

Comments on  
Assessment of Lindane and Other  
Hexachlorocyclohexane Isomers  
EPA-HQ-OPP-2006-0034

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**Executive Summary**

CPF Associates, Inc., an independent scientific research and consulting firm has undertaken a comprehensive review of the potential for human carcinogenicity of the HCH isomers as a response to EPA's February 8, 2006 "Assessment of Lindane and Other Hexachlorocyclohexane Isomers". The results of this review show that in general, HCH isomers are not genotoxic, not carcinogenic in humans, carcinogenic only in male mouse livers, tumorigenic only at high doses, hepatotoxic in laboratory animals, positive in promotion; negative in initiation studies, gap junction intercellular communication inhibitors, and mostly associated with reversible effects. Because of these findings, it is not scientifically appropriate to treat the HCH isomers as human carcinogens with a linear dose-response in the low dose range. If the HCH isomers have carcinogenic potential, they act as cancer promoters by the inhibition of gap junction intercellular communication. Since this phenomenon exhibits a toxicological threshold, the HCH isomers including lindane should be treated in risk assessment using an appropriate NOAEL rather than a linear non-threshold model. These findings have several implications to the Assessment. First, they corroborate EPA's cancer evaluation of lindane. Second, they show that both the qualitative and quantitative carcinogenicity of the other HCH isomers are overstated. Consequently, the risks reported in the assessment are substantially higher than indicated by the toxicological data. It is recommended that EPA revise the Assessment to evaluate all HCH isomers using a NOAEL/margin of exposure approach since low-dose linearity of carcinogenic response is not scientifically justifiable. We also recommend that EPA take this opportunity to publish a reference dose for  $\delta$ -HCH and remove "Technical-HCH" from the IRIS system.

**1. Introduction**

CPF Associates, Inc. ("CPF") is pleased to have this opportunity to submit comments to the US Environmental Protection Agency (EPA) on its "Assessment of Lindane and Other Hexachlorocyclohexane Isomers" ("Assessment") dated February 8, 2006. CPF is an independent scientific research and consulting firm specializing in environmental and occupational health. CPF scientists have been investigating the environmental behavior and effects of chlorinated persistent pesticides for over 20 years with an emphasis on

environmental chemistry and human health effects. CPF received no financial compensation for review of the Assessment or the preparation of these comments and the views presented herein represent those of CPF alone. Since the cancer endpoint was significant in the Assessment (Tables 13 and 14, page 48) and since EPA has specifically requested information on cancer classification (Section V.B., page 50), following a brief introduction focusing on problem identification, we will address issues related to potential human carcinogenicity in detail. For purposes of completeness, information on technical grade HCH (t-HCH) and  $\delta$ -HCH will be included as appropriate.

## 2. Problem Identification

Although currently HCH does not enjoy widespread use as a pesticide or pharmaceutical in the US, the ramifications of this assessment extend beyond the borders of pesticide regulation. ATSDR (2005) has identified  $\alpha$ -HCH,  $\beta$ -HCH,  $\gamma$ -HCH, and  $\delta$ -HCH as chemicals of potential concern at 146, 159, 189, and 126 of 1,662 Ional Priorities List (NPL) sites, respectively. The HCH isomers are additionally chemicals of potential concern at numerous state Superfund or RCRA corrective action sites with an aggregate potential remedial liability exceeding \$100,000,000. HCH isomers have been detected in all environmental media (air, groundwater, surface water, soil) and many biological media (crops, wildlife, human tissue). Although these chemicals have not been manufactured in the US for many years, their environmental persistence renders them significant in the environment and it is important that the most accurate toxicological information be used to evaluate any associated potential human health risks.

A comprehensive risk assessment review of HCH isomers has not been undertaken by EPA for many years. Lindane had been scheduled for IRIS re-evaluation and stakeholders requested EPA to include the other isomers in this undertaking. EPA, however, withdrew the IRIS re-evaluation and deferred risk assessment of lindane to the pesticide registration process. This activity culminated in the issuance of the lindane Reregistration Eligibility Document (RED) in 2002. Since only lindane is actively registered, no further action was taken regarding a toxicological or risk assessment review of the other isomers despite their environmental significance until the publication of this Assessment.

The Assessment accurately identified the isomers and enantiomers of HCH (page 9), however, some of this information is incomplete. Both “technical” (t-HCH) and  $\delta$ -HCH are important environmentally and can be risk drivers in situations involving environmental remediation. In reality, industrial free radical chlorination of benzene produces a total of eight HCH isomers including those mentioned in the Assessment (the other isomers are iota, theta, and eta) in addition to over-chlorinated products (hepta- and octa-chlorocyclohexanes) and under chlorinated products (chlorobenzenes and chlorocyclohexenes)<sup>1</sup>. The non-HCH components may be associated with toxicity or other hazardous properties. For example, Hegyi and Stota (1961) identified delta-

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<sup>1</sup> Hegyi and Stota 1961 reported 4 heptachloro isomers, 2 octachloro isomers, and 2 isomers of pentachlorocyclohexene as components of technical HCH.

1,1,2,3,4,5,6-heptachlorocyclohexane as the dermal allergen in t-HCH even though it constituted only 1.8% of the technical mixture.

In order to enhance insecticidal activity of t-HCH, a product known as fortified BHC (F-BHC)<sup>2</sup> was made that increased the proportion of the gamma isomer (Colson 1979)<sup>3</sup>. Finally, almost pure gamma-BHC (Lindane) has been produced by purification of F-BHC. An intermediate grade, known as technical Lindane, containing 99.5% to 99.7% gamma-BHC has also been produced (Amyes 1990). Lindane has the highest insecticidal activity and least odor.<sup>4</sup> The composition of various BHC products is shown in Table 2-1.

**Table 2-1  
Percent Composition of HCH Products<sup>5</sup>**

Constituent	T-BHC 1 <sup>b</sup>	T-BHC 2	T-BHC 3	T-BHC 4	T-BHC 5	T-BHC 6	FBHC
α-HCH	65-70	61.5	65	60-70	55	29-80	19
β-BHC	5-6	9.1	7	5-12	14	0-14	10
γ-HCH	13	14.4	14 <sup>a</sup>	10-15	12	8-31	44
δ-HCH	6	9	NR	6-10	8	2-16	21
ε-HCH	NR	1	4	3-4	3-4	3-5	1
Other HCH	NR	NR	10	NR	NR	NR	15
8 ClCH	0.6	0.4	NR	NR	NR	NR	NR
7 ClCH	4	4.6	NR	NR	NR	NR	NR
Source	Ramsey & Patterson 1946	Hegyí & Stota 1961	Colson 1979	ATSDR 1998	Kauer et al. 1947	Riemschneider 1952	Colson 1979

In addition to these analyses, Riemschneider (1952) presented analyses showing 29-80% alpha-HCH, 0-14% beta-HCH, 8-31% gamma-HCH, 2-16% delta-HCH, and 3-5% epsilon HCH. It is not clear if these German technical products are produced by the same processes as American HCH products. Although other components (e.g., chlorobenzenes and chlorocyclohexenes) have been qualitatively reported, no quantitative data are available.

It is apparent that there is significant variability among manufactured HCH products. There is even a greater variability of physicochemical, chemodynamic, and toxicological properties among HCH isomers (Mackay et al., Colson 1979, ATSDR 1998, Willet et al.

<sup>2</sup> The former terminology benzenehexachloride or BHC is used in older documents. This terminology is retained here to be consistent with those sources.

<sup>3</sup> Another product known as gamma-fortified BHC has also been produced that contains up to 80% gamma isomer along with alpha, beta, delta-HCH and heptachlorocyclohexane (Hegyí & Stota 1961)

<sup>4</sup> The objectionable odors of T-BHC are associated with the beta isomer and the overchlorination products.

<sup>5</sup> NR = Not reported, 8 ClCH = octachlorocyclohexane, 7 ClCH = heptachlorocyclohexane, a) USEPA 1987 reports this value as 17% rather than 14% citing the same study, b) percent composition based on total identifiable constituents; approximately 2% of the total material was not identifiable.

1998). Table 2-2 illustrates these differences using the Henry's constant as an example of an important chemodynamic property and sub-chronic hepatotoxicity as an example of an important toxicological property.

**Table 2-2**  
**Selected Properties of BHC Isomers**

Isomer	Henry's Constant (Pa-m <sup>3</sup> /mol)	Sub-Chronic Toxicity (mg/kg-day)
alpha-HCH	0.872	18
beta-HCH	0.116	45
gamma-HCH	0.0727	90
delta-HCH	0.149	90

This leads to the conclusion that the mixtures will have different properties based on their composition. For example, a mixture that is predominately alpha-HCH may be more sub-chronically toxic (and volatile) than a mixture that is predominantly gamma-BHC.

HCH isomers in the environment are rarely, if ever, found in the same proportions as t-HCH, F-BHC or Lindane (ATSDR 1998, Deo et al. 1994, Willet et al. 1998). This is due to both differences in the original compositions as well as differential fate, transport, and metabolic processes. For example, the most predominant isomer in human tissue is beta-HCH which is a small component of any HCH commercial product. Alpha and delta HCHs dominate in sediments and some soils, although beta-HCH has been found to dominate in other soils and sediments (Willet et al. 1998).

Various regulatory agencies regulate the HCH isomers differently, resulting in differences in remediation throughout the US and confusion regarding chemical identity. This is despite the fact that these disparate regulations are all based on the same toxicological studies. Although a detailed review of state regulatory approaches is beyond the scope of these comments, a few examples will suffice to demonstrate this problem. The Texas Commission on Environmental Quality (TCEQ) regulates  $\alpha$ -HCH,  $\beta$ -HCH,  $\gamma$ -HCH, and  $\delta$ -HCH in addition to t-HCH. All of these substances are classified as human carcinogens by the TCEQ – all B2 with the exception of  $\beta$ -HCH which is classified in category C. Oral cancer slope factors are used to develop cleanup levels.  $\alpha$ -HCH has a cancer slope factor of 6.3 (mg/kg-day)<sup>-1</sup> cited to IRIS,  $\beta$ -HCH has a cancer slope factor of 1.8 (mg/kg-day)<sup>-1</sup> also cited to IRIS,  $\gamma$ -HCH has a cancer slope factor of 1.3 (mg/kg-day)<sup>-1</sup> cited to HEAST,  $\delta$ -HCH has a cancer slope factor of 1.8 (mg/kg-day)<sup>-1</sup> developed by the TCEQ, and t-HCH has a cancer slope factor of 1.8 (mg/kg-day)<sup>-1</sup> cited to IRIS. In contrast, California's Office of Environmental Health Hazard Assessment (OEHHA) regulates t-HCH with an oral cancer slope factor of 4 (mg/kg-day)<sup>-1</sup>,  $\alpha$ -BHC with a slope factor of 2.7(mg/kg-day)<sup>-1</sup>,  $\beta$ -BHC with a slope factor of 1.5(mg/kg-day)<sup>-1</sup>, and  $\gamma$ -BHC with a slope factor of 1.1(mg/kg-day)<sup>-1</sup>. The carcinogenicity of  $\gamma$ -BHC is cited as a concern under OEHHA's evaluation of California public health goals. Finally, the Florida

Department of Environmental Protection does not consider  $\delta$ -HCH to be a potential human carcinogen, does not regulate t-HCH, and considers  $\gamma$ -HCH to be a Category B2-C carcinogen cited to HEAST.

The Assessment should correct deficiencies and inconsistencies so that there is a coherent national policy toward the HCH compounds. To this end, it is recommended that EPA revise the Assessment to show the variability of t-HCH to justify the subsequent removal of this material from IRIS, HEAST, and any other federal level databases that may be used to assess risk associated with HCH. Since  $\delta$ -HCH is environmentally significant, it should be added to the document. Further, it is recommended that the cancer classifications of all of the isomers be re-evaluated in light of scientific evidence that has accumulated since the original classifications were made. In the remainder of this document, we will consider the cancer hazard identification and quantitative dose-response relationships for each of the isomers.

### 3. Evaluation of Human Carcinogenicity

#### 3.1 Weight-of Evidence Systems

The concept of weight-of-evidence for potential human carcinogens was first developed by the International Agency for Research on Cancer (IARC) to be able to combine evidence from different sources including clinical, epidemiological, toxicological, and biochemical observations to compensate for the lack of definitive and unequivocal studies. In concept, chemicals with the strongest evidence from multiple sources are differentiated from chemicals with weaker evidence using a classification system that is described by a series of alphanumeric codes known as groups. This system of classification at least theoretically allows risk managers to use qualitative evidence concerning potential carcinogenicity in a risk-based decision process. In 1986, the U.S. Environmental Protection Agency (USEPA) developed a weight-of-evidence system that has been widely used throughout the United States. USEPA's system is shown in Table 3-1.

