, 300, 1000 ppm) [c]	30 days	6/group	Spleen weight	Significantly increased at 300 ppm.	58	19	Highest dose (1000 ppm) induced substantial mortality and results were not reported.
							Thymus weight	No significant differences from control.		19	
							Spleen cellularity	No significant differences from control.		19	
							Concentration of RBCs	Significantly increased at 100 ppm, but not at 300 ppm. Not dose dependent.	19		
							Concentration of WBCs	No significant differences from control.		19	
							Absolute PMNs (neutrophils)	No significant differences from control.		19	
							Absolute lymphocytes	Significantly increased at 100 and 300 ppm, but not dose dependent.	19		
							Absolute monocytes	No significant differences from control.		19	
							Antibody PFC response to sheep RBCs	No significant differences from control.		19	
							Splenic-lymphocyte proliferation	Lymphoproliferative response was significantly decreased only in three out of four assays using different mitogens, and at the highest reported dose of 300 ppm.	58	19	
							Cytolytic activity	Activity by cytotoxic T-lymphocytes and natural killer cells was significantly decreased only at the high dose reported (300 ppm) and is of limited biological significance due to functional immune reserves.	58	19	
399	Van Velsen et al. (1986)	Rat, male/ female	Multiple dose dietary bioassay	0, 2, 10, 50, 250 ppm (0, 0.18, 0.89, 4.5, 22 mg/kg-day males; 0, 0.13, 0.66, 3.3, 16 mg/kg-day females)[d]	13 weeks	10/sex/ group	Serum concentration of RBCs	Significantly decreased in highest dose group only (250 ppm) in both males and females.	22 males 16 females	4.5 males 3.3 females	Food consumption rate not reported. Highest dose may exceed maximum tolerated dose. White blood differentials and morphologic features of erythrocytes and thrombocytes were determined microscopically.
							Serum concentration of WBCs	Significantly decreased in highest dose group only (250 ppm) in both males and females.	22 males 16 females	4.5 males 3.3 females	
							Serum concentration of haemoglobin	Significantly decreased in highest dose group only (250 ppm) in both males and females.	22 males 16 females	4.5 males 3.3 females	
							Packed cell volume	Significantly decreased in highest dose group only (250 ppm) in both males and females.	22 males 16 females	4.5 males 3.3 females	
							Concentration of neutrophils	Significantly decreased in females at doses 2, 10 and 50 ppm, but not at 250 ppm. Significantly decreased in males at 250 ppm only. Not dose dependent.	22 males 0.13 females	4.5 males	
							Concentration of lymphocytes	Significantly decreased in highest dose group only (250 ppm) in both males and females.	22 males 16 females	4.5 males 3.3 females	
							Spleen - increased extramedullar hematopoiesis	Observed in both sexes at highest dose only (250 ppm).	22 males 16 females	4.5 males 3.3 females	
							Adrenal glands - cortical hypertrophy	Observed in both sexes at highest dose only (250 ppm).	22 males 16 females	4.5 males 3.3 females	
							Thymus - cortical hypertrophy	Observed in both sexes at highest dose only (250 ppm).	22 males 16 females	4.5 males 3.3 females	
							Relative spleen weight	Significantly increased at 50 ppm, but not at 250 ppm in females. Significantly increased in males at 250 ppm only. Not dose dependent.	4.5 males 3.3 females	0.89 males 0.66 females	
							Relative thymus weight	Significantly decreased in females at 50 and 250 ppm in dose dependent manner. Significantly increased in males at 50 ppm and decreased in males at 250 ppm; not dose dependent in males.	4.5 males 3.3 females	0.89 males 0.66 females	
							Relative adrenal gland weight	Significantly increased in both females and males at highest dose (250 ppm) only.	22 males 16 females	4.5 males 3.3 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
mg/kg-day	= milligram per kilogram per day
NK	= natural killer
NOAEL	= no-observed-adverse-effect level
PFC	= plaque-forming cell
ppm	= part per million
RBC	= red blood cell
WBC	= white blood cell

[a] 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.

[b] Responses were considered significant only for effects reported to be statistically significant at $p<0.05$.

[c] Dietary concentrations in ppm converted to dose in mg/kg-day using estimated food consumption rate of 0.0048 kg/day and average body weight of 0.025 kg.

