PEER REVIEW SUMMARY REPORT

External Peer Review of the *Toxicological Review of Chlordecone (Kepone)*

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I. INTRODUCTION

The Integrated Risk Information System (IRIS) is an U.S. Environmental Protection Agency (EPA) data base containing Agency consensus scientific positions on potential adverse human health effects that may result from chronic (or lifetime) exposure, or in select cases less-than-lifetime exposures, to chemicals in the environment. IRIS currently provides health effects information on over 500 chemical substances.

IRIS contains chemical-specific summaries of qualitative and quantitative health information in support of two steps of the risk assessment process, i.e., hazard identification and dose-response evaluation. IRIS information includes a reference dose for non-cancer health effects resulting from oral exposure (the RfD), a reference concentration for non-cancer health effects resulting from inhalation exposure (the RfC), and an assessment of carcinogenicity for both oral and inhalation exposures. Combined with specific situational exposure assessment information, the health hazard information in IRIS may be used as a source in evaluating potential public health risks from environmental contaminants.

The IRIS program, within EPA's National Center for Environmental Assessment (NCEA), developed a Toxicological Review of Chlordecone, an assessment of which has not previously appeared on the IRIS data base. Chlordecone was nominated for assessment by the Superfund Technical Support Center within NCEA. The draft document slated for the external peer review contains a chronic oral reference dose and a qualitative cancer assessment.

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II. CHARGE TO THE REVIEWERS

The U.S. Environmental Protection Agency (EPA) is seeking an external peer review of the scientific basis supporting the human health assessment of chlordecone that will appear on the Agency's online database, the Integrated Risk Information System (IRIS). IRIS is prepared and maintained by the EPA's National Center for Environmental Assessment (NCEA) within the Office of Research and Development (ORD). There is currently no assessment on the IRIS database for the health effects associated with chlordecone exposure.

The draft health assessment includes a chronic Reference Dose (RfD) and a carcinogenicity assessment. Below is a set of charge questions that address scientific issues in the assessment of chlordecone. Please provide detailed explanations for responses to the charge questions.

(A) General Charge Questions:

1. Is the Toxicological Review logical, clear and concise? Has EPA accurately, clearly and objectively represented and synthesized the scientific evidence for noncancer and cancer hazard?

2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of chlordecone.

3. Please discuss research that you think would be likely to reduce uncertainty in the future assessments of chlordecone.

4. Please comment on the identification and characterization of sources of uncertainty in sections 5 and 6 of the assessment document. Please comment on whether the key sources of uncertainty have been adequately discussed. Have the choices and assumptions made in the discussion of uncertainty been transparently and objectively described? Has the impact of the uncertainty on the assessment been transparently and objectively described?

Chemical-Specific Charge Questions:

(B) Oral reference dose (RfD) for Chlordecone

1. A chronic RfD for chlordecone has been derived from the 2-year dietary study (Larson et al., 1979a) in rats. Please comment on whether the selection of this study as the principal study has been scientifically justified. Has this study been transparently and objectively described in the document? Please identify and provide the rationale for any other studies that should be selected as the principal study.

2. Kidney (glomerular) lesions, liver lesions, and reproductive effects are all sensitive effects of chlordecone exposure. Glomerular lesions in the kidney were selected as the most appropriate critical effect. Please comment on whether the selection of glomerular lesions as the critical effect instead of reproductive endpoints (such as testicular lesions) has been scientifically justified. Is this choice transparently and objectively described in the document? Please provide

detailed explanation. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

3. Some evidence exists to suggest that the mechanism of the critical effect selected for determination of the point of departure (POD) (i.e., glomerular lesions) may be mediated through an autoimmune mechanism. Please comment on whether the available immunotoxicity data support this proposed mechanism of action (MOA). Is this proposed MOA scientifically justified and transparently described?

4. The chronic RfD has been derived utilizing benchmark dose (BMD) modeling to define the POD. All available models were fit to the data for the incidence of glomerulosclerosis in female rats. Please provide comments with regards to whether BMD modeling is the best approach for determining the POD. Has the BMD modeling been appropriately conducted and objectively and transparently described? Has the benchmark response selected for use in deriving the POD been scientifically justified? Is it transparently and objectively described? Please identify and provide rationale for any alternative approaches (including the selection of BMR, model, etc.) for the determination of the POD, and if such approaches are preferred to EPA's approach.

5. Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfD. For instance, are they scientifically justified and transparently and objectively described in the document?

6. An uncertainty factor was considered necessary to account for deficiencies in the chlordecone toxicity database (e.g. absence of standard two-generation reproduction studies and immunotoxicity studies). Please comment on whether the rationale and justification for the application of the database uncertainty factor has been scientifically justified and transparently described in the document. Please comment on whether the available immunotoxicity data for chlordecone indicate that additional immunological studies could result in a different POD.

(C) Carcinogenicity of Chlordecone

1. Under the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=116283), there is *suggestive evidence of the human carcinogenic potential* of chlordecone. This characterization lies at the high end of the continuum for this weight of evidence descriptor. Please comment on the scientific justification for the cancer weight of the evidence characterization. Has the scientific justification for the weight of evidence characterization been sufficiently, transparently, and objectively described? A quantitative cancer assessment has not been derived for chlordecone. Do the data support an estimation of a cancer slope factor for chlordecone? Please comment on the scientific justification for not deriving a quantitative cancer assessment considering the uncertainty in the data and the suggestive nature of the weight of evidence of carcinogenic potential.

III. GENERAL IMPRESSIONS

Harvey J. Clewell

The toxicological review for chlordecone appears to be thorough, given the surprisingly limited amount of data on this controversial compound, and I found the various conclusions in the document to be well considered and reasonable. The document is well-written and in my opinion serves as a reliable basis for the evaluation of this compound. The discussion in the various sections of the document, while somewhat repetitive, allows each section to stand separately, increasing the clarity of the exposition. I am impressed with the candor of the authors in transparently describing the considerations that lead to their recommendations for the risk assessment approach. Overall, I am very satisfied with the Agency's risk assessment, which makes the best use possible of the surprisingly limited data available on the compound.

The section on toxicokinetics is informative and for the most part demonstrates a good understanding of the implications of the data. The Hazard Assessment also does a good job of describing the important information that can be gleaned from the various studies and their limitations. I was particularly pleased with the directness of the discussions in the sections on mechanistic data and mode of action. I agree in general with the approach used for the noncancer dose-response assessment, although I have a few concerns. I also agree with the decision not to conduct a cancer dose-response assessment.

My principal concern is why body weight to the three-quarters (BW^3/4) scaling was not used for cross-species dosimetry. I thought that the Agency had adopted BW^3/4 scaling for both cancer and noncancer dose-response assessment. In this case that would involve replacing the UF of 10 for animal to human extrapolation with a reduced UF of 3 for uncertainty in pharmacodynamics and a cross-species dosimetric adjustment of about 4 for differences in rat and human kinetics. This would clearly be appropriate for this chemical: the kinetic data in rats (three studies described on pages 8 and 13) are consistent with a half-life on the order of 20 days (ranging from about 7 to 40 days), while the human worker data (p.13) suggests a half-life on the order of 95 days (average of range from 63 to 128 days).

A secondary concern is the selection of a single model result for the BMD analysis based on an extremely small difference in the AIC. The selected model has an AIC of 84.3 and four other models have AICs of 84.7. The other models produce a consistent BMDL10 of 0.045 mg/kg/d as compared to the selected model BMDL10 of 0.076. I would probably have averaged the results for these five models.

Both of these concerns would have relatively small impacts on the noncancer risk assessment. On the other hand, I believe the uncertainty factor for database should be increased from 3 to 10 to reflect uncertainties associated with the potential estrogenicity of this compound, particularly considering its long environmental and biological persistence, and the presence of many other potential endocrine disruptors in the environment. A two-generation developmental toxicity study would be of particular

value to improve confidence in the risk assessment for this compound, for both cancer and non-cancer endpoints.