**Table 3-1  
USEPA (1986) Carcinogen Weight of Evidence Classification System**

<b>Group</b>	<b>Description</b>
A	Known human carcinogen.
B1	Probable human carcinogen based on limited human data.
B2	Probable human carcinogen based on sufficient evidence in animals and inadequate or no evidence in humans.
C	Possible human carcinogen
D	Not classifiable as to human carcinogenicity
E	Evidence of noncarcinogenicity in humans

In the early to mid 1980s USEPA classified the HCH isomers according to this system as shown in Table 3-2 (EPA 1987).

**Table 3-2**  
**USEPA Weight of Evidence for BHC Isomers**

<b>Isomer</b>	<b>Weight of Evidence</b>
alpha ( $\alpha$ )	B2
beta ( $\beta$ )	C
gamma ( $\gamma$ )	B2/C
delta ( $\delta$ )	D
Technical	B2

USEPA's classification was primarily based on a series of bioassays in mice which exhibited primarily liver tumors in male mice. The classification was somewhat ambiguous and there appears to be no clear basis for assigning B2 or C codes to the individual isomers. These guidelines have been the basis for most regulatory action on HCH in the past 20 years and are adhered to in the Assessment.

In 2005, EPA finalized new carcinogenic risk assessment guidelines. In essence, these guidelines allow for the use of more biological data in carcinogen classification and tend away from numerical classification schemes. The primary focus of the guidelines is on a weight-of-evidence narrative that summarizes the full range of available evidence. The mode of action of a potential carcinogen is emphasized by EPA as an important way to understand how a particular chemical should be treated according to the guidelines. The narratives are accompanied by a series of descriptors that succinctly summarize the conclusions reached in the narratives:

- 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.

In the RED (EPA 2002) EPA evaluated lindane under the new guidelines and used the descriptor "suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential". The classification was based on an increased incidence of benign lung tumors in female mice only and dose-response quantification of carcinogenicity was not performed.

An alternative to USEPA's former or current method is the system published by Ashby et al (1990). The Ashby et al system classifies chemicals according to the weight of evidence that they pose a potential human cancer hazard and builds upon approaches

developed and used by IARC, USEPA, and an independent group of industrial scientists (Tripartite Group). Ashby et al developed this system to address advances in the state of knowledge of animal carcinogenicity, human carcinogenic models, and the interpretation of carcinogenic studies. The previous systems relied heavily on the determination of the strength of evidence for animal carcinogenicity and the observation of carcinogenicity in humans. Little attention was paid to the relevance or “predictivity” of animal carcinogenicity data in supporting or not supporting because there was a lack of understanding regarding these models. Since these models and relationships are now more clearly understood, this scheme was developed as a tool to assist scientists in determining the supportive or non-supportive animal carcinogenic data relevant to predicting carcinogenicity in humans. Thus, the weight of evidence classification developed by Ashby et al provides a more reliable description of the scientific data available and its interpretation.

In performing this analysis, three types of information are considered: human evidence of carcinogenicity, animal bioassay results, and corroborative information. Corroborative information can be from animal bioassay studies and in vivo experiments. This information is used to determine if the animal bioassay results support or do not support the predictability of the animal bioassay results for human response. The codes and descriptions used in the Ashby system are shown in Table 3-3.

**Table 3-3**  
**Ashby et al. (1990) Carcinogen Weight of Evidence System**

<b>Weight of Evidence Code</b>	<b>Description</b>
1	Known human carcinogen
2	Carcinogenic activity in animals; probable human carcinogen
3	Possible human carcinogen
4	Equivocal evidence for human carcinogenicity
5	Evidence inadequate for classification
6	Carcinogenic in animals, probably not a human cancer hazard
7	Carcinogenic activity in animals; considered not a human carcinogen
8	Evidence of non-carcinogenicity

Classification of the HCH isomers according to the Ashby et al. system has been performed by CPF and will be discussed below.

Recently, a European perspective has been developed as an alternative system for carcinogen classification (Bolt et al. 2004). This system relies on a primary classification of potential human carcinogens as genotoxic or non-genotoxic. Further evaluation yields a fourfold categorization:

- Non-threshold genotoxic carcinogens. Dose-response data will be presented using a linear non-threshold approach.
- Genotoxic carcinogens for which the existence of a threshold cannot be sufficiently supported. In these cases, a linear non-threshold dose-response model is used as a default based on the precautionary principle.
- Genotoxic carcinogens for which a practical threshold is supported by studies on mechanisms and/or toxicokinetics. Dose-response is evaluated using a NOAEL.
- Non-genotoxic and non-DNA reactive carcinogens for which a real threshold is assumed. Dose-response information is presented using a NOAEL.

The carcinogenicity of lindane and the HCH isomers has been evaluated by CPF using the European system. The results will be presented below.

### 3.2 Causation

In addition to weight of the evidence schemes for assigning categories to carcinogens, methodologies adopted to evaluate causation are also useful, especially in the evaluation of epidemiologic and clinical data. These include the causation criteria developed by Bradford Hill and the newer concepts of evidence based toxicology (Guzelian et al. 2005).

A critical element in evaluating claims associated with alleged exposure to a chemical substance is whether the exposure caused the disease. Causation may be thought of as a chain of events that links an injury to toxic substance exposure. This chain must not be broken for causation to be demonstrated. In evaluating a chain of causation for a specific injury or illness, analysts usually start by evaluating the illness and then determining whether the subject was actually exposed to the agent of concern. The exposure analysis is based on biomonitoring, dosimetry, environmental monitoring, mathematical modeling, questionnaires, or a combination of these methods. Once it has been determined that exposure has occurred, a toxicology/microbiology/epidemiology review is conducted to determine if a health hazard exists. The existence of a health hazard is then linked to the exposure through risk assessment concepts such as dose-response quantification. Finally, confounding causes of the illness are investigated. Only when exposure has occurred at a level sufficient to elicit an adverse health effect that is not explainable by other causes can the exposure be causally linked to the disease.

In 1965, Sir Austin Bradford Hill developed the first general criteria for evaluating causation in epidemiologic studies (Bradford Hill 1965). Since then consensus criteria have evolved in the scientific literature (Doll 1984, Guzelian et al. 2005) for evaluating claims of causation. These criteria may be distilled into a few general principles for assessing causation in individuals:

**Hazard identification/qualitative toxicology.** Is the chemical capable of causing the alleged disease in the person claiming damage?

**Exposure and Dose Response.** Did the person claiming the disease contact the hazardous chemical at a sufficient level (duration, frequency, intensity) to result in an injury?

**Time course of disease.** Was exposure temporally related to the injury given appropriate considerations of disease latency?

**Confounders/differential diagnosis.** Are there possible alternative causes for the disease?

**Analysis of scientific plausibility.** Do toxicologic, epidemiologic, chemical, and clinical data present an internally consistent and coherent view of the disease?

Epidemiologic studies deal with populations rather than individuals; for example, a population consisting of all people potentially exposed to a commonly applied pesticide. Epidemiologic studies typically involve a comparison of the incidence of an effect in an exposed group to the incidence in a control group. In addition to the individual criteria mentioned above, epidemiological criteria for causation include the numerical strength of association between exposure and health effect, consistency of human associations among populations, and agreement with experimental evidence (e.g., from animal studies). Epidemiologic studies are often used to support what is known as general causation – is the exposure capable of eliciting a response in the general population? They cannot be used to evaluate the exposure of a specific individual

Epidemiologic studies are conducted in accordance with specific protocols and scientifically controlled to eliminate biases and confounding factors. In particular, the doses of the substance are defined and/or controlled. Other human toxicological data may be derived from laboratory experiments. Although much human experimentation is precluded by ethical concerns, at times volunteers may elect to undergo exposure to potentially toxic substances for research purposes. As with epidemiologic studies, the results are conducted in accordance with a defined protocol and controlled to eliminate bias and confounding. In addition, human information concerning the effects of exposure to potentially toxic substances may be derived from clinical case reports. In these cases, clinicians report their observations and results of clinical testing on patients who have reported exposure to a potential toxicant. Although these reports may yield some useful information, they are not controlled, subject to bias and the experience of the particular clinician, and the exposures are often not measured, controlled or defined. When investigating health effects, environmental health scientists typically put more weight on properly designed and implemented epidemiologic studies, followed by a controlled laboratory experiment. Less weight is given to clinical reports, which, because of the unevenness of their scientific quality, must be evaluated on an individual basis.

Guzelian et al. (2005) have distilled the collected experience of health scientists dealing with causation into a methodology known as evidence-based toxicology. This methodology consists of three general steps with individual elements designed to fulfill the requirements of a classical causation analysis.

- Collecting and evaluating the relevant data
  - Source

- Exposure
- Dose
- Diagnosis
- Collecting and evaluating the relevant knowledge
  - Frame the question
  - Assemble the relevant literature
  - Assess and critique the literature
- Joining data with knowledge to arrive at a conclusion
  - General causation (answer to the framed question)
  - Dose-response
  - Timing
  - Alternative cause
  - Coherence

### **3.3 Use of Different Methodologies**

None of the methods for determining the ability of a chemical to cause human cancer is adequate by itself. Each of the methods has advantages and disadvantages. For example, neither the EPA method nor the European method can make use of clinical data. For a pharmaceutical such as lindane with a long history of clinical use, these data by themselves may be determinative. The concept of a cancer promoter is not treated explicitly in most of the systems, nor is the concept of hormesis. Since the HCH isomers have been shown to act as promoters and have a hormetic effect on endpoints associated with their carcinogenic mode of action, these concepts are arguably important in reaching a determination regarding the potential to cause cancer at low doses. Due to the drawbacks of the individual methods, the remainder of these comments will utilize concepts from each of the methods as appropriate accompanied by additional information from the scientific literature that may be relevant to a determination of the ability of the agents to cause human cancer. Lindane will be discussed in the next section since there is more information available on lindane. Structure-activity analogies to lindane may be used in the subsequent discussions of  $\alpha$ -HCH,  $\beta$ -HCH, and  $\delta$ -HCH. Due to the prominence of mode of action in the EPA guidelines, a separate section will be used to present information relevant to mode of action for all the isomers.

## **4. Potential Human Carcinogenicity of lindane ( $\gamma$ -HCH)**

Section V.B. of the Assessment requests additional information regarding the cancer classification of lindane. In order to address this concern, we review and analyze human, animal and supporting data in the context of the weight-of-evidence systems presented in Section 3.

### **4.1 Human Evidence**

In 1983, Vesselinovitch and Carlborg concluded that the probability of developing a human cancer from exposure to lindane was infinitesimally small (on the order of  $10^{-10}$ ) and that environmental exposure to lindane did not pose a threat to human health. ATSDR

(2005) reviewed epidemiologic studies that had been conducted to investigate the potential association between exposure to lindane and human cancer and concluded that there was no clear evidence from epidemiologic studies to support the hypothesis that lindane is a human carcinogen. All of the positive associations reported were numerically weak, subject to confounding, and many lacked statistical significance. Calle et al (2002) reviewed the available data on the hypothetical relationship between body burden or exposure to organochlorine compounds and human breast cancer and concluded that “organochlorine exposure is not believed to be causally related to breast cancer”. Cornell (1998) also reviewed the evidence for an association of lindane with breast cancer and concluded that “there is currently no evidence to show that lindane exposure is associated with increased risk for breast cancer in humans”. In addition they noted that, while lindane is a hormone disruptor, there is no evidence that disruptions caused by lindane are the type that increase breast cancer risk. An ecologic study reported by Muir et al. (2004) found weak associations between lindane exposure and breast cancer in one location, but no association in a neighboring location. Although this study had many limitations, it can be used to rule out the existence of a strong association. Most recently, Mills & Yang (2006) examined the relationship between lindane exposure and breast cancer in the California Latina farm worker community. They failed to find an association or a dose-response relationship. In another study, Mills & Yang (2003) found weak associations between lindane exposure and prostate cancer in a California farm worker population. This study suffers from many deficiencies, however, the fact that exposure was never demonstrated in any of the positive cancer cases severely limits the utility of the results. Blair et al. (1998) concluded that lindane was not a major etiologic factor in the development of non-Hodgkins lymphoma (NHL). Lee et al (2004) found a non-significant association (odds ratio of 1.3 95% CI = 0.97-1.8) for exposure of non-asthmatics to lindane. In contrast, McDuffie et al. (2001) found a weak association between lindane exposure and NHL (OR = 2.05 95% CI = 1.01-4.16). It should be noted, however, that McDuffie et al (2001) found similar or stronger associations with 14 other compounds to which the study group had been exposed.