[d] Dietary concentrations in ppm converted to dose in mg/kg-day using estimated food consumption rates of 0.034 kg/day (males) and 0.025 kg/day (females) and default average body weight for both sexes of 0.38 kg.

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

Reference [a]		Dose (exposure)					Response				
		Species, Sex	Study Design	Dose Range (mg/kg-day)	Exposure Duration	Sample Size	Test Employed/ Effects Tested	Observed Response [b]	LOAEL (s)	NOAEL (s)	Major Study Limitations
518	Cornacoff et al. (1988)	Mouse, female	Multiple dose dietary bioassay	0, 19, 58, 192 (0, 100, 300, 1000 ppm) [c]	30 days	6/group	Ataxia	Signs of ataxia within 1 week of exposure duration at 58 and 192 mg/kg-day. Ataxia resolved in a few days for the 58 mg/kg-day group, but persisted in the 192 mg/kg-day group to effects resulting in mortality.	58	19	Highest dose (1000 ppm) induced substantial mortality and results were not reported.
399	Van Velsen et al. (1986)	Rat, male/ female	Multiple dose dietary bioassay	0, 2, 10, 50, 250 ppm (0, 0.18, 0.89, 4.5, 22 mg/kg-day males: 0, 0.13, 0.66, 3.3, 16 mg/kg-day females) [d]	13 weeks	10/sex/ group	Ataxia	Several males and females in the highest dose group showed ataxia and became progressively inactive, resulting in mortality.	22 males 16 females	4.5 males 3.3 females	Food consumption rate not reported. Highest dose may exceed maximum tolerated dose.

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
mg/kg-day = milligram per kilogram per day
NOAEL = no-observed-adverse-effect level
ppm = parts per million

[a] 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.

[b] Responses were considered significant only for effects reported to be statistically significant at $p < 0.05$.

[c] Dietary concentrations in ppm converted to dose in mg/kg-day using estimated food consumption rate of 0.0048 kg/day and average body weight of 0.025 kg.

[d] Dietary concentrations in ppm converted to dose in mg/kg-day using estimated food consumption rates of 0.034 kg/day (males) and 0.025 kg/day (females) and default average body weight for both sexes of 0.38 kg.

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

		Dose (exposure)				Response				
	Reference [a]	Species, Sex	Study Design	Dose Range (mg/kg-day)	Exposure Duration	Sample Size	Test Employed/ Effects Tested	Observed Response [b]	LOAEL (mg/kg-day)	NOAEL (mg/kg-day)	Major Study Limitations
518	Cornacoff et al. (1988)	B6C3F1 Mice, female	Multiple dose dietary bioassay	0, 19, 58 (0, 100, 300 ppm) [c]	30 days	6/group	Thymus weight	No significant differences from control.		58	Highest dose (1000 ppm) induced substantial mortality and results were not reported.
							Histopathology	No significant differences from control in ovarian development (oogenesis, corpora lutea) at 300 ppm. No significant differences in endometrial epithelium of uteri at 300 ppm.		58	
399	Van Velsen et al. (1986)	Wistar Rat, male/ female	Multiple dose dietary bioassay	0, 2, 10, 50, 250 ppm (0, 0.18, 0.89, 4.5, 22 mg/kg-day males; 0, 0.13, 0.66, 3.3, 16 mg/kg-day females) [d]	13 weeks	10/sex/ group	Organ weights	Relative weight of ovaries was significantly increased in females at 10 ppm and significantly decreased at 250 ppm. Relative weight of testes was significantly decreased in males at 250 ppm.	0.66	0.13	Food consumption rate not reported. Highest dose may exceed maximum tolerated dose. White blood differentials and morphologic features of erythrocytes and thrombocytes were determined microscopically.
							Histopathology	Atrophy of testes, prostate and ovaries at 250 ppm. Reduced size of seminiferous tubules, lower number of Leydig cells, absence of spermatogonia at 250 ppm. Absence of corpora lutea in ovaries at 250 ppm. Hyperplasia of endometrium epithelium at 250 ppm.	16	3.3	

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
mg/kg-day = milligram per kilogram per day
NOAEL = no-observed-adverse-effect level
ppm = part per million

[a] 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.

[b] Responses were considered significant only for effects reported to be statistically significant at P<0.05.

[c] Dietary concentrations in ppm converted to dose in mg/kg-day using estimated food consumption rate of 0.0048 kg/day and average body weight of 0.025 kg.