George P. Daston

I believe this to be a well conducted review and risk assessment. Chlordecone is a well-studied chemical. The database on chlordecone includes both human observational information (from workers exposed for an extended period) and animal studies. The human toxicity included neurological, male reproductive, hepatic and dermatological effects. All of these were observed in animal studies, as well as renal and immunological effects. Effects on the kidney and immune function should be considered to be human relevant despite the fact that no effects were observed on these systems in the exposed workers. While the human data are sufficient to demonstrate the potential for chlordecone to produce toxicity in humans, they are not sufficient to rule out the possibility of additional effects given more widespread or longer-term exposure.

The IRIS review summarizes a large and complex data set in a concise and readable way. The information is laid out logically and the interpretations are reasonable. The critical effect selected was a glomerular lesion that was observed to increase in incidence in a dose-related way in a chronic rat study. The background incidence for this lesion is high in male rats, but is much lower in females; however, the dose-related increase in the lesion was observed in both sexes, lending credence to the conclusion that it was treatment-related. The review considered using other effects as the critical effect for risk assessment, and RfDs were presented that were based on using these alternates. However, these alternates were either less sensitive than the glomerular effects or had much greater uncertainty associated with them. Therefore, I believe that the correct decision was made to use the glomerular lesions as the critical effect for risk assessment as it provides a scientifically defensible RfD that should be protective for all aspects of chlordecone toxicity.

There are a number of unanswered questions about chlordecone toxicity, particularly about the mode of action for the neurotoxic, reproductive, and immunotoxic effects. It would be useful to have additional information about mode of action, but it is unlikely that the information would change the process by which the RfD was calculated, or change the critical effect. Questions about the carcinogenic potential remain. Several chronic studies have been conducted, with one study indicating the potential to cause liver tumors. Chlordecone was also shown to promote DEN-induced liver tumors. However, the chronic studies were not of sufficient quality to support a definitive determination of carcinogenicity. There is sufficient data on genotoxic potential to conclude that chlordecone is not genotoxic.

In summary, I believe that the IRIS assessment on chlordecone is well written. The calculation of the RfD is scientifically justified.

Gary L. Ginsberg

Please see response to General Charge Question #1.

Michael I. Luster

Overall, this is a well written, accurate and complete review on the toxicity and potential adverse effects of chlordecone. There are some minor inconsistencies and information gaps in the document which can be easily rectified (see Specific Comments below). Based upon animal studies and limited human data, chlordecone produces toxicities in a number of organ systems and this occurs at dose levels similar to those in the kidney. Other sensitive effects include liver lesions, reproductive effects, testicular atrophy and neurotoxicty. The multiple targets provide a challenge to choose the most appropriate 'critical effect/target' to establish an RfD, although selection of most of these targets would probably have resulted in a similar RfD. That said, I agree with the report that kidney lesions, based upon a chronic study by Larson et al. (1979a), likely represent the most appropriate critical effect (glomerulosclerosis at 0.3 mg/kg-day LOAEL). However, I would have also included sperm parameters based upon the data from Linder et al. (1984) (particularly sperm number) in the comparisons of PODs for the various endpoints (see Table 5.1). I also concur with the report that there are some weaknesses (uncertainties) in the use of the kidney lesions as the critical effect, although the report discusses only several of them These additional uncertainties are listed in detail below, but include a high background incidence in control male rats (56%) necessitating the use of data only from female rats and a lack of evidence in humans of kidney damage.

I think it is likely that increasing severity of glomerulosclerosis is an adverse effect in itself resulting potentially in decreased kidney function. Thus, the lesions could represent a stand-alone adverse effect, and although it is obviously beneficial to establish the MOA, it is not necessary that an autoimmune etiology be established to confirm an adverse effect. I concur with the conclusions regarding the modeling approach used for establishing the POD; benchmark seems appropriate for this type of data. In addition, I agree with the report of the uncertainty factors utilized and the establishment of the RfD although I could also support a 10-fold rather than 3-fold for weaknesses in the data set for the reasons I just mentioned above and describe in detail later.

Regarding cancer, I think the bioassay data, even with its flaws, provides evidence for carcinogenicity. However, due to the flaws in the study and other studies suggesting that it may act only as a promoter and not initiator, I agree that the data should not be used for quantitative risk assessment.

Lauren Zeise

The document is well written and in general lays out the data, conclusions and supporting arguments in a clear, understandable fashion. The document does a pretty good job presenting in summary form the mechanistic information and interpretations, along with the standard toxicity study data. Where individual study authors do not provide statistical

analyses, the EPA's independent analyses are presented, also a plus. However, the format for the document results in a good deal of repetition, and in some spots the document is more repetitious than need be even within the prescribed format.

There are some conclusions and issues that require attention, as discussed at greater length in response to the charge questions:

- The conclusion of suggestive evidence of human carcinogenicity does not follow from the evidence presented and should be reconsidered. As discussed below, the evidence appears consistent with a finding of "likely to be carcinogenic to humans."

- The xenoestrogenic properties of the compound should be discussed in the context of background exposures to other similarly acting xenobiotics and natural hormones. The implications for translation to humans of animal findings, dose response and the sensitivity of available in vivo studies for certain effects should be discussed. While the mechanism of toxic action is unknown, xenoestrogenicity is a potential mechanism for certain endpoints, including hepatocellular carcinoma.

- The statement is made that fetal effects may have been the direct result of maternal toxicity. How depressed weight gain and other observed maternal toxicities could have lead to the developmental toxicity endpoints such as undescended testes requires some explanation. Undescended testes (also seen after in utero DES exposure) is a major risk factor for testicular cancer in humans.

- The weight to place on the findings from occupational poisonings at the Hopewell plant is unclear. Clearly they do provide qualitative information on target sites and some obvious clinical effects and the Toxicological Review does a good job considering it. However, more quantitative evaluation appears warranted. There is a wide range of blood levels reported to be associated with adverse effects in the occupational study (e.g., neurological effects observed at blood concentrations 0.009 to 11.8 ppm). Because this compound has a long half life and effects were observed months rather than years after initial exposure, the blood concentrations are unlikely to substantially understate levels causing adverse effects. This could be given more discussion. Also due to the long half life, route specific issues regarding the occupational exposures are also less important than may be considered at first glance, especially for target sites distant from the site of compound administration. Blood levels could provide a reasonable context for considering the confidence and uncertainties in applying the findings from animal studies.

- It would be of interest to compare and discuss the blood levels associated with effects in humans with those calculated for benchmark doses and LOAELs from the animal studies, using pharmacokinetic approaches.

- There are a few fairly low quality studies (in terms of providing quantitative information for dose response characterization) that are given too much weight and

discussion. The Larson dog study stands out. At some dose levels, the dose group consisted of just one dog.

- The overall adequacy of the data base warrants a database uncertainty factor larger than 3: A two-generation study is lacking; the study for establishing the non-cancer chronic RfD is of low power given the small group sizes at the end of the study; female toxicity is inadequately explored, and without an established NOAEL; and the NCI findings of hepatocellular carcinoma in male and females of two species are treated as inadequate for the classification of the compound as likely because of study design and reporting issues.

IV. RESPONSE TO CHARGE

A. General Charge Questions

1. Is the Toxicological Review logical, clear and concise? Has EPA accurately, clearly and objectively represented and synthesized the scientific evidence for noncancer and cancer hazard?

Harvey J. Clewell

The toxicological review is well written and commendably clear and transparent. While there is some repetition from one section to another, I believe this adds to the document's clarity. The authors have done a particularly good job of describing the considerations underlying their conclusions and recommendations regarding the mode of action information and the selection of the critical endpoint. The analysis is balanced and the decision-making is logical.