Most of the epidemiologic studies are conducted in occupational settings where exposure is difficult to demonstrate and there is a high potential for confounding, especially among such groups as agricultural workers and pesticide applicators. In contrast, the use of lindane as a pediculocide involves direct exposure and less opportunity for confounding<sup>6</sup>. The concentration of lindane in lice treatments is typically 1%(10,000 ppm) (EPA 2002) which is significantly higher than concentrations involved in environmental exposures. Friedman (1997) investigated the potential association between lindane therapy and cancer in a cohort of 1146 HMO patients who received treatment with lindane. After ruling out confounders such as Kaposi’s sarcoma associated with AIDS and strong cancer risk factors, there was no convincing evidence that lindane was a human carcinogen. As noted in the RED (EPA 2002), FDA collects information on adverse drug reactions

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<sup>6</sup> FDA (2002) states that for pediculosis, 1 ounce of lindane shampoo is applied to the hair and allowed to remain for 4 minutes after which water is added to make a lather and the hair is rinsed. Treatment may be repeated in 7 days. As a scabicide, lotion is applied to the entire body from the neck down and left on for 8-12 hours.

associated with substances regulated by the FDA<sup>7</sup>. FDA (2002) reported 488 reports of adverse effects associated with lindane use. The most common adverse effect was drug ineffectiveness (111 cases) followed by convulsions (65), dermatitis (34) and dizziness (29). Of the total 488 reports, there were very few cancers. A malignant brain tumor and perianal cancer were reported in adults; one case each of acute lymphocytic leukemia, acute myelocytic leukemia and beta cell lymphoma of the brain were reported in children. All of these cancers have numerous etiologies and none of them are considered to be rare in the population. FDA did not report that attending physicians had linked any of these cancers to lindane exposure<sup>8</sup>.

There are several reports of the occurrence of aplastic anemia in humans exposed to  $\gamma$ -BHC only and to  $\gamma$ -BHC and other compounds. There are also two reports of the occurrence of leukemia in cousins exposed to  $\gamma$ -BHC. In addition, an increased incidence of lung cancer was found in a number of workers who applied pesticides containing many compounds including  $\gamma$ -BHC (IARC 1987).

Considering all the evidence available from epidemiologic studies and case reports, the associations of  $\gamma$ -HCH with human cancer are weak, often statistically insignificant and confounded by the presence of other potential carcinogens. Exposure to lindane or other HCH isomers was not actually measured in most epidemiologic or case study reports and, in many cases; researchers relied upon weak surrogates of exposure (such as historical records of pesticide use in a broad geographical location). The reports of cancer are not consistent with respect to tumor type and dose-response relationships have not been established. In the context of the Bradford Hill criteria or evidence-based toxicology, the data obtained from epidemiologic or case studies do not support a hypothesis that lindane causes human cancer.

#### **4.2 Animal Bioassay**

Lindane has been studied in many animal bioassays. In the NCI study (NCI 1977), lindane was found not to be carcinogenic in Osborne-Mendel rats fed doses up to 23.6 mg/kg/day for up to 80 weeks. In a more recent study conducted according to Good Laboratory Practices, (Aymes 1990), lindane was found not to be carcinogenic in Wistar rats fed up to 20 mg/kg/day.

Prior to EPA's (2001) cancer assessment, 13 animal bioassays in which  $\gamma$ -BHC was administered in the diet of mice and/or rats were reported in the literature (presented in Appendix 1). Eight of the studies were negative for cancer at any dose (Ito et al. 1973a, Nagasaki et al. 1972a, Life Science Research 1989, Fitzhugh et al. 1950, Ito et al. 1973b, Truhaut 1954, Ito et al. 1975, Weisse & Herbst 1975). Tumor activity was observed in five of the studies in mice only (Thorpe and Walker 1973, NCI 1977, Hanada et al. 1973, Wolff et al. 1987, Goto et al. 1972). The results of the NCI (1977) study must be

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<sup>7</sup> These data are of variable quality. The sources of the data include consumers, attorneys, scientific literature, regulatory agencies, and associations.

<sup>8</sup> Of the serious adverse effects, the label use was exceeded in 34 cases, there was oral administration in 8 cases, and application was contraindicated in 5 cases.

considered to be equivocal in that treatment-related tumors were only found in the low dose group of male mice and not in the high dose. The NCI study was negative for female mice. Many of these studies were considered to be of poor quality because of substantive study limitations (presented in Appendix 1). The primary tumor types were hepatocellular adenomas and carcinomas, hyperplastic nodules, and hepatomas. There was significant evidence of liver damage in some of the studies.

In 2001, EPA evaluated the results of several new studies including:

- CD-1 mice (50/sex/dose) at 0, 10, 40, or 160 ppm for 78 weeks.
- Female Agouti, Pseudoagouti, and Black mice (36-96 animals per strain) at 0 or 160 ppm for 24 months.
- B6C3F1 mice (50/sex/dose) at 0.1, 10, 100, or 400 ppm for two years.
- Osborne-Mendel rats (50/sex/dose) variable dosing regime for 80 weeks.

The only treatment-related effect noted in any of these studies was an increase in lung adenomas for female CD-1 mice. On the basis of these findings, EPA classified lindane as “suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential”. Burin (2000) points out that the incidence of the female alveolar-bronchiolar tumors was within the historical rate at the test laboratory, there was no evidence in the incidence of carcinomas, the lung has not historically been considered as a target organ for lindane in the mouse, and that this particular tumor type is commonly occurring in mice and highly variable in incidence.

### **4.3 Corroborative Information Regarding Human Relevance**

In general, data from the bioassays do not support the hypothesis that lindane is associated with human cancer. In the bioassays, tumors were observed at only at one site, the liver, in those studies which were positive. In addition, tumors were observed only in organs with cellular toxicity -- liver toxicity was observed in all livers that had tumor activity. In addition, the majority of liver tumors reported were benign.

Non-bioassay information was also reviewed for corroborative evidence regarding human relevance.

Although a few studies have reported positive genotoxic results (Kumar et al., 1995; Iverson et al., 1984; Nybom and Knutsson, 1947; Rocchi et al., 1980; and Ishidate and Odashima, 1977), the majority of the genotoxicity studies are negative (Király et al., 1979; Dzwonkowska and Hubner 1986; Jenssen and Ramal, 1980; Moriya et al. 1983; Nagy et al., 1975; Moriya et al., 1983; Shirasu et al., 1976; Shahin and von Borstel, 1977; Kar and Singh, 1979; and Alhmed et al., 1977). The World Health Organization reviewed the available information and concluded, “The mutagenicity of lindane has been adequately studied. This compound has been extensively investigated for its ability to induce gene mutation in both bacteria and mammalian cells, and for its activity in the assay for sex-linked recessive mutation in *D. melanogaster*. Negative results were

obtained consistently. Its ability to induce chromosomal damage and sister chromatid exchange has been investigated in mammalian cells both *in vitro* and *in vivo*, again with negative results. Both assays for DNA damage in bacteria and studies *in vivo* to investigate covalent binding to DNA in the liver of rats and mice following oral administration also gave negative results. The few studies in which positive results were obtained involved invalid study designs or lindane of unknown purity.”

The metabolic pathway of  $\gamma$ -HCH appears to be similar in humans and rats.  $\gamma$ -HCH is primarily excreted in the urine, the major metabolites are chlorophenols. In humans, it is metabolized in the liver through dechlorination, dehydroxylation, and dehydrogenation to hexachlorocyclohexane, pentachlorohexane, and chlorophenols. *In vitro*,  $\gamma$ -HCH has been found to form an epoxide.  $\gamma$ -HCH appears to be detoxified by the P-450 oxidative system because an increase of hepatic enzymes by induction increased the elimination of  $\gamma$ -BHC.

One mechanism that has been postulated for the tumor promotional activity of several classes of chemicals is inhibition of gap junction intercellular communication (GJIC). Inhibition of GJIC by cancer promoters can result in disruption of regulated cell division and, ultimately, in proliferation. A considerable amount of evidence has been developed to support the hypothesis that  $\gamma$ -HCH is a GJIC inhibitor.

Tsushimoto et al. (1983) found that  $\gamma$ -HCH was not mutagenic (6-thioguanine and diphtheria toxin resistance assays), promoted skin tumors in mice, and inhibited GJIC in Chinese hamsters *in vitro*. GJIC inhibition was dose-related and showed clear evidence of a threshold. The authors concluded that  $\gamma$ -HCH could be a weak promoter. Ruch et al. (1987) found that  $\gamma$ -HCH inhibited GJIC between mouse hepatocyte cells in a dose-responsive manner. The activity of  $\gamma$ -HCH was lower than that of the known promoter 12-O-tetradecanoylphorbol-13-acetate (TPA).

Leibold and Schwarz (1993) found inhibition of communication in rat hepatocyte cells in a dose-responsive fashion. It should be kept in mind that  $\gamma$ -HCH has not been found to be carcinogenic in rats. Vitamin E prevented inhibition of communication by  $\gamma$ -HCH. Leibold and Schwarz postulated that  $\gamma$ -HCH affected IC via formation of free radical intermediates such as pentachlorocyclohexyl radical. Other researchers have found that  $\gamma$ -HCH induces oxidative stress through increases in superoxide radical generation which could also be inhibited by Vitamin E.

Criswell and Loch-Carus (1995) found that  $\gamma$ -HCH inhibited GJIC in rat uterine monocytes through an arachidonic acid-sensitive cAMP-independent mechanism. The production of cAMP was dose-responsive and showed clear evidence of a threshold.

Upham et al. (1997) found that inhibition of GJIC by lindane was both dose- and time-dependent. Inhibition as measured by the scrape loading/dye transfer (SL/DT) technique followed a sigmoid dose-response relationship with a threshold occurring at lindane concentrations less than 25 mg/L. When the lindane concentration was kept constant, inhibition progressed rapidly and reached a plateau between 25 and 50 minutes following

initiation. These investigators also noted that the cytotoxicity of lindane measured by viable uptake of neutral red of F-344-WB cells also followed a sigmoid dose-response relationship with a clear threshold at about 50 mg/L lindane. The cells recovered to their initial activity in about 200 minutes following cessation of treatment.

Guan et al. (1995) evaluated the impact of lindane treatment on Connexin43 (Cx43) in rat liver epithelial cells. Short term treatment resulted in loss of gap junction permeability. Longer term treatment resulted in the loss of permeability and number and phosphorylated Cx43 proteins. The data followed a sigmoid dose-response relationship with a threshold being evident at a lindane concentration of 5  $\mu$ M.

Overall, the evidence points to a dose-responsive mechanism of the following nature:

No effect  $\rightarrow$  loss of gap permeability  $\rightarrow$  loss of gap number  $\rightarrow$  loss of gap expression  $\rightarrow$  cytotoxicity  $\rightarrow$  frank hepatotoxicity.

#### **4.4 $\gamma$ -BHC Conclusion**

Considering the weight-of-evidence for the human carcinogenicity of lindane, the most appropriate classification using the EPA (2005) system is that it is “not likely” to be carcinogenic in humans. Based on the Ashby system, the relevant weight-of-evidence conclusion is Category 6, “carcinogenic in animals, probably not a human cancer hazard”, although word carcinogenic might better replaced by the word “tumorigenic” to reflect the fact that the predominant tumors were benign. There is sufficient evidence to show that lindane is non-genotoxic and probably acts by a mode characterized by inhibition of gap junction intercellular communication. If lindane is to be considered as a carcinogen, it probably acts as a promoter at high doses. These high dose regimes may have been associated with the elevated incidence of liver tumors in some of the rodent assays, but are unlikely to occur through environmental exposure to humans. The data are consistent with the European system classification of a non-genotoxic carcinogen acting through a threshold mechanism which should be assessed by comparison to a NOAEL or LOAEL using a margin of exposure or similar approach.

#### **5. Risk Assessment of $\alpha$ -HCH**

Currently, USEPA has designated  $\alpha$ -HCH as a category B2, or probable human carcinogen. Only one epidemiologic study presenting results regarding the potential association of  $\alpha$ -HCH and human cancer was located in the literature (Mathur et al. 2002). These researchers investigated body burdens of chlorinated pesticides in women who had been diagnosed with breast cancer compared to normal women. Mathur et al. found that women with breast cancer had significantly higher blood levels of  $\alpha$ -HCH. This study also found that these women had significantly higher levels of seven other organochlorine pesticides. Mathur et al. (2002) did not seek to measure any additional chemicals in the women’s blood, nor did they investigate blood lipid levels. Since fat intake is associated with both breast cancer and body burden of lipophilic compounds, their results may have been an artifact of fat consumption. USEPA’s cancer classification

for  $\alpha$ -HCH on IRIS is based on 10 rodent bioassays, all conducted prior to 1981. Three additional studies have been identified and discussed by ATSDR (1994), two of which show either negative results or benign tumors. A summary of the relevant toxicity studies is presented in Appendix 1. Many of these studies were not designed to be used in risk assessment. In all of these studies, mice showed consistent responses for liver tumors; rats and hamsters showed predominantly negative results. The relevance of mouse liver tumors to human risk assessment has been questioned in recent years (Frith et al. 1994, ILSI 1995). This is because mice exhibit a very high spontaneous liver tumor incidence which makes them particularly susceptible to the development of liver tumors under laboratory study conditions.