[d] Dietary concentrations in ppm converted to dose in mg/kg-day using estimated food consumption rates of 0.034 kg/day)males) and 0.025 kg/day (females) and default average body weight for both sexes of 0.38 kg.

Table 9. Selection of Endpoints for Critical Effect: Beta-HCH.

	Reference [a]	Study Design	Observed Response in Liver [b]	Selected for Evaluation of POD [c]	Included in BMD Evaluation [d]
382	Fitzhugh et al. (1950)	Male and female rats (Wistar), dietary exposure at multiple doses, exposure of ~107 weeks	Relative liver weight	Yes	No [1d]
			Microscopic liver changes	Yes	No [1d]
			Gross macroscopic liver changes	Yes	No [1d]
390	Schroter et al. (1987)	Female rats (Wistar), dietary exposure at multiple doses following a known initiator, exposure of 15 or 20 weeks	Increase in liver DNA	Yes	Yes
			Liver mass	Yes	Yes
			P450 activity	No [1c]	--
			Area of hepatic foci	Yes	Yes
			Number of hepatic foci	Yes	Yes
399	Van Velsen et al. (1986)	Male and female rats (Wistar), dietary exposure at multiple doses, exposure of 13 weeks	Absolute and relative liver weight	Yes	Yes
			Hepatic glycogen concentration	Yes	Yes
			P450 activity and total P450 levels	No [1c]	--
			Liver histology incidence (hyalinization of centrilobular cells, mitoses, focal cell necrosis, periportal fat accumulation)	Yes	Yes
			Kupffer cell hyperactivity	Yes	No [2d]

Notes:
BMD = benchmark dose
DNA = deoxyribonucleic acid
HCH = hexachlorocyclohexane
mg/kg-day = milligram per kilogram per day
POD = point of departure
-- = not relevant, endpoint not selected for POD evaluation

[a] 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.

[b] Inclusive list of observed effects associated with the liver.

[c] Endpoints were not considered to be appropriate for the POD evaluation for the following reasons.

[1c] Endpoint is an early precursor that is not closely linked with an adverse effect, and is therefore not necessarily indicative of an adverse effect.

[d] Endpoints that were considered for the POD evaluation were additionally explored using BMD modeling where possible. Data for some endpoints/studies was not amenable to BMD modeling. The following reasons for exclusions are noted:

[1d] Number of animals evaluated was not reported.

[2d] Only one dose level evaluated or no dose-response trend observed.

Table 10. Results from BMD Analysis for Deriving a Toxicity Criterion for Beta-HCH.

	Reference	Test System	Endpoint	Variable Type	Best-Fit Model [a]	Variation Modeling [b]	BMD [c]	BMDL [c]
Low-Dose Studies								
390	Schroter et al. (1987)	Wistar rat (female)	DNA content	C	--	--	--	--
			Foci area	C	--	--	--	--
			Foci number	C	--	--	--	--
			Relative liver weight	C	--	--	--	--
399	Van Velsen et al. (1986)	Wistar rat (male and female)	Female mitoses	D	Log-probit	NA	3.53	0.90
			Male focal necrosis	D	Multiple	NA	8.32	3.13
			Female absolute liver weight	C	Linear	Constant	4.90	3.93
			Male mitoses	D	gamma	NA	13.84	4.11
			Female hyalinization	D	Weibull	NA	18.86	4.67
			Male glycogen content	C	Polynomial	Non-constant	22.90	21.15
			Female liver glycogen content	C	--	--	--	--
			Female relative liver weight	C	--	--	--	--
			Female periportal fat	D	--	--	--	--
			Male periportal fat	D	--	--	--	--
			Male absolute liver weight	C	--	--	--	--
			Male hyalinization	D	--	--	--	--
			Male relative liver weight	C	--	--	--	--

Notes:
BMD = benchmark dose
BMDL = lower 95% confidence interval on BMD
BMR = benchmark response
C = continuous
D = dichotomous
DNA = deoxyribonucleic acid
HCH = hexachlorocyclohexane
NA = not applicable
SD = standard deviation
-- = modeling was unsuccessful

[a] Criteria used for selection of best-fit model are described in the text.
[b] Applicable only for continuous variables.
[c] BMR for continuous data was 1 SD; BMR for dichotomous data was 10% change.

ATTACHMENT A

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