George P. Daston

The Toxicological Review was laid out in a logical manner and was easy to read. The data base for chlordecone is voluminous and includes detailed case reports and clinical follow-up from workers who were exposed to the chemical for an extended period. The review does a nice job of presenting the clinical observations along with the animal data that both supports the causal role of chlordecone in human adverse effects and presents information about modes of action. The animal data largely conforms to the human observations, but not always. For example, chronic rodent studies indicate the potential for renal toxicity but no signs of renal toxicity were observed in the exposed workers. The review does a good job of presenting possible explanations for the disparities and is not overly speculative. The synthesis of the large volume of information on chlordecone toxicity was concise and logical.

Gary L. Ginsberg

Overall, the document is put together well and easy to follow. It lays out the key toxicological information from a hazard characterization and dose response context in the manner needed for risk characterization and IRIS file development. The mechanistic information provided at various points in the document was useful (e.g., the discussion of potential estrogenic MOA for autoimmune effects in mice on pages 54-55), but should be summarized into one coherent discussion of all key endpoints and what we know of their mechanism, perhaps in Section 4.5. As it now stands, Section 4.5 is primarily concerned with cancer MOA. I have not read most of the primary Kepone literature so cannot speak to the accuracy of the technical presentation.

The purpose of the document would be clearer if the introduction explained why is it necessary to develop an IRIS file at this time given that it is being developed 30 yrs after Kepone has been removed from the market and after all this time of not having an RfD.

Some effort to integrate environmental fate, exposure potential, toxicology information and other factors (e.g., action on similar chlorinated chemicals such as mirex, emerging science on these types of structures; new tox data) would be very helpful to show the rationale for moving forward with this IRIS file at this point in time.

In terms of accurate and objective analysis, there are a number of points where the analysis relies upon professional judgment that can be questioned in terms of the selection of points of departure (PODs) for non-cancer assessment and in terms of judging the significance and utility of the cancer dose-response information. The current draft is unsatisfying in these regards and needs improved approaches for analyzing the discarded options and explaining their risk implications (e.g., discounting the NCI positive cancer bioassay on the basis of study irregularities leaves the assessor without a viable cancer risk assessment approach). The document appears to have a bias against a finding of Kepone carcinogenicity, labeling the evidence as only "suggestive" (Page 71) and using speculation and conflicting rationale to discount this evidence. The National Toxicology Program (NTP) lists Kepone as a carcinogen that is "reasonably anticipated to be a human carcinogen," it is listed as a 2B carcinogen by IARC, and it is regulated as a carcinogen in California with that state deriving a low dose slope for quantitative cancer risk assessment for Kepone. Kepone has been shown to be a liver tumor promoter in a specialized initiation-promotion protocol at low doses that are unlikely to be associated with cytotoxicity. While the mechanism for Kepone's promotional effect is not known (possibly estrogenic effect, immunotoxicity, gap junction inhibition), it is difficult to deny the consistent evidence of oncogenicity of this compound, and of its close structural analogue mirex.

While most of the document is generally well organized, Table 4-18 (Page 62) is a somewhat disorganized compilation of non-cancer dose response information. The table should be reorganized based upon either study type, exposure duration and endpoint. Further, the table is missing autoimmune findings mentioned in the text (pages 52-53); this is an important omission given that some autoimmune effects may occur at lower levels than LOAELs defined for other endpoints.

Michael I. Luster

Overall this is a well written, accurate and complete report on the toxicity and potential adverse effects of chlordecone. The toxicity data were presented clearly and objectively. The report follows a logical discussion and justified conclusions. The choice for the selection of the critical effect and the development of the POD and RfD are clear and adequately discussed.

Lauren Zeise

The Toxicological Review is logical and the writing is clear, but the format is such that it is not always concise. The same logic and information appears in multiple places, and rather than add clarity it can make the logic harder to follow. Overall the scientific evidence is objectively represented and synthesized but there are a few places where

improvements can be made. These include the arguments leading up to the conclusion regarding carcinogenicity, the discussion of the occupational data and its relationship to animal findings and the compounds' xenoestrogenicity. Several more specific points in this regard are presented in the General Impressions section above. Kidney toxicity, while not reported in the occupational poisoning incident, was likely not adequately investigated. Thus, it is not correct to state that it did not occur in the poisoning victims, and a more nuanced discussion of lack of observation of kidney toxicity is needed instead. Another minor point, on page 94 the statement is made that because similar effects are seen in animals and humans toxicodynamics in the two species is similar. Qualitatively this may be the case, but this does not address the potential for quantitatively different toxicodynamics (e.g., same incidence caused at significantly different internal dose levels among species).

2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of chlordecone.

Harvey J. Clewell

As far as I know, the relevant studies have been included in the review.

George P. Daston

I found the review to be comprehensive. I know of no other literature that would materially affect the outcome of the assessment.

Gary L. Ginsberg

Possible utility for mechanistic evaluation of the lupus effect:

Wang, F et al. (2007) Diminished prolactin from chlordecone tx in ovariectomized mice. Int. Immunopharmacol 7: 1808-1812.

Estrogenic effects – interaction across estrogen mimics in culture:

Benachour, et al. (2007) Cytotoxic effects and aromatase inhibition by xenobiotic endocrine disrupters alone and in combination. TAP 222: 129-140.

Michael I. Luster

The report may want to consider referencing another recent study by Wang et al. (Int. Immunopharmacol. 7:1808-12, 2007) which sheds some light on the MOA of chlordecone autoimmune disease and the role of prolactin.

Lauren Zeise

There are two different types of studies that should be considered - first, authoritative reviews of the compounds carcinogenicity; second, studies that may provide greater insight on the implications of the compounds' xenoestrogenicity for humans. Examples of the first are:

- Report on Carcinogens, Eleventh Edition; U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program.
- International Agency for Research on Cancer, IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 20. Some Halogenated Hydrocarbons. IARC, World Health Organization, Lyon, France. (While the IARC Monograph is referenced in the discussion of chemical and physical characteristics of the compound, it is not discussed in the section evaluating the carcinogenicity information.)
- National Institute of Occupational Safety and Health's Carcinogen List.

- Additional reports are noted in response to charge question C below.

Examples of the second are:

Hodges LC, Bergerson JS, Hunter DS, Walker CL (2000). Estrogenic effects of organochlorine pesticides on uterine leiomyoma cells in vitro. Toxicol Sci. 54(2):355-64.

Wu F, Safe S (20070. Differential activation of wild-type estrogen receptor alpha and C-terminal deletion mutants by estrogens, antiestrogens and xenoestrogens in breast cancer cells. J Steroid Biochem Mol Biol. 103(1):1-9.

Ray S, Xu F, Li P, Sanchez NS, Wang H, Das SK (2007). Increased level of cellular Bip critically determines estrogenic potency for a xenoestrogen Kepone in the mouse uterus. Endocrinology. 148(10):4774-85.

3. Please discuss research that you think would be likely to reduce uncertainty in the future assessments of chlordecone.

Harvey J. Clewell

I would definitely recommend a two-generation exposure study that included neurobehavioral and repro-developmental endpoints. There has not been an adequate cancer bioassay, but I would not recommend conducting one at this point. Given the evidence for a purely promotional mode of action for carcinogenicity, I think the RfD should be considered protective against cancer. A two-generation developmental toxicity study would be of value to improve confidence in the risk assessment for this compound for both cancer and non-cancer endpoints.

George P. Daston

Despite considerable research, there is still much to be done in characterizing the modes of action of chlordecone. My interpretation of the literature on chlordecone is that we have good leads on why it produces reproductive, hepatic, neurological, renal, and carcinogenic effects, but that none of these can be considered definitive. That said, I don't believe this additional research to be critical to the development of a risk assessment for chlordecone because it is unlikely that more definitive information would either change our opinion on the potential for chlordecone to be a human health hazard, or would change our quantitative approach in establishing reference values.