Many of the tumors observed in the studies were benign and were seen only at high doses (e.g., 250 and 500 ppm in food). In one of two positive studies in the rat, benign tumors were observed at a 1,000 ppm dose level after 48 weeks, but not after 24 weeks; malignant tumors were not observed until 1,000 ppm had been fed for 72 weeks. At the highest dose (1,500 ppm) benign tumors outweighed malignant tumors by over 3:1 (Ito et al. 1975). Tryphonas and Iverson (1983) concluded that there was little or no evidence for progression of benign tumors induced by  $\alpha$ -HCH to malignancies and stated that the transformation, if it did occur, must progress very slowly. In two of the animal bioassays, Siglin et al. 1991 and Fitzhugh et al. 1950, no tumors were observed.

In a number of the bioassays, there was also evidence of toxicity and mortality at some of the high dose levels (Ito et al. 1975, Thorpe and Walker 1973). These are important findings because they suggest that the maximum tolerated dose (MTD) was exceeded and that the resulting toxicity may have enhanced tumorigenicity in the bioassay. In other words, the formation of tumors is likely a response from damage inflicted due to an excessive and intolerably high dosage, a situation which should not occur in animal studies. Results under these conditions should not be used in hazard identification or dose-response quantification.

All of the studies were considered to have limitations, which are also presented in Appendix 1, and none conformed with current bioassay protocols. Two studies were not considered in this evaluation because of study limitations, Sugihara et al. (1975) and Tsukada et al. (1979).

### **Non-Bioassay Information**

The weight of the evidence suggests that  $\alpha$ -HCH is not genotoxic.  $\alpha$ -HCH was found to be weakly genotoxic (causing DNA fragmentation and repair) in rat and human hepatocytes (Mattioli et al., 1996) and in mouse liver cells (Iverson et al. 1984).  $\alpha$ -HCH caused mitotic disturbances in rat liver cells (Hitachi et al., 1975). DNA reactivity was observed only at high doses (NOAEL = 300 mg./kg) (Kitchin and Brown 1994).  $\alpha$ -HCH was found to be negative for mutagenicity in yeast and did not cause DNA damage in bacteria (IARC 1998)

From a mechanism of action standpoint,  $\alpha$ -HCH has been shown to inhibit gap junction intercellular communication with a clear threshold and hormesis at low doses (Fukushima et al 2005). Masuda et al. (2001) also showed that  $\alpha$ -HCH showed a clear threshold with respect to tumor promoting activity in diethylnitrosamine-initiated liver foci in rats. Tryphonas and Iverson (1983) further found that  $\alpha$ -HCH initiated hepatocellular adenomas were unlikely to progress to hepatocellular carcinomas.

$\alpha$ -HCH is an isomer of  $\gamma$ -HCH which is the most studied of HCH isomers. From a structure-activity relationship (SAR) standpoint, many observations on the biological activity of  $\gamma$ -HCH may also be relevant to  $\alpha$ -HCH.

Based on the available data,  $\alpha$ -HCH is not likely to be a human carcinogen. Using the Ashby classification,  $\alpha$ -HCH would fit into Category 6: Carcinogenic activity in animals; probably not a human cancer hazard. Based on the European system,  $\alpha$ -HCH would be classified as a non-genotoxic carcinogen with a no effect level.

## 5.2 Dose-Response Relationships

USEPA currently provides a slope factor of  $6.3 \text{ (mg/kg/day)}^{-1}$  in IRIS for  $\alpha$ -HCH based on the occurrence of liver tumors in male dd mice (Ito et al. 1973a). This slope factor is used in the Assessment and represents the highest slope factor calculated by USEPA for this chemical in the Health and Environmental Effects Profile (HEEP) (USEPA 1987), where toxicity information was originally reported for all the HCH isomers. In this document, USEPA calculated three slope factors ranging from 1.33 to 6.34  $(\text{mg/kg/day})^{-1}$ . The lowest slope factor estimate of the range is from the single viable chronic rat study (Schulte-Hermann and Parzefall 1981) reviewed by USEPA (1987), while the two remaining values are based on mouse liver tumor incidence, a questionable end-point (Nagasaki et al. 1972a, Ito et al. 1973a). The two mouse studies are based on a less-than-lifetime duration, and were scaled to compensate for this deficiency. Such scaling incorporates considerable uncertainty; alternate approaches may be used with proposed cancer guidelines, if scientifically supported. Often, when multiple studies of similar duration and quality report consistent tumor types across species, USEPA will use the geometric mean of the slope factors calculated for the individual studies (e.g., DDT, benzo(a)pyrene). If this is done with the three existing slope factors for  $\alpha$ -HCH reported in the HEEP, the resulting value would be  $3.4 \text{ [mg/kg-day]}^{-1}$ .

### 5.2.1 Recalculation of the Cancer Slope Factor

The existing slope factor is subject to substantial uncertainty that can be reduced by the incorporation of scientific developments and USEPA policy changes that have occurred in the last 10 years. These developments include consideration of study- and strain-specific animal survival, updated default parameters for animal lifetime, a new inter-species extrapolation parameter, and acceptance of study aggregation.

In our analysis, we evaluated the three studies discussed above (Nagasaki et al. 1972a, Ito et al. 1973a, Schulte-Hermann and Parzefall 1981), in addition to other studies of similar quality that USEPA summarized in the HEEP document (USEPA 1987). We selected two additional studies for inclusion in a recalculated slope factor for  $\alpha$ -HCH. We included the Ito et al. (1973b) study because it evaluated multiple doses (i.e., 0, 50, 100 and 250 ppm) and more than 20 animals per dose. The Ito et al. (1975) study was selected because it evaluated multiple dose levels in rats (i.e., 0, 500, 1,000 and 1,500 ppm), and was of longer duration relative to the mouse studies (72 weeks). Other studies discussed in the HEEP were not selected primarily due to small sample sizes (i.e., < 20 animals per dose), one dose level, or high mortality.

### 5.2.2 Errors in IRIS record

The IRIS record for  $\alpha$ -HCH discusses the positive tumor response in mice reported in Nagasaki et al. (1975), but neglects to discuss the lack of tumor development in rats and hamsters in this same study. In addition, a dietary concentration of 250 ppm appears to be equivalent to a dose of 32.5 mg/kg/day in mice assuming a food factor of 0.13, not 37.5 mg/kg/day as reported on IRIS.

We recalculated the study-specific slope factors calculated by USEPA and originally presented in the HEEP for the five most critical bioassays using Tox\_Risk software. Study-specific and/or scientific literature values were incorporated into these quantitative estimates where possible, and in their absence the software default values were used (e.g., 130 weeks for rat lifetime). We recalculated the study-specific slope factors using the total tumor incidence data (benign and malignant) in the Tox\_Risk software exactly as they appear in the HEEP document or on IRIS [i.e., some data entered as dose (mg/kg/day), and other data entered as dietary concentration (ppm)]. For the Ito et al (1973a) study, the dose of 37.5 mg/kg/day does not appear to be equivalent to 250 ppm multiplied by the food factor of 0.13 noted in the IRIS record (but rather should be 32.5 mg/kg/day). This same discrepancy was noted for the Nagasaki et al. (1972a) study presented in the HEEP. Nevertheless, for both studies we used 37.5 mg/kg/day in the Tox\_Risk software.

A number of parameters were modified in recalculating the cancer slope factor for each study. First, we used the  $BW^{0.75}$  inter-species scaling factor as previously proposed by USEPA (1992b) and also noted in the new cancer guidelines. Second, we used a more accurate lifetime for the dd mouse based on survival curves found in the literature (Suzuki et al. 1981). This study reports an average lifetime of 64.5 weeks for dd mice which was used instead of the mouse default lifetime of 79 weeks in the Tox\_Risk software. Third, we used study-specific lifetime estimates when they differed from the

default values (e.g., an average rat lifetime of 125 weeks was used for the Schulte-Hermann and Parzefall 1981 study, rather than the default of 130 weeks). Fourth, in the absence of study- or strain-specific information, we relied on the updated default parameters in the Tox\_Risk software. Lastly, we combined the individual slope factors from five studies and calculated the geometric mean value. The use of an aggregate slope factor is considered appropriate for  $\alpha$ -HCH to compensate for the individual study deficiencies, to reflect species and sex variation, and because all five studies report liver tumors. As noted above, USEPA has frequently reported slope factors that represent the geometric mean of multiple studies (e.g., DDT, benzo(a)pyrene). This analysis gives the best estimate cancer slope factor still assuming, as a worst-case conceptual model, that  $\alpha$ -HCH functions as a complete carcinogen and taking the 95% upper confidence limit on cancer slope factor from the linearized multistage model. The resulting recalculated slope factor for  $\alpha$ -HCH is  $0.62 \text{ (mg/kg/day)}^{-1}$ , which is an order of magnitude lower than the existing slope factor of  $6.3 \text{ (mg/kg/day)}^{-1}$  in IRIS.

Since  $\alpha$ -HCH apparently is a non-genotoxic carcinogen acting through a mechanism of inhibiting gap junction intercellular communication, an alternative dose-response relationship for  $\alpha$ -HCH and cancer may be developed based on the data presented by Fukushima et al. (2005). These researchers measured GST-P foci number and area as an indicator of gap junction intercellular communication (See Section 8, below for a more detailed discussion of the mode of action). A clear NOAEL was located at  $0.1 \text{ mg/kg-day}$ . Applying an uncertainty factor of 100 would result in a reference dose of  $0.001 \text{ mg/kg-day}$  which is identical to the value derived in the Assessment (Table 8). Thus, this reference dose may be used to evaluate both carcinogenic and non-carcinogenic effects of  $\alpha$ -HCH.

## **6. Risk Assessment of $\beta$ -HCH**

### **6.1 Cancer Classification**

$\beta$ -HCH is classified as a category C, or possible human carcinogen; there is no evidence of mutagenicity or tumorigenicity in humans. Zheng et al (1999) evaluated the potential association between  $\beta$ -HCH and breast cancer in a group of 490 Connecticut women (304 cases and 186 controls). They concluded that the results of the study did not support a hypothesis that increasing adipose levels of HCH were associated with an increased risk of breast carcinoma. Weiderpass et al. (2000) evaluated the potential for a relationship between serum concentrations of  $\beta$ -HCH and endometrial cancer in 154 cases compared to 205 controls of Swedish women. They found a very weak and non-statistically significant association (OR = 0.9) with no dose-response relationship. These researchers concluded that exposure to organochlorine pesticides did not increase the risk of endometrial cancer. USEPA based its classification in IRIS and in the Assessment on 7 bioassays, and 1 additional study is presented by ATSDR (ATSDR 1994). Of the eight rodent bioassays, only two studies reported tumors in mice (Thorpe and Walker 1973, Goto et al. 1972). Dietary administration was associated with liver tumors in one strain of mice receiving one dose of  $\beta$ -BHC, in one experiment (Thorpe and Walker 1973). A dose-response relationship could not be established in this study and high mortality

occurred in dosed animals. This study is the basis of the cancer classification and slope factor reported in the Assessment. The Goto et al. (1972) study found benign tumors only. Several studies reported an absence of tumor activity in the dosed animals; these studies were also considered to be of poor quality (Ito et al. 1975, Ito et al. 1973a, Fitzhugh et al. 1950, Hanada et al. 1973, Nagasaki et al. 1972a, Ito et al. 1973b).

As seen with the other isomers, the tumors associated with the positive studies were predominantly benign and occurred only at high dose levels with evidence of toxicity and mortality. Again, toxicity observed under these conditions should not be used in hazard identification or dose-response quantification. In addition, many of the studies were not designed to be used in risk assessment. For example, the Goto et al. (1972) study, published in German, evaluated only 10 male mice exposed to one concentration of 600 ppm. A summary of the relevant toxicity studies is presented in Appendix 1.

The evidence supporting a hypothesis of potential human carcinogenicity of  $\beta$ -HCH is weak, if not non-existent. Tumor activity was observed in mice only; there is no evidence of tumorigenicity in rats. Tumors were observed only in organs (livers) evincing cellular toxicity. The majority of liver tumors observed were benign. There was also a high background liver tumor frequency in the control mice.