The lack of a modern two-generation study for reproductive toxicity is of some concern, as it is possible that a two-generation study would produce a lower BMD. The early study by Good (1965) suggested permanent effects on fertility in offspring whose parents were exposed to chlordecone, irrespective of whether the offspring were directly exposed. However, it is worth noting that, when considered in aggregate, the reproductive studies conducted on chlordecone include virtually all of the endpoints that are now measured in EPA's modern reproductive toxicity guidelines, including sperm production, estrous cyclicity, and careful histological evaluation of the gonads, including morphomeric analysis of ovarian follicles.

Additional information on the immunotoxic potential of chlordecone would be of use, particularly given that some of the chronic toxicity in animal studies appears to be the result of chronic inflammation (especially the renal effects that were used as the critical effect in the risk assessment). Immunotoxicity studies would be unlikely to alter the point of departure, as it is likely that the manifestations of altered immune function were observed at the end of the chronic studies. However, having more information on immunotoxic potential would provide a more complete picture of the range of potential human health effects.

The question as to whether chlordecone is carcinogenic still appears to be unresolved. Despite the fact that the chronic toxicity of chlordecone has been evaluated in a number of studies, the overall conclusions that can be drawn from these studies is not definitive.

That said, it is not clear whether anything would be gained by conducting additional chronic studies. The dose levels that need to be investigated for potential hepatic carcinogenesis overlap those that produce neurotoxicity, which is a limiting factor in the conduct of chronic studies. I fear that any new chronic study will encounter the same problems that plagued the earlier studies on chlordecone. Perhaps when the science of toxicogenomics is a little more advanced, experiments can be designed that address carcinogenic potential in studies of shorter duration; however, the ability to conduct and appropriately interpret such studies is not imminent.

At the peer review meeting, the suggestion was made that the toxicity findings for mirex could be used to supplement the chlordecone data set. I caution against this. Although mirex and chlordecone have many structural similarities, their chemistry and biological activity are different enough that it is not possible to use mirex as a surrogate for chlordecone. The kinetics of the two differ substantially, as evaluated by comparing two similar pharmacokinetics studies on chloredecone (Egle et al., 1978, Drug Metab. Disposition 6: 91) and mirex (Byrd et al., 1982, Toxicol. Appl. Pharmacol. 66: 182). Mirex (Mehendale, 1977, Drug Metab. Disposition 5: 56) but not chlordecone (Mehendale, 1977, Toxicol. Appl. Pharmacol. 40: 247) affects liver function (biliary excretion) in short-term dosing studies. Chlordecone was found to have estrogenic activity in rats in vivo, but not mirex (Gellert, 1978, Environ. Res. 16: 131). The CNS toxicity (tremors) produced by chlordecone was not observed with similar dosing regimens of mirex (Reiter et al., 1976, Toxicol. Appl. Pharmacol. 38: 130). There are just too many differences between the two compounds to rely on the toxicology data of one to support conclusions about the effects of the other.

One might also argue that studies that relate tissue and blood concentrations with adverse effects in animal studies might facilitate comparisons to the human data, but the human data are too incomplete to support such comparisons with confidence.

Gary L. Ginsberg

1) Reanalysis of the cancer dose response for Kepone by comparison to the dose-response for mirex. They produce liver tumors in rats and mice at similar doses across chemicals. Could the irregular Kepone study be strengthened and made more useful by consideration of the mirex cancer database? Why did USEPA's IRIS evaluation of mirex also not derive a cancer slope factor? Why did CalEPA derive slope factors for both mirex and Kepone? This may also involve re-evaluation of the histopathology slides from the Kepone NCI studies to determine whether hepatotoxicity from inappropriately high doses at the outset of the study may have led to a cancer dose response that is different from a uniform dosing schedule at the lifetime TWA dose.

2) Further exploration of Kepone immunomodulation and autoimmune effects in mice given that this is a potent, low dose effect in the SLE model system, the mechanism is unknown (possible to be partially due to estrogenic agonist effects but clearly other

mechanisms as well) and it may be possible that sensitive immunotoxic endpoints could be the driver in RfD derivation.

3) Further exploration of Kepone's endocrine effects and possible early life imprinting of a modified phenotype that affects later sexual maturation or reproductive performance in both male and female offspring. An adequately designed multi-generational study is missing from the database. Further, low dose effects of Kepone on sperm parameters may be of functional consequence in appropriately designed multi-gen studies and perhaps in more sensitive animal model systems given the insensitivity of the rat model to the functional effects of male gonadal toxicants.

4) Further exploration of Kepone neurotoxicity, particularly in developmental neurotoxicity protocols, given that Kepone is a potent neurotoxicant with unexplained mechanism (structural analogue mirex does not have this property). The one developmental neurotoxicity study is dated (Squibb and Tilson, 1982) and does not conform to modern developmental neurotoxicity protocols. The mixed (mainly negative) results in that study bear further investigation.

5) Toxicokinetic evaluation – what is the mechanism for Kepone accumulation in the liver in rodents and is that likely to occur in humans? What is the active hepato- and neurotoxicants – parent compound or metabolite?

Please also note that this reviewer requested 7 studies to be sent electronically to finalize my review. These studies were all cited in the draft Kepone IRIS file document by U.S. EPA. Unfortunately, U.S. EPA was not able to provide 5 of these 7 articles. No explanation was provided. If this signifies that these articles were not actually evaluated by EPA but only identified from secondary literature sources, then the uncertainties in the draft risk evaluation may be improved based upon closer inspection of the primary literature. Either way, this reviewer's comments are limited by the fact that access to the primary literature was not provided initially as part of the review materials and was further not provided upon request.

Michael I. Luster

The statement that glomerulosclerosis observed in the Larson studies may result from an autoimmune response is speculative (inferred from Sobel's NZB studies). What would be needed to better establish this association would be monitoring for the presence of autoantibodies and IgG immune complexes in kidney of experimental animals exposed chronically (i.e., that develop kidney lesions from chlordecone). The presence of immune complexes in the kidneys of chronically exposed animals would strongly support that an autoimmune process is occurring even in non-autoimmune prone animals.

It would have been beneficial to have follow-up studies of former plant workers and local residents from Hopewell for prevalence of autoimmune diseases (including measurement of serum autoantibody titers), kidney diseases and other possible outcomes (cancers, liver disease, reproductive outcomes, etc).

A two-generation study for reproductive effects would be beneficial.

There is still a lack of animal cancer bioassay data to allow for quantitative evaluation for cancer.

Lauren Zeise

Various research initiatives could reduce the uncertainty in future assessments. However, given the limited current exposure it is hard to envision any research initiatives with any amount of substantial funding. If such funding were to be made available, valuable insights for risk assessment could result from research directed at:

1. Understanding cross species differences in pharmacokinetics.
2. How small increments of environmental exposure to chlordecone might add to or otherwise impacts other hormonally mediated processes causing human disease (e.g., uterine leiomyoma; liver, postmenopausal breast, and endometrial cancers; testicular effects).
3. Cancer dose response relationships, including large multi-dose cancer bioassays in multiple strains.
4. Cancer time-response relationships. The NCI chronic studies were conducted in adult animals. For xenoestrogens, sites affected are dependent on timing of exposures. For example, in rats adult exposure can lead to liver cancer (in humans as well) but exposure early in life can lead to uterine and other cancers.
5. Cancer mode of action.
6. Understanding and quantifying the human variability in response suggested by the occupational poisonings.

4. Please comment on the identification and characterization of sources of uncertainty in sections 5 and 6 of the assessment document. Please comment on whether the key sources of uncertainty have been adequately discussed. Have the choices and assumptions made in the discussion of uncertainty been transparently and objectively described? Has the impact of the uncertainty on the assessment been transparently and objectively described?