Evidence for genotoxicity is weak.  $\beta$ -BHC was not mutagenic to bacteria nor did  $\beta$ -BHC cause DNA damage in bacteria (IARC 1997)  $\beta$ -BHC caused chromosomal aberrations in a genotoxicity study in rat bone marrow (Shimazu et al., 1972).

There is evidence of potential estrogenic activity of  $\beta$ -BHC (Steinmetz et al., 1996) These investigators found that  $\beta$ -BHC exerts estrogen-like effects in human breast cancer cells *in vitro*, however, it does not activate the estrogen receptor (ER), nor is it converted to an ER ligand. It is possible that the stimulation of proliferation observed in this experiment is common to both HCHs and estrogen. In other words, although they may be both cancer promoters, they act through different mechanisms. This hypothesis seems to be supported by the lack of association between  $\beta$ -HCH exposure and either breast or endometrial cancers in epidemiologic studies as both of these cancers are hormonally mediated.

Based on the weight of the evidence, including structure-activity relationships, there is little to suggest that  $\beta$ -HCH is carcinogenic in humans. The most appropriate EPA classification would be “not likely” and the most appropriate Ashby et al. classification would be Category 6. Under the European system,  $\beta$ -HCH should be treated as a non-genotoxic carcinogen with a threshold and NOAEL.

## **6.2 Discussion of the Existing Cancer Slope Factor**

USEPA's slope factor on IRIS for  $\beta$ -HCH of  $1.8 \text{ (mg/kg/day)}^{-1}$  is based on effects in livers of male mice in Thorpe and Walker (1973) (USEPA 1996). In addition to the problem with this end-point, only one dose group (200 ppm) was used in calculation of the slope factor. There was high mortality in exposed mice during the first 3 months, and

relatively few animals were tested. There was also a high incidence of tumors in the controls and benign tumors predominated in both the control and dosed group.

### **6.3 Recalculation of the Cancer Slope Factor**

The existing  $\beta$ -HCH slope factor is subject to substantial uncertainty that can be reduced by the incorporation of scientific developments and USEPA policy changes that have occurred in the last 10 years. The developments that are applicable to  $\beta$ -HCH include consideration of the new inter-species extrapolation parameter, and aggregation of sex-specific responses from the same study.

Despite the limitations of the Thorpe and Walker (1973) study, we re-analyzed the data from this study using the linearized multi-stage model for cancer low dose extrapolation in the context of the existing cancer risk assessment guidelines. First, we used the  $BW^{0.75}$  inter-species scaling factor as previously proposed by USEPA and also noted in the new cancer guidelines. Second, we calculated a slope factor based on the female mouse data presented in the Thorpe and Walker (1973) study that had been discussed by USEPA, but not included in the calculation. Third, we combined the male and female slope factors to calculate a geometric mean slope factor. This analysis gives the best estimate cancer slope factor still assuming, as a worst-case conceptual model, that  $\beta$ -HCH functions as complete carcinogen and taking the 95% upper confidence limit on cancer slope factor from the linearized multistage model. The resulting recalculated slope factor for  $\beta$ -HCH is  $0.64 \text{ (mg/kg/day)}^{-1}$ . Even this recalculated slope factor should be used with caution, however, since the evidence does not support the hypothesis of human carcinogenicity of  $\beta$ -HCH.

### **6.4 Errors in IRIS Record**

We also identified a number of inaccuracies in the IRIS record for  $\beta$ -HCH that should be corrected. Specifically, the Nagasaki et al. (1972b) study did not evaluate the toxicity of  $\beta$ -HCH, but rather tested technical grade HCH (which consists of 67%  $\alpha$ -HCH, 11%  $\beta$ -HCH, 15%  $\gamma$ -HCH, and 6%  $\delta$ -HCH). Therefore, the calculated slope factor of  $4.7 \text{ (mg/kg/day)}^{-1}$  from this study, noted in the discussion of confidence section, should be omitted from the record. The slope factor of  $6.3 \text{ (mg/kg/day)}^{-1}$  calculated based on Ito et al. (1973b), and cited in the discussion of confidence section should also be removed. A critical review of this study revealed that mice fed dietary concentrations of up to 250 ppm  $\beta$ -HCH did not develop any tumors. Another study published by Ito et al. (1973a) corroborated the lack of carcinogenicity of  $\beta$ -HCH in mice fed up to 500 ppm  $\beta$ -HCH, but was not mentioned in the IRIS record; we suggest that USEPA discuss the negative data results from these studies. The Ito et al. (1973a) study should be discussed in the  $\beta$ -HCH record because it was selected as the basis of the  $\alpha$ -HCH cancer slope factor on IRIS.

## **7. Risk Assessment of $\delta$ -HCH**

In all of the literature reviewed, no epidemiologic studies, case reports, or clinical studies were found that indicated evidence of carcinogenicity in humans. Four animal bioassays were found that evaluated dietary administration of  $\delta$ -BHC (presented in Appendix 1). All of the studies were negative (no tumor activity) and had considerable study limitations (noted in Appendix 1).

## 7.1 Discussion of $\delta$ -HCH Toxicology

USEPA has officially placed  $\delta$ -HCH in category D based on inadequate evidence of carcinogenicity. There is no evidence for mutagenicity or tumorigenicity in humans (ATSDR 1994), and  $\delta$ -BHC has been negative for carcinogenicity in three studies (two mouse and one rat bioassay) (Ito et al. 1973a, 1975, Nagasaki et al. 1972a).

Several studies are available that describe the toxicity of  $\delta$ -HCH in animals. Based on the available data, the liver appears to be the most sensitive target organ for  $\delta$ -HCH in rodents following oral exposure. No adverse liver effects were observed in rats exposed to dietary concentrations up to 500 ppm (25 mg/kg/day) for 24 or 48 weeks (Ito et al. 1975) or in mice fed up to 500 ppm (65 mg/kg/day) for 24 weeks (Ito et al. 1973a, Nagasaki et al. 1972a). Slight liver cell hypertrophy was noted in rats fed 1,000 ppm (50 mg/kg/day) for 48 weeks (Ito et al. 1975), therefore 500 ppm (25 mg/kg/day) was conservatively designated as a no-observed-adverse effect level (NOAEL) from this study. Higher dietary concentrations of 1,500 ppm  $\delta$ -HCH were reported to be toxic to rats. These two studies also evaluated the other HCH isomers at comparable levels and demonstrated that  $\delta$ -HCH is less toxic to the liver than the  $\alpha$ -,  $\beta$ - and  $\gamma$ -HCH isomers (Ito et al. 1973a, 1975, Nagasaki et al. 1972a).

The only other toxicological information available for  $\delta$ -HCH was located in a review article (Reuber 1980) that reported the results of an unpublished FDA study in rats (Nelson 1949). In this study, male rats (n=5/dose) were fed 800 to 3,200 ppm  $\delta$ -HCH for 104 weeks. Survival was reduced in all dose groups. Adverse liver effects, characterized by severe diffuse cirrhosis and portal vein thrombosis were observed in rats fed 1,600 ppm, while higher doses of 3,200 ppm were associated with focal necrosis of the skeletal muscle. Testicular atrophy was also noted in all treated rats, but was most severe in rats receiving the highest dose level. Rats exposed to 800 ppm developed fibrosis of the heart.

## 7.2 Calculation of a Reference Dose

Since there is no evidence to support the carcinogenicity of  $\delta$ -HCH, it is reasonable to derive a toxicity criterion based on non-cancer effects. We selected the Ito et al. (1975) for derivation of an RfD, because it has the longest duration (48 weeks), tested multiple dose levels at higher concentrations than Ito et al. (1973a) and provides data for a comparative analysis of the HCH isomers. ATSDR (1994) reviewed this study and reported that 1,000 ppm (50 mg/kg/day) is a NOAEL for hepatic effects in rats in the text, but identifies a lowest-observed-adverse-effect level (LOAEL) of 500 ppm (25 mg/kg/day) on Table 2-2 in the ATSDR report (Ito et al. 1975). Due to these

inconsistencies, we conducted an independent review of this study and determined that 25 mg/kg/day is actually a NOAEL for liver effects because no histopathologic observations were noted at this dose level. Applying an uncertainty factor of 300 to account for extrapolation of inter- and intra-species variation and study duration, yields a provisional RfD of 0.083 mg/kg/day.

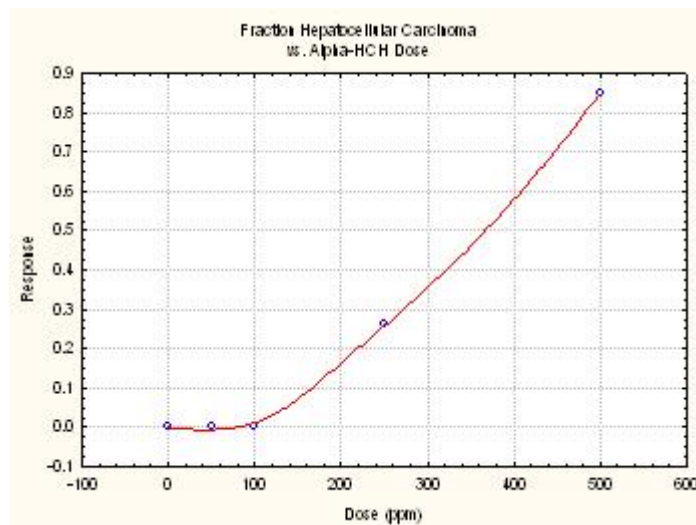
## **8. Discussion and Mode of Action Considerations**

As would be expected with isomers, there is a great deal of commonality in the biological response to the HCH isomers. This response includes equivocal or negative human data, negative cancer bioassay responses in rats and hamsters, and positive cancer responses in male mouse livers at high doses. Although not discussed explicitly in these comments due to chemical ambiguity, the data available for t-HCH is similar to that available for the individual isomers. EPA (1987) notes that t-HCH is negative for cancer in rat and Syrian golden hamsters. EPA (1987) further notes that t-HCH did induce hepatomas in mice at 300 ppm in the diet, but not at 100 ppm. In another study, t-HCH produced a significantly elevated incidence of hyperplastic nodules and hepatomas in male dd mice at 660 ppm in the diet, but not at 66 ppm. Other studies reported in EPA (1987) are similar in dose-response, predominance of male liver tumors and significant contribution of benign tumors to the overall count.

Although a comprehensive discussion is beyond the scope of these comments, a substantial amount of evidence is available regarding the mode of action of HCH isomers. This evidence includes lack of genotoxicity, tumor promotional ability, existence of thresholds in the observed dose-response range, and information about the ability of HCH to inhibit GJIC.

Extensive evidence was found that supports the theory that HCH is a tumor promoter and not a tumor initiator. In fact, HCH is routinely identified as a tumor promoter in the literature (Ito et al., 1980; Wolff et al. 1987, Schultz-Hermann, R. and Bursch, W. 1990; Schoter et al., 1987 and Gerlyng et al., 1994). In promotion studies with HCH, tumor yields were reversible when treatment stopped (Shulte-Herman and Parzefall 1981). HCH has been found to promote both liver tumors initiated with diethylnitrosamine and with *N*-nitrosomorpholine (Siglin et al. 1991; Lueback et al. 1995) and also to promote skin tumors (Tsushimoto et al 1983). The World Health Organization evaluated mechanistic data for lindane (WHO 1991) and concluded that “The results of studies on initiation-promotion, on mode of action, and on mutagenicity indicate that the tumorigenic effects of gamma-HCH in mice results from non-genetic mechanisms”, i.e. it is a non-genotoxic carcinogen.

The animal bioassay data for HCH dose-carcinogenic response show a threshold in the observed region when there are sufficient data to ascertain the dose-response. Figure 8-1 presents the dose-response curve for hepatocellular carcinoma found in dd male mouse livers (Ito et al., 1973a,b) administered  $\alpha$ -HCH.



These data show a classical threshold similar to that shown for hepatotoxicity. Similar dose-response curves are found when data for the other HCH isomers are plotted. The similarities in the locations of the threshold (around 100 ppm or greater) support the hypothesis that tumor genesis of lindane only occurs when there is metabolic saturation (Chadwick et al. 1987).

Inhibition of GJIC has long been thought to be a factor in carcinogenesis, particularly where promoters are concerned (Troski & Goodman, 1994, Chipman et al. 2003, Klaunig et al. 2000). As noted above, HCH isomers have been found to inhibit GJIC and/or apoptosis in a variety of experimental environments (Kureta et al. 1982; Criswell et al., 1995a; Criswell et al., 1995b; Zhong-Xiang et al. 1986, Ruch et al., 1987; Guan et al., 1995 ; Leibold and Schwartz, 1993; and Tsushimoto et al., 1983, Lueback et al. 1995). In general, these studies show the existence of a dose-response relationship that involves a threshold. Guan et al. (1995) observed that short exposures to HCH resulted in loss of gap permeability whereas longer exposures lead to a loss in gap junction number; still longer treatment affected Cx43 protein and mRNA. In addition, inhibition of GJIC is reversible upon cessation of treatment and may also be reversed by administration of antioxidants. When tested under similar circumstances, the GJIC inhibition potency of lindane was less than other well known promoters such as DDT and Phenobarbital (Ruch et al. 1987).

## 9. Summary and Conclusions

To reiterate the principle findings of the research upon which these comments are based, in general, the HCH isomers are:

- not genotoxic,
- not carcinogenic in humans
- carcinogenic only in male mouse livers
- tumorigenic only at high doses,

- hepatotoxic in laboratory animals
- positive in promotion; negative in initiation studies
- gap junction intercellular communication inhibitors
- mostly associated with reversible effects.

From the standpoint of formal causation using criteria developed by Bradford Hill (1965) or Guzelian et al. (2005), the available data do not support an hypothesis that HCH isomers are carcinogenic in humans. The weight of the evidence suggests that the HCH isomers are “not likely” to cause human cancer as per EPA’s classification system or may be classified in Category 6: Carcinogenic in animals, probably not a human cancer hazard in Ashby et al’s (1990) system. Perhaps the most useful classification for the HCH isomers is the European classification of non-genotoxic carcinogens. Both mechanistic data and dose-response curves within the observed region strongly point to the existence of a toxicological threshold for these compounds. Since the threshold appears to be related to phenomena that may also be associated with frank hepatotoxicity, it is possible that the existing (or proposed) reference doses may be adequate to assess risk and regulate these materials for both carcinogenic and non-carcinogenic effects.

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**APPENDIX 1**  
**SUMMARY OF BIOASSAY RESULTS CONSIDERED IN THE CARCINOGENESIS WEIGHT OF EVIDENCE**  
**CLASSIFICATIONS**

Chemical	Mice		Rats		Hamsters		Exposure Regimen	Study Findings/Comments	Reference
	—	—	—	—	—	—			
***** α-BHC	+						<b>Duration:</b> 24 weeks; <b>Sample size:</b> 20-40 male dd mice; <b>Dose level:</b> 0, 100, 250, 500 ppm	<b>Results:</b> Liver nodule hyperplasia was observed in treated mice (0/20, 0/20, 30/38 and 20/20, respectively); the incidences of hepatocellular carcinoma were 0/20, 0/20, 10/38, 17/20, respectively. No metastatic changes or tumors in other organs were noted. Increased liver weight was noted in all exposed mice, and was 3.6 times higher in 500 ppm group than controls. Severe liver cell hypertrophy was observed in the 250 and 500 ppm groups. Necrotic change was rarely noted in 500 ppm group, and increased smooth endoplasmic reticulum (SER) was noted. <b>Study limitations:</b> No statistical analysis; only examined liver histologically; mortality data not presented or discussed except that mice dying during the experiment were excluded; only males tested.	Ito et al. (1973a)
α-BHC	+						<b>Duration:</b> 24 weeks <b>Sample size:</b> 26-30 male dd mice (20 controls); <b>Dose level:</b> 0, 50, 100, 250 ppm	<b>Results:</b> Benign and malignant tumors were observed only in the 250 ppm group at 76% (23/30) and 26.7% (8/30), respectively. The incidences of nodular hyperplasia were 0/20, 0/28, 0/26, and 23/30 for the 0, 50, 100 and 250 ppm groups, respectively. The incidences of hepatocellular carcinoma were 0/10, 0/28, 0/26 and 8/30, respectively. Liver cellular hypertrophy was observed in the 100 and 250 ppm groups. No cirrhotic changes of the liver or metastatic changes in other organs were noted. Liver weight, as a percentage of body weight was increased. Body weight did not appear to be affected in the treated mice. <b>Study limitations:</b> Only liver examined microscopically. Mortality data not discussed, except that mice dying during the experiment were excluded. No statistical analysis. Only males tested.	Ito et al. (1973b)
α-BHC	+						<b>Duration:</b> 36 weeks exposure and 36 weeks of observation; <b>Sample size:</b> 12-20 male ddy mice sacrificed per interval	<b>Results:</b> 20/20 and 14/14 (100%) of mice exposed for 24 and 36 weeks had liver tumors. 14/20 (70%) of mice exposed for 20 weeks had tumors (incidence for benign and malignant tumors not presented). No liver tumors were in controls. The incidence of liver tumors increased	Ito et al. (1976)

Chemical	Mice		Rats		Hamsters		Exposure Regimen	Study Findings/Comments	Reference
	—	—	—	—	—	—			
							(controls: 20); <b>Dose level:</b> 0 and 500 ppm	progressively with continuous exposure to alpha-BHC, but some tumors disappeared following exposure cessation. Nodular hyperplasias were reversible, whereas hepatocellular carcinomas were irreversible. After 24 weeks and up to 60-72 weeks most tumors were nodular hyperplasia, but after 60-72 weeks, most were hepatocellular carcinomas. <b>Study limitations:</b> Only one dose; no statistical analysis; only reported incidence for total liver tumors; study focused on liver effects; mortality data not discussed, and body weight data not discussed (i.e., difficult to determine if body weights were reduced because control body weight only reported for 72 weeks); only males tested.	
α-BHC			+				<b>Duration:</b> 24-72 weeks <b>Sample size:</b> 18-24 male W rats (5-16 rats effective rats per interval); <b>Dose level:</b> 0, 500, 1000, 1500 ppm	<b>Results:</b> Hepatocellular carcinoma was observed only in rats of the 1000 and 1500 ppm groups at 72 weeks (1/16 and 3/13, respectively). The incidences of nodular hyperplasia were as follows: control--0/8 (72 weeks), 500 ppm-- 0/6 (24 weeks), 0/5 (48 weeks), 1000 ppm--5/12 (48 weeks), 12/16 (72 weeks), and 1500 ppm--10/13 (72 weeks). No metastases were observed. Liver weight was increased as a percentage of body weight in all dose groups at all duration (no statistics). Histopathologic changes were observed in the liver at all doses. Body weight appeared to be significantly reduced relative to controls (20-27% reduction in 1000 and 1500 ppm groups, respectively at 72 weeks relative to controls). Mortality appeared to be high in exposed animals, but limited details were presented. <b>Study limitations:</b> No statistical analysis. Mortality appears to be high, although mortality data not discussed, other than rats dying were excluded. Small sample size. Only examined liver in detail. Rats dying during the experiment were not examined. Only males tested.	Ito et al. (1975)
α-BHC	+	+					<b>Duration:</b> 32 weeks followed by 6 weeks observation; <b>Sample size:</b> 10-11/sex dd mice (controls 20 male/21 female); <b>Dose level:</b> 0, 100, 300, 600 ppm	<b>Results:</b> Hepatoma incidences for males were: 0/14, 1/8, 7/7, 7/7, and for females were: 0/15, 0/8, 2/3, 6/8, respectively. Tumor size increased with increasing dose. No peritoneal invasions or metastases were noted in any mice. Atypical proliferation in liver (associated with liver cell damage) was noted in all treated male and female mice. <b>Study limitations:</b> no statistical analysis; high mortality in both controls and exposed groups (25-33% for controls; and 30-70% for exposed mice) after 36 to 38 weeks. Small sample size. Only mice that survived 36	Hanada et al. (1973)

Chemical	Mice		Rats		Hamsters		Exposure Regimen	Study Findings/Comments	Reference
	—	—	—	—	—	—			
								weeks were examined.	
$\alpha$ -BHC	+						<b>Duration:</b> 24 weeks <b>Sample size:</b> 20 male dd mice; <b>Dose level:</b> 0, 100, 250, 500 ppm	<b>Results:</b> All mice in the 500 ppm group had liver tumors (hepatomas) consisting of multiple yellow nodules up to 2 cm in diameter. In the 250 ppm group, 9/20 mice had smaller nodules (up to 0.3 cm). In the 250 and 500 ppm groups, nodules showed adenomatous pattern, and carcinomatous area consisting of irregular and atypical neoplastic cells. Mice exposed to 100 ppm had no remarkable changes in the liver (0/20 liver nodules). <b>Study limitations:</b> This study is an abstract, and therefore has no study details. Small sample size, and no mortality data discussed. The incidence of benign and malignant tumors was not specified.	Nagasaki et al. (1972a)
$\alpha$ -BHC	+	+	-	-	-	-	<b>Duration:</b> 24 weeks <b>Sample size:</b> exp 1: 20 male Wistar rats; 16 male Golden Syrian hamsters; 36 ddy male mice (16 controls and 20 exposed); exp 2: 80 M/F dd mice; 80 M/F CH3/He mice; 64 M/F DBA/2 mice; 88 M/F ICR mice; 88 M/F C57BL/6 mice; <b>Dose level:</b> 0, 500 ppm	<b>Results:</b> In experiment 1: no neoplastic changes were observed in rats and hamsters, although minimal liver cell hypertrophy was noted. Male dd mice exhibited liver cell hypertrophy; 20/20 had nodular hyperplasia, and 6/20 had hepatocellular carcinoma. No neoplasms were in controls. Liver weight as a percentage of body weight was increased in all three species. In experiment 2: liver nodules were observed in all 5 strains of mice, DDY, ICR, DBA/2, C3H/He and C57BL/6 mice, but their incidence varied and was highest in ddy mice (20/20 males and 16/20 females) and lowest in C57BL/6 mice (4/21 males and 3/18 females or 16.7-19%), and was higher in males than females. No neoplasms were in controls. Carcinomas were noted in DDY (25-65%), ICR (20.7-39%), DBA/2 (6.3-6.7%), and C3H/He (10%) mice, but not in C57BL/6 (0%). Body weight did not appear to differ between treated and control mice. <b>Study limitations:</b> Only livers examined histologically. One dose level. No mortality data discussed, except that animals dying were excluded. No statistical analysis. Treatment and control groups varied in size. No statistical analysis. Liver hypertrophy was observed in all treated mice but was most pronounced in male ddy mice.	Nagasaki et al. (1975)
$\alpha$ -BHC	+						<b>Duration:</b> 6 months <b>Sample size:</b> 10 male ICR-JCL mice <b>Dose level:</b> 600 ppm	<b>Results:</b> Hepatomas were observed in 10/10 mice, some of which were malignant. The incidence of benign and malignant tumors was not reported. Liver weight was increased. <b>Study limitations:</b> Only one dose; small sample size; only males tested; minimal details on histopathology. Mortality data not discussed. No	Goto et al. (1972)

Chemical	Mice		Rats		Hamsters		Exposure Regimen	Study Findings/Comments	Reference
	—	—	—	—	—	—			
								statistical analysis conducted. Only examined the liver. Study did not discuss findings in control animals. (German)	
$\alpha$ -BHC				+			<p><b>Duration:</b> up to 26 months;  <b>Sample size:</b> 15 female Wistar rats (3-7 rats per sacrifice interval) (controls: 32 or 3-5 per interval);  <b>Dose level:</b> 0, and initial p.o. dose of 100 mg/kg followed by 18.4 mg/kg/day</p>	<p><b>Results:</b> Rats exposed for 11-16 months had grayish white foci in the liver. After 2 yrs of exposure, tumorous nodules up to 2 cm in diameter were detected, histologically these were foci of altered hepatocytes, neoplastic hepatic nodules, or hepatocellular carcinoma. In rats exposed continuously for 20-26 months, 6/6 had microscopic liver lesions (5/6 had liver nodules, and 1/6 had hepatocellular carcinoma), while 6/6 controls also had microscopic lesions (5/6 foci and 1/6 nodules). 10/22 control animals had microscopic liver lesions, mostly foci of altered cells; one control had nodules. Body weight gain was reduced at 4.5 months (13% reduction), 13.5 months (13% reduction), and 23.5 months (22% reduction), but was not statistically significant at 4.5 and 23.5 months. In the same study, rats pretreated with DENA and intermittently exposed to 420 mg/kg alpha-BHC every 3 weeks displayed marked acceleration of tumor formation compared to rats treated with DENA only. This suggests that alpha-BHC may be a tumor promoter. This study failed to detect evidence suggesting that alpha-BHC is an initiator.  <b>Study limitations:</b> tumors and foci of altered cells also in controls; one dose tested; small sample size; only females tested. Body weight was significantly reduced, indicating exceedance of the MTD. No mortality data were presented.</p>	Schulte-Hermann and Parzefall (1981)
$\alpha$ -BHC	—	—					<p><b>Duration:</b> 24 weeks;  <b>Sample size:</b> 15/sex B6C3F1 mice;  <b>Dose level:</b> 0, 250 ppm</p>	<p><b>Results:</b> No hepatic foci or adenomas were observed in controls or treated mice. In same study, alpha-BHC exposure following diethylnitrosamine (DENA) initiation resulted in a decreased number of liver adenomas in males compared to DENA treated mice, and an increase in liver adenomas in females compared to DENA treated mice. The ability of alpha-HCH to stimulate synthesis in hepatocellular foci of B6C3F1 mice correlated with its ability to promote or inhibit hepatic tumorigenesis. These results suggest a causal relationship between the ability of nongenotoxic carcinogens to induce cell proliferation with their ability to promote or inhibit tumor formation in the mouse liver.  <b>Study limitations:</b> Only liver examined, only one dose tested, small sample size, no body weight or mortality data discussed. The results are</p>	Siglin et al. (1991)