Harvey J. Clewell

The uncertainties in sections 5 and 6 arise primarily from the lack of definitive data on the dose-responses and modes of action for the toxicity and rodent carcinogenicity of chlordecone. The database is surprisingly inadequate for such a controversial compound. The authors do a good job of discussing the uncertainties associated with the limitations of the available studies. I was gratified by the transparency and objectivity of the discussion.

George P. Daston

I found the discussion of sources of uncertainty to be logical and comprehensive. I have a quibble or two about the numerical value assigned to one or two of the UFs, which I will discuss in the next section. However, overall the discussion of uncertainty makes sense and is scientifically defensible.

Gary L. Ginsberg

Chapters 5 and 6 could do more to describe the uncertainty in the toxicology database for Kepone. There is no well organized uncertainty discussion in either Chapters 5 or 6. Given that for several endpoints (immunotoxicity, male gonadal toxicity, endocrine disruption, and developmental neurotoxicity) there is a lack of mechanistic information, NOAELs are not clearly established and the potential exists for more subtle toxic effects at lower doses, there is considerable uncertainty in the Kepone database. This is not reflected well in the current description. For example, the significance of effects on sperm parameters in rats and humans is dismissed on the basis that this was not associated with functional effects in either case. However, the male rat has a large sperm surplus that does not exist in humans and the human evidence for lack of functional effect comes from a few workers who may have tried to father children while having oligospermia (Page 92). The significance of this endpoint, for which no NOAEL was identified and BMD analysis was not undertaken, should, at a minimum, be treated as an important uncertainty rather than discounted on the incomplete evidence available.

In addition, an uncertainty raised in Chapter 4 (early life vulnerabilities) is not further discussed in Chapter 5. The treatment of this subject in Chapter 4 (Page 75) is incomplete. This section appropriately focuses upon the limited database describing postnatal effects from in utero and/or lactational exposures. However, the importance of these findings for risk assessment are unclear, and the message that “developing animals may be more susceptible to subtle neurological effects of chlordecone….” is unresolved.

This section should discuss the adequacy of the available studies to describe the developmental neurotoxicity of Kepone, whether the database provides information on the hormonal effects of Kepone on sexual maturation, autoimmunity and other effects in association with early life exposure, and whether there are any toxicology triggers that suggest early life exposures may be of prime importance in risk assessment of this compound.

Toxicokinetics and cross-species extrapolation is a large uncertainty, also not described well in these chapters. The mechanism for Kepone concentration in liver and for cross-species differences in half life and metabolism are unknown. An important uncertainty is Kepone kinetics in humans. The implications of all of these cross-species kinetic differences for risk assessment is unclear. This should be described in the document.

Michael I. Luster

I believe the authors did an excellent job describing sources of uncertainty. Assumptions are justified and their impact accurately described. I particularly found Figs 5-1 and 6-1 (RfD comparison array for alternate PODs) extremely useful. I agree with the confidence in the RfD as 'medium' based upon limitations in the Larson study, although I think there are some additional uncertainties in the Larson data set that should be discussed (see response to question #6). However, any overall concerns I have are diminished by the fact that the RfD would probably not differ significantly whether reproductive outcomes or liver lesions were used in lieu of kidney lesions.

Lauren Zeise

There is a reasonably good discussion of the uncertainties in sections 5 and 6, but it could be improved. The RfD array is a good visual for understanding how the different choices impact on the identification of the RfD. In general the choices and assumptions made are well described. There are some key sources of uncertainty and issues that have not been addressed or require greater discussion:

- The wide range of blood levels associated with findings of adverse effects in the human poisonings, including some levels that were fairly low.
- More detailed discussion of uncertainties in human and test animal pharmacokinetic differences and similarities. This coupled with the low blood concentration associated with effects in humans is cause for some concern. The compound has a long half life in humans and there is uncertainty in the half life in the test animals providing the basis for the RfD calculation.
- How low doses of this xenoestrogenic compound might contribute to ongoing disease processes that other similarly acting endogenous and exogenous compounds affect.
- Potential for carcinogenicity from in utero or early life exposure.
- How background exposures to similarly acting compounds could/might impact human variability and susceptibility to Kepone.

- Lack of adequate study in animals of dose response relationships for female reproductive toxicity. Existing data are not adequate for understanding experimental no adverse effect levels.
- The impact of model choice on the BMD – a number of models fit the data adequately and were not significantly different in fit from one another, but were in the $BMDL_{10}$ values.

B. Oral Reference Dose (RfD) for Chlordecone

1. A chronic RfD for chlordecone has been derived from the 2-year dietary study (Larson et al., 1979a) in rats. Please comment on whether the selection of this study as the principal study has been scientifically justified. Has this study been transparently and objectively described in the document? Please identify and provide the rationale for any other studies that should be selected as the principal study.

Harvey J. Clewell

I agree with the choice of the Larson study as the basis for the RfD. It is far more suitable than the other available studies and was well conducted.

George P. Daston

I believe that the choice of Larson et al. as the principal study is justified. It is of chronic duration; it included a sufficient number of animals and dose groups to have good resolving power; it comprehensively evaluated the potential for chlordecone to affect organs and organ systems; its design was sufficiently robust to allow the identification of apparent LOAEL and NOAEL for the critical effect. I believe that the study has been objectively described and that the interpretation in the IRIS document is correct. The authors of the IRIS document considered a number of other studies and critical effects and even calculated alternate reference doses from these, but provided a good rationale for using the renal effects in Larson as the critical effect.

Gary L. Ginsberg

The Larson, et al. 1979 study has merit as the key study for RfD derivation on the basis of large numbers of animals (40/sex/grp), large number of doses (6 plus control) and length of study (2 years). A variety of toxic effects occurred along a dose response. Other studies that are candidates for RfD derivation have been evaluated in Section 5.1 (Figures 5-1 and 6-1, which is unnecessary to have the same figure appear twice a few pages apart). However, this assessment is not comprehensive as studies and endpoints which may have yielded a more sensitive dose response are anti-sperm effects (Linder, 1983; USEPA, 1986c: LOAEL in Linder study either 0.26 or 0.83 mg/kg/d), autoimmune effects (potentiation of SLE in mice, Sobel series of studies with LOAEL of 0.2 mg/kg/d) and developmental neurotoxicity (Squibb and Tilson, 1982: apparent LOAEL at 0.07 mg/kg/d according to analysis on Page 75). The reason for not including the sperm effects is questionable functional relevance, which is speculative rationale, and instead a cruder endpoint, testicular atrophy, is chosen for comparative analysis. The reason for not including a quantitative analysis of the autoimmune endpoint as a candidate for RfD derivation was not stated. The neurodevelopmental endpoint was not even brought up in Chapter 5 as a candidate for RfD development; while the evidence for this effect was perhaps weakest, it still should be raised as a candidate for RfD derivation. As currently written, the document is unclear regarding the relevant study for this endpoint (Squibb and Tilson, 1981), describing it as basically negative (e.g., Table 4-18, Page 63), yet a

later assessment (pg 75) states that this study provides a suggestion of effect on dopaminergic systems at the low dose (0.07 mg/kg/d). These interpretations should be harmonized.

Regarding additional endpoints, Table 4-14 (Page 43) indicates effects on sperm parameters that were clearly affected at doses as low as 0.83 mg/kg/d but the text calls the next lower dose the NOAEL (0.26 mg/kg/d). However, the data for the 10 rats per group were suggestive of effects at the lowest dose level (0.26 mg/kg/d), with decreased sperm viability, motility and epididymal reserve at this dose. A larger number of rats may have shown the effect to be significant. The text (pg 44) should not dismiss this dose as a NOAEL so quickly, especially since the BMD for a cruder endpoint (testicular atrophy) is estimated at 0.1 mg/kg/d.