Chemical	Mice		Rats		Hamsters		Exposure Regimen	Study Findings/Comments	Reference
	—	—	—	—	—	—			
								discussed in the text, but not presented on tables or figures.	
$\alpha$ -BHC	+	-					<b>Duration:</b> 24 weeks; <b>Sample size:</b> 15/sex B6C3F1 mice; <b>Dose level:</b> 0, 250 ppm	<b>Results:</b> No liver adenomas were observed in females, but 4/15 (27%) males developed liver adenomas. The majority of hepatocellular lesions exhibited eosinophilic staining. In males and females, 4/15 (27%) and 1/15 (7%) developed hepatocellular foci. No foci were observed in controls. Terminal body weight for males was significantly lower than controls. In the same study, alpha-BHC exposure following DENA initiation resulted in a decreased number of liver adenomas in males compared to DENA treated mice, and an increase in liver adenomas in females compared to DENA treated mice. Therefore, alpha-BHC appeared to inhibit hepatic tumorigenesis in males while promoting hepatic formation in females. The greater susceptibility of males to liver tumors relative to females has been attributed to the tumor-promoting effects of testosterone in males and tumor-inhibiting effects of estrogens in females. In conclusion, the ability of $\alpha$ -HCH to promote or inhibit hepatic tumorigenesis appeared to correlate with its ability to induce DNA synthesis in hepatocellular foci following short-term treatment. <b>Study limitations:</b> Only liver examined. Only one dose tested, and small sample size.	Siglin et al. (1995)
$\alpha$ -BHC	+						<b>Duration:</b> 50 weeks; <b>Sample size:</b> 4-9/group male HPB black mice (75 total with interval sacrifice) (48 controls); <b>Dose level:</b> 0, 500 ppm	<b>Results:</b> All treated mice developed both gross and histologic nodules of the liver by 33 weeks of exposure. All nodules were benign (adenomata), and did not progress. No nodules were observed until 21 weeks of exposure. No hepatocellular carcinoma or metastases in the lungs were detected. The authors conclude that the transformation of hepatocellular adenoma to hepatocellular carcinoma must progress very slowly, if at all. Hepatic focal necrosis, enlargement of mesenteric lymph nodes (leukemia), and alveogenic tumors were present in a few exposed and control mice. Liver weight increased in exposed mice. Liverless body weight was reduced relative to controls at all time and was approximately 60% of controls by 50 weeks (no statistics). Therefore, it appears that the maximum tolerated dose (MTD) was exceeded. The authors suggest that the incidence of carcinoma in dd mice may result from promotion of pre-existing spontaneously initiated hepatocytes. <b>Study limitations:</b> One dose level, small sample size, only males tested,	Tryphonas and Iverson (1983)

Chemical	Mice		Rats		Hamsters		Exposure Regimen	Study Findings/Comments	Reference
	—	—	—	—	—	—			
								only examined the liver and lungs. No statistical analysis. It appears that the MTD was exceeded based on reduced body weight in exposed mice (> 10%).	
α-BHC			—	—			<b>Duration:</b> up to 56 weeks on average <b>Sample size:</b> 10/sex Wistar rats (20/sex controls); <b>Dose level:</b> 0, 10, 50, 100, 800 ppm	<b>Results:</b> No tumors were reported or discussed. A few pale foci (< 1 mm diameter) and suggestive necrosis were observed in the 800 ppm group. Rats exposed to 800 ppm had significantly reduced body weight gain and increased mortality relative to controls. Rats fed up to 100 ppm lived on average 56 weeks vs. 58 weeks for controls. The liver to body weight ratio was significantly increased in the 50, 100 and 800 ppm groups. Nearly all animals died or were sacrificed in extremis. <b>Study limitations:</b> Small sample size; minimal details on histopathology. Not all rats were examined histologically.	Fitzhugh et al. (1950)
***** β-BHC	+	+					<b>Duration:</b> 110 weeks; <b>Sample size:</b> 30/sex CFI mice (45/sex controls); <b>Dose level:</b> 0, 200 ppm	<b>Results:</b> In males, the incidence of liver tumors was 40% for benign tumors (simple nodular growth or type a), and 33% for malignant tumors with papilliform and adenoid growth, with occasional lung metastasis (type b). The incidences in females were: 30% and 13%, respectively. The total liver tumor incidence was 12/30 and 22/24 for females and males, respectively. Male controls exhibited tumor incidences of 20% and 4% for type (a) and (b), respectively. For female controls the incidences were 23% and 0%, respectively. Enlarged livers were noted in female mice by week 50 and in males by week 60. Gross liver lesions were observed, with occasional yellow necrotic areas. Tumors were observed at other sites (e.g., lung, lymphoid tissue, testes/ovaries). Mortality was significantly elevated in both sexes of mice exposed for 17 and 21 months. Some mice showed signs of ataxia before death. <b>Study limitations:</b> Only one dose, high mortality in exposed mice (12% and 25% for males and females, respectively) within 3 months, which was statistically significant. Controls had a high incidence of liver tumors (36-45% in mice examined at 26 months; 23-24% total)	Thorpe and Walker (1973)
β-BHC			—				<b>Duration:</b> 24-48 weeks <b>Sample size:</b> 18-24 male W rats (5-8 rats effective rats per interval);	<b>Results:</b> No liver nodules (nodular hyperplasia or hepatocellular carcinoma) were observed in any of the exposed rats. Liver weight was increased as a percentage of body weight in all dose groups at all duration (no statistics). Histopathologic changes (cell hypertrophy) were noted in	Ito et al. (1975)

Chemical	Mice		Rats		Hamsters		Exposure Regimen	Study Findings/Comments	Reference
	—	—	—	—	—	—			
							<b>Dose level:</b> 0, 500, 1000 ppm	the liver of rats exposed to 500 ppm for 48 weeks and 1000 ppm for 24 weeks. Mortality appeared to be high in exposed animals, but limited details were presented. <b>Study limitations:</b> No statistical analysis. Mortality appears to be high, although mortality data not discussed, other than rats dying were excluded. Small sample size. Only examined liver in detail. Unable to determine if body weight was significantly reduced because control body weight data were only available for 72 weeks. Only males tested.	
β-BHC	—						<b>Duration:</b> 24 weeks; <b>Sample size:</b> 20-40 male dd mice; <b>Dose level:</b> 0, 100, 250, 500 ppm	<b>Results:</b> No nodular hyperplasia or hepatocellular carcinoma were observed in any of the exposed or control mice. Increased liver weight was noted in exposed mice at all dose levels. <b>Study limitations:</b> no statistical analysis; only examined liver histologically, mortality data not presented or discussed except that mice dying during the experiment were excluded. Only males tested.	Ito et al. (1973a)
β-BHC			—	—			<b>Duration:</b> up to 52 weeks on average <b>Sample size:</b> 10/sex Wistar rats (20/sex controls); <b>Dose level:</b> 0, 10, 100, 800 ppm	<b>Results:</b> No tumors were reported or discussed. Rats exposed to 800 ppm had significantly increased mortality relative to controls (all died by 10 weeks). Rats fed 100 ppm had pale foci of necrosis (< 1 mm diameter) in the liver and had reduced mean age at death, which was not significant because of a few late survivors. Females fed 100 ppm had significantly reduced body weight gain. The liver to body weight ratio was significantly increased in the 10, 100 and 800 ppm groups. Nearly all animals died or were sacrificed in extremis. <b>Study limitations:</b> Small sample size; minimal details on histopathology. Body weight data not presented for 10 ppm group. Not all rats were examined histologically.	Fitzhugh et al. (1950)
β-BHC	—	—					<b>Duration:</b> 32 weeks followed by 6 weeks observation; <b>Sample size:</b> 10-11/ sex dd mice (controls: 21 male/20 female); <b>Dose level:</b> 0, 100, 300, 600 ppm	<b>Results:</b> No hepatomas were observed in exposed or control mice of either sex. Atypical proliferation in liver (associated with liver cell damage) was noted in males: 0/14, 0/9, 4/8 and 8/8 respectively and for females: 0/15, 0/9, 2/8, 3/4, respectively. <b>Study limitations:</b> no statistical analysis; high mortality in controls (25-33%) and female mice at 600 ppm (60%); lower mortality in male mice and lower dose females (10-20%) after 36 to 38 weeks.	Hanada et al. (1973)

Chemical	Mice		Rats		Hamsters		Exposure Regimen	Study Findings/Comments	Reference
	—	—	—	—	—	—			
β-BHC	—						<b>Duration:</b> 24 weeks; <b>Sample size:</b> 20 male dd mice; <b>Dose level:</b> 0, 100, 250, 500 ppm	<b>Results:</b> No remarkable changes were observed in the liver of mice. <b>Study limitations:</b> This study is an abstract, and therefore has no study details. Small sample size, and no mortality data discussed. Only males tested.	Nagasaki et al. (1972a)
β-BHC	—						<b>Duration:</b> 24 weeks; <b>Sample size:</b> 26-28 male dd mice (20 controls); <b>Dose level:</b> 0, 50, 100, and 250 ppm	<b>Results:</b> No hyperplastic nodules or hepatocellular carcinoma were observed in mice exposed to 50, 100 or 250 ppm. Minimal cellular hypertrophy was noted in the 250 ppm group. Liver weight as a percentage of body weight was elevated (no stats). <b>Study limitations:</b> No statistical analysis, mortality data were not discussed, except that mice dying during the experiment were not included. Only liver was examined microscopically. Only males tested.	Ito et al. (1973b)
***** γ-BHC	+	+					<b>Duration:</b> 110 weeks; <b>Sample size:</b> 30/sex CFI mice (45/sex controls); <b>Dose level:</b> 0, 400 ppm	<b>Results:</b> In males, the incidences of liver tumors were 38% for simple nodular growth (type a), and 55% (16/29) for tumors with papilliform and adenoid growth, with occasional lung metastasis (type b). In females the incidences for type (a) and type (b) tumors were: 34% and 34% (10/29), respectively. Male controls exhibited tumor incidences of 24% (20% and 4% (2/45) for type (a) and (b), respectively). For female controls the incidence was 23% (exclusively type a). Enlarged livers were noted in both sexes of mice by week 50. Gross liver lesions were observed, with occasional yellow necrotic areas. Tumors were observed at other sites (e.g., lung, lymphoid tissue, testes/ovaries). Mortality was significantly elevated in female mice exposed for 17 and 21 months. During a re-examination of the slides by Vesselinovitch and Carlborg (1983), it was concluded that lindane did not affect the incidence of hepatocellular carcinomas in either sex, but lindane did enhance liver adenomas and hyperplastic nodules in male mice. <b>Study limitations:</b> Only one dose, high mortality in exposed mice (10% and 20% for males and females, respectively) within 3 months, which was statistically significant only for females. Only 3% of females and 17% of males exposed to gamma-BHC survived the experiment, suggesting that the MTD was exceeded. Associated liver enlargement and hepatomas may have resulted from nonspecific toxic effects (USEPA 1980). Controls had a high incidence of liver tumors (36-45% in mice examined at 26 months; 23-24% total)	Thorpe and Walker (1973)