Michael I. Luster

I am in agreement that the Larson et al. (1979a) report is the most appropriate study to establish an RfD as, arguably, it provides the best data available for quantitative risk assessment. I also agree with the use of kidney lesions to establish the critical effect (glomerulosclerosis at 0.3 mg/kg/day LOAEL). However, some limitations are present in the Larson study which could be better stated. A minor issue is that only one species (rats) was examine but of more concern was that there was a high background incidence in control male rats (56%) of kidney lesions necessitating the use of data only from female rats. The report could provide a better discussion for the rationale for the high background incidence of kidney lesion, particularly in males? Also, in contrast to the hepatic and male reproductive effects, nephrotoxicity has not been observed in humans. I agree that the human data lack sufficient exposure information suitable for quantitative risk assessment. However, it does provide a level of assurance, particularly regarding the issue of ‘adverse effects’, in that similar liver and male reproductive pathologies have been observed in workers.

I also have some concerns with the use of the studies by Chetty et al. (1993) as supportive for using glomerulosclerosis as the critical target. While supporting the Larson studies in that kidney damage was observed by Chetty et al., these authors found that the liver effects (i.e., SGPT enzymes changes) were more sensitive (>1.0mg/kg) than altered kidney parameters (nitrogen at > 4.9 mg/kg) - see pg 80. Can the report provide better support that kidney biomarker tests are less sensitive than liver biomarkers (including references)?

Lauren Zeise

The selection of the Larson et al. study has been scientifically justified. The selection of a study for derivation of an RfD is made difficult by the limited database available, given the seriousness of health effects observed in human poisonings. The Larson et al. rat study has quite limited power due to group size. Although at first glance it appears to be a substantial study, with 40/sex/group, and is indeed a chronic study, the sizes of groups available for examination are quite small. Groups sizes used for statistical analysis are for

animals "analyzed between 1 and 2 years." The numbers lasting until the end of study are not provided and it is unclear why those not surviving were not analyzed. Group sizes for those analyzed between one and two years, in order of increasing dose, in males are 22, 11, 6, 9, 4, and in females, 34, 13, 17, 12 and 4. Thus, a caveat regarding group size should accompany the many descriptions of this study as a 40/sex/group study, given that well over half the animals were not exposed and examined for the full two years. Further, the number in each dose group surviving to two years should be noted along with the disposition of rats surviving to age one year, but not lasting until the study end.

The only other chronic experimental studies with reasonable group sizes are the NCI in male and female rats and mice. These studies did have greater group sizes, but used higher (and variable) dose levels and the degree of attention paid to non-neoplastic endpoints is unclear. The adverse effects on sperm parameters found in the Linder et al. study should also be presented in summary form in Figure 5-1.

Finally, there are the blood concentration levels associated with effects in the human poisonings. It was not clear from the write-up whether or not data were available for individuals that might be tabulated along with the health effects seen in these individuals. It possible this kind of comparison could provide additional insights on the appropriateness of study selection.

Table 4-18 is a good display of possible studies. Including group size at the end of study, perhaps in parentheses under the dose groups, would make some of the problems in study selection more transparent and would further help justify study selection. The Larson et al. 1979 dog study draws interest, but the dose group sizes were either one or two animals, so the findings have very limited meaning. In fact, the reporting of the NOAEL and LOAEL for this study in the table and on page 32 is problematic for this reason.

2. Kidney (glomerular) lesions, liver lesions, and reproductive effects are all sensitive effects of chlordecone exposure. Glomerular lesions in the kidney were selected as the most appropriate critical effect. Please comment on whether the selection of glomerular lesions as the critical effect instead of reproductive endpoints (such as testicular lesions) has been scientifically justified. Is this choice transparently and objectively described in the document? Please provide detailed explanation. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

Harvey J. Clewell

I agree with the selection of the kidney effects as the critical effect. It is protective for all of the other endpoints considered. The only endpoint that results in a lower RfD is testicular atrophy, but that is an artifact of the use of an uncertainty factor for use of a subchronic study in the face of evidence from the chronic study that the effect resolves rather than progresses over time.

George P. Daston

I believe that the choice of chronic glomerular effects as the critical effect was an appropriate choice and is adequately justified in the review. The effect was produced at a low dose and was observed after chronic exposure. Testicular atrophy would also have been a reasonable choice, as it is clear from both the human reports and other animal studies that chlordecone has the potential to affect male reproductive function. However, in Larson et al. testicular atrophy was only observed at the 13-week sacrifice, not at the 2-year timepoint. The reason for this is not explained in the review or in Larson's paper, but my guess is that the background incidence was high at the end of the study, which would have obscured the treatment-related response. (The only mention of testicular findings after the 3-month sacrifice was an increase in relative testis weight at one year in the highest dose group.) The LOAEL for the glomerular lesions was lower than the LOAEL for the testicular effect at 13 weeks. Therefore, I believe that the use of the glomerular effect as the critical effect was appropriate, and that using it as a point-of-departure will be protective for other health effects, including testicular toxicity. The comparison of RfDs in Table 5-2 makes it appear that the RfD calculated for testicular atrophy is lower than that for the glomerular toxicity; this is attributable to the application of a 10x UF to correct for the use of a subchronic observation to establish a chronic RfD. However, I believe that this is an inappropriate use of this factor. There may be some justification for using this factor when no chronic data exist, but chlordecone had been evaluated for chronic effects in the same study, as well as two others, without an observation of more significant effects (and particularly nothing like an observation of a 10x lower LOAEL) at later observation points.

Note added following the peer-review meeting: It would be worthwhile to model the sperm concentration data from Linder et al. (Table 4-14 in the IRIS document) among the other endpoints considered in the suite of possible critical effects. The preferred parameter to model would be epididymal sperm concentration (i.e., the sperm count

divided by the weight of the tissue). If these data are not available, then evaluating the sperm count would be sufficient. I would recommend against using the sperm motility or viability data for modeling. The control values for each of these fall well below the accepted range of normal (see Morrissey et al., 1988, Fundam. Appl. Toxicol. 11: 343).

Gary L. Ginsberg

As stated in response to the previous question, spermatotoxic, autoimmune/renal toxicity and possibly also developmental neurotoxicity effects may occur along a similar if not more sensitive dose response than that identified for the endpoints chosen for primary analysis: glomerular lesions, testicular atrophy, liver lesions and reduced reproductive success (Figure 5-1). Further, several of these additional effects may be upstream indicator endpoints for the more overt toxic manifestations (e.g., autoimmune as upstream for glomerular toxicity, spermatotoxic for reproductive). While one can't say whether any of these additional endpoints would become the leading effect for RfD development, it is appropriate that they be given more complete consideration including quantitative evaluation of POD and uncertainty factors.

Michael I. Luster

I agree with the report that kidney (glomerulosclerosis) lesions are the most sensitive endpoint. The report clearly justifies the basis for this decision. Liver effects, testicular atrophy and reproductive success all show similar PODs but are justified for not being used to develop the RfD based upon small differences in the LOAEL or lack of chronic studies. The only concern that I have is that the report excluded the sperm count data by Linder in the POD comparisons. The argument that the decrease in sperm counts observed may not be of sufficient magnitude to represent a biological effect might need to be better supported. My understanding (not being a reproductive expert) is that normal sperm counts in humans are already closer to a critical number than in rodents to affect performance. One could use the same argument regarding kidney lesions: e.g., "was there a sufficient amount of scarring at the LOAEL to affect kidney function?"

There was also a discussion that for many of the potentially critical outcomes (e.g., reproductive), experimental animal data were only available as an average and, thus, variance could not be determined. I agree that this would limit its applicability for quantitative risk assessment, but I wonder if it is the responsibility of EPA to attempt to contact the study investigators to determine whether individual data were collected and, if so, whether it is available for analyses, although it would be very unlikely that the individual data are still available given the studies were conducted in 1965.