Chemical	Mice		Rats		Hamsters		Exposure Regimen	Study Findings/Comments	Reference
	—	—	—	—	—	—			
γ-BHC	—						<b>Duration:</b> 24 weeks; <b>Sample size:</b> 20-40 male dd mice; <b>Dose level:</b> 0, 100, 250, 500 ppm	<b>Results:</b> No nodular hyperplasia or hepatocellular carcinoma were observed in any of the exposed or control mice. Increased liver weight was noted only in 500 ppm group. <b>Study limitations:</b> no statistical analysis; only examined liver histologically, mortality data not presented or discussed except that mice dying during the experiment were excluded. Only males tested.	Ito et al. (1973a)
γ-BHC	—						<b>Duration:</b> 24 weeks; <b>Sample size:</b> 20 male dd mice; <b>Dose level:</b> 0, 100, 250, 500 ppm	<b>Results:</b> No remarkable changes were observed in the liver of mice. <b>Study limitations:</b> This study is an abstract, and therefore has no study details. Small sample size, and no mortality data discussed. Only males tested.	Nagasaki et al. (1972a)
γ-BHC			—	—			<b>Duration:</b> 104 weeks; <b>Sample size:</b> 50/sex rats; <b>Dose level:</b> 0, 1, 10, 100 and 400 ppm (correspond to 0.05, 0.47, 4.81, 19.66 mg/kg/day for males and 0.06, 0.59, 6, 24.34 mg/kg/day for females)	<b>Results:</b> EPA identified a no observed effect level (NOEL) of 10 ppm from this study for systemic effects. The lowest effect level (LEL) of 100 ppm (4.81-6 mg/kg/day) is based on liver pathological changes. EPA noted that 10 ppm is a possible threshold level, but that consistent effects were observed at 100 ppm. At 100 ppm, the observed effects were as follows: periportal hepatocyte hypertrophy, increased liver and spleen weights. At 400 ppm the following were observed: decreased survival in females (trend in males), convulsions in females, decreased body weight gain, increased inorganic phosphorous, calcium, urea and cholesterol and decreased albumin/globulin ratio and RBC parameters. The RfD committee derived an RfD of $4.7 \times 10^{-3}$ mg/kg/day from this study based on a NOEL of 0.47 mg/kg/day. <b>Study limitations:</b> Good study with multiple doses, conducted statistical analysis, evaluated time trends, histopathology of multiple organs, presented body weight and mortality data, and discussed clinical findings.	Life Science Research (1989) (summarized in OPP Docket # 009909)
γ-BHC	+/-	—	—	—			<b>Duration:</b> 70-80 weeks; <b>Sample size:</b> 50/sex Osborne Mendel rats; 50/sex B6C3F1 mice (controls: 10/sex for matched rats and mice ; 40/sex pooled mice; 45/sex pooled rats); <b>Dose level:</b> Rats: males TWA	<b>Results:</b> Rats: No statistically significant treatment-related tumors were observed in rats treated for 80 weeks and observed for 29-30 weeks. Body weight in treated rats did not differ significantly from controls. Mice: Only low dose male mice had a significant increased incidence of hepatocellular carcinoma (19/49), which was only significant when compared to pooled controls. The incidence in the high-dose group was not significant (9/49). The liver tumor incidences for pooled controls, matched controls and historical controls were 5/49, 2/10, and 75/360,	NCI (1977)

Chemical	Mice		Rats		Hamsters		Exposure Regimen	Study Findings/Comments	Reference
	—	—	—	—	—	—			
							0, 236 and 472 ppm; females TWA 0, 135 and 270 ppm. Mice: 0, 80 and 160 ppm	respectively. Therefore, there was no tumor dose response for male mice. No hepatic hyperplasia was observed in mice of either sex. Control mice had a 23% liver tumor incidence (hepatocellular carcinoma and neoplastic nodules). Treated mice did not exhibit significant changes in body weight relative to controls. <b>Study limitations:</b> Poor survival rates in rats, especially in female controls; small number of matched controls (10/sex). Dosing to rats was reduced after 38 weeks due to deaths among treated animals. It is possible male rats did not receive the MTD.	
γ-BHC	+	+					<b>Duration:</b> 32 weeks followed by 6 was observation; <b>Sample size:</b> 10-11/ sex dd mice (controls: 21 male/20 female); <b>Dose level:</b> 0, 100, 300, 600 ppm	<b>Results:</b> Hepatomas were observed only in 600 ppm group. The hepatoma incidences for males were 0/14, 0/10, 0/9, and 3/4, for the 0, 100, 300 and 600 ppm groups, respectively and for females were 0/15, 0/8, 0/7, and 1/3, respectively. No peritoneal invasions or metastases were observed in any mice. Atypical proliferation in liver (associated with liver cell damage) was noted in males as follows: 0/14, 0/10, 5/9 and 4/4, respectively and for females as follows: 0/15, 0/8, 1/9, 3/3, respectively. <b>Study limitations:</b> no statistical analysis; high mortality in controls (25-33%), male mice at 600 ppm (60%) and female mice at all doses (20-70%); lower mortality in male at 100 and 300 ppm (0-10%) after 36 to 38 weeks; small sample size.	Hanada et al. (1973)
γ-BHC			—	—			<b>Duration:</b> up to 70 weeks on average <b>Sample size:</b> 10/sex Wistar rats (20/sex controls); <b>Dose level:</b> 0, 5, 10, 50, 100, 400, 800 and 1600 ppm in oil added to diet; and 10, 100 and 800 ppm dry gamma BHC	<b>Results:</b> No tumors were reported or discussed. A few pale foci (< 1 mm diameter) and suggestive necrosis were observed in rats of the 800 and 1600 ppm groups. Rats exposed to 800 and 1600 ppm gamma BHC in oil had significantly increased mortality relative to controls, based on mean age at death. These rats exhibited nervous symptoms and convulsions. Rats fed 400 ppm had reduced mean age at death, which was not significant due to a few late survivors. Rats fed 800 ppm dry gamma BHC had similar mean age at death as control rats. Body weight gain was significantly reduced only at 1600 ppm. The liver to body weight ratio was significantly increased in the 100, 800 and 1600 ppm (in oil) and 800 ppm dry gamma BHC groups. At 800 and 1600 ppm, liver cell hypertrophy (fat degeneration and necrotic changes) were noted. Nearly all animals died or were sacrificed in extremis. <b>Study limitations:</b> Small sample size; minimal details on histopathology.	Fitzhugh et al. (1950)

Chemical	Mice		Rats		Hamsters		Exposure Regimen	Study Findings/Comments	Reference
	—	—	—	—	—	—			
								Not all rats or organs were examined histologically.	
γ-BHC	—						<b>Duration:</b> 24 weeks; <b>Sample size:</b> 26-28 male mice; <b>Dose level:</b> 0, 50, 100 and 250 ppm	<b>Results:</b> No hyperplastic nodules or hepatocellular carcinomas were observed in mice exposed to 50, 100 or 250 ppm. Minimal liver cellular hypertrophy was noted in the 250 ppm group. <b>Study limitations:</b> Only liver was examined microscopically; no statistical analysis. Mortality data not discussed, except that mice dying during the experiment were excluded. Only males tested.	Ito et al. (1973b)
γ-BHC			—	—			<b>Duration:</b> 2 years <b>Sample size:</b> not specified; <b>Dose level:</b> 0, 25, 50 and 100 ppm	<b>Results:</b> No significant increase in tumors was observed. <b>Study limitations:</b> Not specified.	Truhaut (1954) as cited in USEPA 1980
γ-BHC		+					<b>Duration:</b> 24 months <b>Sample size:</b> 36-96 female Obese mottled yellow A <sup>vy</sup> /a, lean pseudogouti A <sup>vy</sup> /a and lean black a/a female mice; <b>Dose level:</b> 0, 160 ppm	<b>Results:</b> Hepatocellular adenomas were in 12% pseudoagouti mice, 3% black mice, and 35% in yellow mice. Hepatocellular carcinomas were presented in the same species at 5%, 1%, and 17%, respectively. Control incidences for adenomas were 9%, 5%, and 6%, respectively and for carcinomas were 13%, 2% and 3%, respectively. The A <sup>vy</sup> yellow mice have a proclivity to form hepatocellular adenomas and lung tumors, which is augmented by exposure to lindane. The authors conclude that the A <sup>vy</sup> gene, in the absence of obesity, sensitizes cells to transformation. This study demonstrates that certain genetic lines derived from a common strain are more susceptible to induction of liver tumors and possibly lung tumors. <b>Study limitations:</b> High tumor incidence in controls. Susceptible strain. Only one dose.	Wolff et al. (1987)
γ-BHC			—				<b>Duration:</b> 24-48 weeks <b>Sample size:</b> 18-24 male W rats (6-8 rats effective rats per interval); <b>Dose level:</b> 0, 500 ppm	<b>Results:</b> No liver nodules (nodular hyperplasia or hepatocellular carcinoma) were observed in any of the exposed rats. Liver weight was increased as a percentage of body weight at 24 and 48 weeks (no statistics). Histopathologic changes (cell hypertrophy) were noted in the liver of rats exposed to 500 ppm for 48 weeks. Mortality appeared to be high in exposed animals, but limited details were presented. <b>Study limitations:</b> Only one dose tested and sex tested. No statistical analysis. Mortality appears to be high, although mortality data not discussed, other than rats dying were excluded. Small sample size. Only	Ito et al. (1975)

Chemical	Mice		Rats		Hamsters		Exposure Regimen	Study Findings/Comments	Reference
	—	—	—	—	—	—			
								examined liver in detail. Unable to determine if body weight was significantly reduced because control body weight data were only available for 72 weeks.	
γ-BHC	+						<b>Duration:</b> 6 months <b>Sample size:</b> 10 male ICR-JCL mice <b>Dose level:</b> 0,300, 600 ppm	<b>Results:</b> Benign hepatomas were observed in 5/10 mice exposed to 600 ppm. No tumors were observed in the 300 ppm group. Body weight was reduced 12% in the 600 ppm relative to controls, indicating the exceedance of the maximum tolerance dose (MTD). A marked increase in liver weight was observed. <b>Study limitations:</b> Small sample size; minimal details on histopathology. Mortality data not discussed. No statistical analysis conducted. Only examined the liver. Study did not discuss findings in control animals. Only males tested. (German)	Goto et al. (1972)
γ-BHC	—	—					<b>Duration:</b> 80 weeks; <b>Sample size:</b> 50/sex/dose Chbi NMRI mice (100/sex controls); <b>Dose level:</b> 0, 12.5, 25, and 50 ppm (equivalent to 2.1, 4.1 and 8.2 mg/kg/day for males and 2, 3.9, and 7.8 mg/kg/day for females)	<b>Results:</b> No evidence of lindane-related tumor formation was observed in treated mice. The incidence of liver cell adenomas was 5/200, 2/100, 0/100 and 2/100, in the 0, 12.5, 25 and 50 ppm groups, respectively. The incidence of other tumors was 41/200, 22/100, 13/100 and 22/100, respectively. No significant differences in body weight, or mortality were observed. No microscopic changes attributable to lindane exposure were observed in the liver cells. <b>Study limitations:</b> Lung tumors were observed in both control and exposed mice. Control mice had a higher incidence of lymphocytic leukemia/lymphosarcoma than exposed mice. Limited details on histopathology.	Weisse and Herbst (1977); Herbst et al. (1975)
δ-BHC	—						<b>Duration:</b> 24 weeks; <b>Sample size:</b> 20-40 male dd mice; <b>Dose level:</b> 0, 100, 250, 500 ppm	<b>Results:</b> No nodular hyperplasia or hepatocellular carcinoma in any of the exposed or control mice. Increased liver weight was noted in 500 ppm group. <b>Study limitations:</b> no statistical analysis; only examined liver histologically, mortality data not presented or discussed except that mice dying during the experiment were excluded. Only males tested.	Ito et al. (1973a)
δ-BHC	—						<b>Duration:</b> 24 weeks; <b>Sample size:</b> 20 male dd mice; <b>Dose level:</b> 0, 100, 250, 500	<b>Results:</b> No remarkable changes were observed in the liver of mice. <b>Study limitations:</b> This study is an abstract, and therefore has no study details. Small sample size, and no mortality data discussed. Only males	Nagasaki et al. (1972a)

Chemical	Mice		Rats		Hamsters		Exposure Regimen	Study Findings/Comments	Reference
	—	—	—	—	—	—			
							ppm	tested.	
δ-BHC			—				<b>Duration:</b> 24-48 weeks <b>Sample size:</b> 18-24 male W rats (5-8 rats effective rats per interval); <b>Dose level:</b> 0, 500, 1000 ppm	<b>Results:</b> No liver nodules (nodular hyperplasia or hepatocellular carcinoma) were observed in any of the exposed rats. Liver weight was increased as a percentage of body weight in all dose groups at all duration (no statistics). Histopathologic changes (cell hypertrophy) were noted in the liver of rats exposed to 1000 ppm for 48 weeks. Mortality appeared to be high in exposed animals, but limited details were presented. <b>Study limitations:</b> No statistical analysis. Mortality appears to be high, although mortality data not discussed, other than rats dying were excluded. Small sample size. Only examined liver in detail. Unable to determine if body weight was significantly reduced because control body wt data were only available for 72 weeks. Only males tested.	Ito et al. (1975)

\*\*\*\* = Basis of the USEPA Cancer Slope Factor.