Lauren Zeise

The choice of endpoint was carefully described. There is the nagging concern for this endocrine disrupting chemical that reproductive endpoints were not sufficiently studied. Given the plethora of human exposures to similarly acting compounds – endogenous and exogenous - and ongoing human health status and pathological processes related to such

exposures, this may well be a more important endpoint for humans and the testicular atrophy endpoint, and a more relevant surrogate for possible human outcomes from incremental environmental exposures. It would be good to see a more thorough discussion of this issue. Ultimately the draft has made a reasonable policy call, but more careful consideration and discussion of reproductive endpoints is recommended. The discussion should include recent mechanistic work (not cited in the current draft) examining the xenoestrogenic effects of the compound.

3. Some evidence exists to suggest that the mechanism of the critical effect selected for determination of the point of departure (POD) (i.e., glomerular lesions) may be mediated through an autoimmune mechanism. Please comment on whether the available immunotoxicity data support this proposed MOA. Is this proposed MOA scientifically justified and transparently described?

Harvey J. Clewell

I believe the available data is suggestive but not definitive regarding an immune component in the mode of action for the kidney effects. Nevertheless, I felt the discussion was reasonable.

George P. Daston

The immunotoxicity data for chlordecone provide some support for the conclusion that it has the potential to promote inappropriate immune responses, but this support is not definitive. The studies that indicate that chlordecone has the potential to produce autoimmune responses are unusual in their design and difficult to interpret (e.g., the use of a model in which all animals develop autoimmunity). Other studies suggest that chlordecone has the potential to suppress the immune response. This observation could either support or refute the link between chlordecone and autoimmunity: the support is in the observation that the immune system may be a target for chlordecone, the refutation is in the observation that the immune response is in the opposite direction. I believe that the IRIS review uses the MOA information as suggestive support, but does not over interpret. In any case, I don't believe that definitive knowledge of the MOA is necessary to complete the risk assessment.

Gary L. Ginsberg

This concept is supported from data on a particular mouse model of immunotoxicity that mimics human lupus and produces glomerulosclerosis (Sobel, et al., 2005). The fact that a similar lesion was found in rats by Larson et al. 1979 may point to a common underlying mechanism although the mouse studies suggested that Kepone's effect occurred against a certain genetic background that confers sensitivity to the immunotoxic effect. Therefore its not clear the renal effects in rats was in a particularly sensitive species and strain for the immunotoxic renal effect, which is one of the reasons that use of the Larson dose response as the most sensitive for RfD derivation contains a degree of uncertainty. Other, immunotoxic endpoints need to be tested in sensitive animals.

Michael I. Luster

It is quite plausible that the kidney lesions described in the Larson studies are a result of an autoimmune response. However, these lesions (i.e., glomerulosclerosis) are not specific for autoimmune disease but may occur in other kidney diseases that are not associated with autoimmunity. Nonetheless, I would suspect that glomerulosclerosis, if

present at sufficient severity, represents an adverse effect as it destroys glomeruli, independent of autoimmune disease leading to potential loss in kidney function.

That said, I think the studies by Sobel et al. (2005), showing that similar doses expedite the development of autoimmune disease in a SLE-prone mouse strain (NZB mice), provide a strong argument (plausibility) for hypothesizing an autoimmune MOA for the kidney lesions. The strength of the Sobel studies are that multiple tests for autoimmune disease were conducted including findings of enhanced IgG immune complexes in chlordecone treated mice (an excellent indication of autoimmune disease). However, it might be worth noting that there are currently no acceptable or validated models for studying the ability of xenobiotics to produce autoimmunity (an area of active research), although, there is a general consensus among the immuntox community that autoimmune-prone mice are the most appropriate model (e.g., NZB for lupus and NOD for diabetes).

It might also be worth noting that other studies have shown that certain environmental chemicals and therapeutics can exacerbate autoimmune disease development in autoimmune prone mice and several of these have epidemiological support (see reviews by CG Parks & GS Cooper 'Occupational exposure and risk of SLE' published in Autoimmunity 38(7):497-506, 2005 and A. Veraldi et al., 'Immunotoxic effects of chemicals: A matrix of occupational and environmental chemicals' published in Am J. Ind. Med. 49(12)1046-55, 2006). Most notable, are a series of recent studies with trichloroethylene (TCE) using MLR autoimmune prone mice which show very similar glomerular lesions as described for chlordecone.

Lauren Zeise

The document contains a transparent description of this MOA. I defer to those on the Panel with expertise in this area for substantive comment.

4. The chronic RfD has been derived utilizing benchmark dose (BMD) modeling to define the POD. All available models were fit to the data for the incidence of glomerulosclerosis in female rats. Please provide comments with regards to whether BMD modeling is the best approach for determining the POD. Has the BMD modeling been appropriately conducted and objectively and transparently described? Has the benchmark response selected for use in deriving the POD been scientifically justified? Is it transparently and objectively described? Please identify and provide rationale for any alternative approaches (including the selection of BMR, model, etc.) for the determination of the POD, and if such approaches are preferred to EPA's approach.

Harvey J. Clewell

I am not completely comfortable with the selection of a single model result for the BMD analysis based on an extremely small difference in the AIC. The selected model has an AIC of 84.3 and four other models have AICs of 84.7. The other models produce a consistent BMDL10 of 0.045 mg/kg/d as compared to the selected model BMDL10 of 0.076. I would probably have averaged the results for these five models. I believe this approach would be more consistent with the recommendations of the Benchmark Dose Technical Guidance Document. I certainly agree with the selection of a BMR of 10% for this endpoint. It would be informative to perform benchmark analysis on the induction of tumors to determine whether the RfD is likely to be protective against the promotional effects of this compound.

George P. Daston

BMD modeling is appropriate for the data and is the accepted default approach for identifying the POD. BMD methodology was used appropriately in this assessment. I believe that the selection of the log-probit model for the glomerular lesions was an appropriate choice and conforms with EPA guidance on choice of models when multiple models fit the data. The BMR is appropriately justified and is also consistent with EPA guidance. The choices are transparently described in the text. It would be possible to independently reproduce the modeling if necessary.

Gary L. Ginsberg

BMD analysis used to derive the 95% lower confidence level on the 10% response level is a valid approach to handle the dose response data as long as sufficient data exist for this procedure. The Larson 1979 dataset appears to be suitable for this purpose based upon the dose response and the numbers of animals on test.

Michael I. Luster

I support the use of the BMD to define the POD for these data and I believe it is consistent with current EPA guidelines. The authors provide adequate justification for the

use of this approach. As a side note: I found it useful and a good example of transparency that a POD was developed and discussed for other reported sensitive endpoints (Figs 5-1 and 6-1).

Lauren Zeise

There is a good write-up of the benchmark dose calculations in Appendix B. It includes a clear presentation of the data used, some statistics on pair-wise comparisons that the Agency calculated because study authors did not (well done), and an adequate description of the methods and procedures. However, the model selected is not clearly better than the other models employed and the BMD and BMDLs for it are out of line with a number of other models that fit the data adequately. Statistically, the fit for the selected model is not significantly different from the others, and the selection rationale, although well described, appears somewhat arbitrary. While acknowledging that the selected fit does have the smallest AIC, it differs from most others by an inconsequential amount. By a different criteria (e.g., χ^2), a different model would be selected with a lower BMD. Some additional rationale for model selection would be more satisfying, or selection of a model that is less than an outlier. This would be preferable to selecting a model that has only a very slightly better AIC, or model averaging.

As noted above, a careful consideration should be given to possible human – rat differences in half-lives and tissue levels. Further exploration regarding whether there is a sufficient basis for making a pharmacokinetic adjustment should be undertaken. Consideration could be given to an adjustment based on half-life alone. Development of a PBPK human model may be beyond what is supported by current data, and could result in considerable delays in release of the Toxicological Review, and so may not necessarily be the best approach. However, adjustment of doses applied in the animal study to a human equivalent dose (if half-life data in the animals and humans are found adequate to support such an adjustment) could be done. Then, model fitting could be performed to derive a benchmark dose. A pharmacodynamic factor would still be required to complete the interspecies adjustment.

5. Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfD. For instance, are they scientifically justified and transparently and objectively described in the document?

Harvey J. Clewell

I agree with the use of uncertainty factors of 10 for human variability and sensitive subpopulations, as well as with the UF of three for the obvious database limitations. I might even feel more comfortable with a UF of 10 for the lack of adequate multi-generational study. I would prefer that the UF of 10 for animal to human extrapolation be replaced with a reduced UF of 3 for uncertainty in pharmacodynamics and a cross-species BW^3/4 dosimetric adjustment for differences in rat and human kinetics. This would clearly be appropriate for this chemical: the kinetic data in rats (three studies described on pages 8 and 13) are consistent with a half-life on the order of 20 days (ranging from about 7 to 40 days), while the human worker data (p.13) suggests a half-life on the order of 95 days (average of range from 63 to 128 days). Alternatively, a PBPK model for Kepone could be used to estimate average blood concentration as the dose metric. The average blood concentrations estimated in animal exposures associated with BMDLs, NOAELs, or LOAELs could also be compared with the human data on blood levels in workers showing effects. It is also possible a simple two-compartment model similar to those for dioxin could be developed in the human.

George P. Daston

I found the choice of UFs to be scientifically justified and reasonable. There is not enough information about interspecies sensitivity or range of human variability to alter the default values for these UFs. It is possible to quibble with the 3x factor for database insufficiency, which appears to be predicated largely on the lack of a modern multigeneration study. While I agree that this is a data gap for a compound that clearly has the potential to cause reproductive toxicity, it does not seem to me that, given all of the data from the various reproductive and chronic studies on chlordecone that we would find anything unexpected, or that the LOAEL for reproductive toxicity would be 3-fold lower than that for chronic glomerular toxicity. That said, I believe that this is a minor quibble and does not make that much of a difference in the assessment.

I already noted my objection to the use of a 10x subchronic-to-chronic UF for testicular atrophy as an alternative POD (see response to Charge Question #2).

Gary L. Ginsberg

The 10 fold UF for animal to human extrapolation is appropriate. The 10 fold UF for inter-individual variability is appropriate as well. However, the 3 fold UF for database deficiencies should be increased to 10 fold on the basis that the dose response for frank toxicity for a variety of endpoints is still unresolved with the possibility that NOAELs have not been identified for renal and immunotoxicity (Sobel series of studies), spermatotoxic effects (Linder, 1983), or developmental neurotoxicity (Squibb and Tilson,

1982). Further, there may be upstream sensitive endpoints that interact with background disease or vulnerability conditions (immunotoxicity and enhancement of lupus; estrogenic and spermatotoxic effects; developmental neurotoxic effects) which have yet to be identified or fully explored. Given the importance of each of these endpoints as ongoing disease processes in the human population (autoimmune dx, reproductive dysfunction, learning disorders), it is critical that these toxic effects of Kepone and the uncertainties they present be more fully weighed than is presented in the current document.

Michael I. Luster

For the most part, I think the selection of UFs were appropriate. I am in agreement with including the UFs for animal to human and human variability for all the endpoints including kidney lesions. The addition of a UF for LOAEL to NOAEL for reduced reproductive success and a subchronic to chronic for testicular atrophy seems justified. Regarding the UF applied for the weakness in the Larson study, I do agree with including an UF for weakness in the data set but for additional reasons as stated in the report. I believe that weaknesses in the data are that lesions were observed in only one gender due to the very high background incidence that occurred in the other gender. Also, as mentioned previously, in addition to a lack of human evidence for kidney damage, the studies by Chetty et al., indicated that liver effects were seen at lower doses than kidneys effects (although this may be a matter of difference in biomarkers used).

As stated previously, I am not sure I understand the argument for addition of the UF based upon lack of immunotoxicity studies. While it would be beneficial to have evidence of autoimmune disease in the Larson study (e.g., IgG immune complexes or elevated autoantibody titers), I would suspect that glomerulosclerosis, if severe enough, would constitute an adverse effect independent of the MOA.

Although I would not go as far as to suggest as increasing the UF from 3 to 10 for data weaknesses, it might be a good idea for the authors to state their bases for selecting a 3-fold rather than a 10-fold.

Lauren Zeise

The selection of uncertainty factors is well justified, with the exception of the database uncertainty factor. It should be increased. In addition to immunological effects and lack of an adequate two-generation reproductive study, the female reproductive endpoints have not been adequately studied, and the Agency has ultimately found the cancer study inadequate for making a call of “likely” in the presence of multi-species and sex findings. If the final Toxicological Review still takes this position (in contrast to several other authoritative institutions – see response to charge question C below), the database is clearly deficient and a factor of 10 is justifiable. If the Agency changes its mind and develops a cancer unit risk value, it should still consider increasing the database uncertainty factor given the limitations of the Larson et al. study and the inadequacies in the data base for understanding no effect levels, noted above, for the variety of endpoints

of concern. Also, as noted elsewhere, examination of half-life information for humans and animals may provide a sufficient basis for a cross species pharmacokinetic adjustment for interspecies differences. To this, one would apply a pharmacodynamic adjustment based on scaling of ratios of bodyweight or using a fixed default PD adjustment factor. These two adjustments would be used in lieu of a cross species uncertainty factor.

6. An uncertainty factor was considered necessary to account for deficiencies in the chlordecone toxicity database (e.g. absence of standard two-generation reproduction studies and immunotoxicity studies). Please comment on whether the rationale and justification for the application of the database uncertainty factor has been scientifically justified and transparently described in the document. Please comment on whether the available immunotoxicity data for chlordecone indicate that additional immunological studies would likely result in a lower POD.

Harvey J. Clewell

I agree with the use of an uncertainty factor of at least three for the obvious database limitations. I would feel more comfortable with a UF of 10 considering the lack of an adequate multi-generational study for a compound with evidence of neurological and estrogenic effects. I do not believe that additional immunological studies are likely to provide evidence that would support a different POD.

George P. Daston

I already answered part of this in my response to Charge Question #5. The other reason given for the 3x database insufficiency factor was that the mode-of-action information for immunotoxicity was not definitive. I disagree with this as the basis for a conclusion that the database was insufficient for risk assessment. Having this information would not make us more certain that the glomerular toxicity observed in the chronic study was human-relevant, nor would it change the BMDL, nor affect the way that the RfD was calculated. How then would the resolution of the MOA question result in a 3x increase in our certainty?

Gary L. Ginsberg

These uncertainties are important to the RfD derivation process. As stated in my response to the previous question, the UF due to these and additional endpoints which may yield a more sensitive dose response upon detailed study merits the use of a 10 fold database UF instead of 3 fold. Given that a NOAEL has not been identified for the immunotoxic effect in the sensitive mouse species (LOAEL estimated at 0.2 mg/kg/d which is 2.5 times higher than the BMDL from the Larson study), it appears entirely possible that immunotoxicity and renal toxicity in Lupus-susceptible individuals may merit a greater than 10 fold inter-individual UF. Along these lines, it is noteworthy that Wang, et al. (2007) state: “However, comparison of chlordecone doses producing various adverse effects in mice indicate that autoimmune effects are among the most sensitive, i.e., they occur at doses of chlordecone at or below those required to produce other forms of toxicity.”

Other endpoints without clear NOAELs (spermatotoxic, developmental neurotoxic) are additional reasons for extending the UF to 10 fold.

Michael I. Luster

Please see response to Charge Questions #5.

Lauren Zeise

The database uncertainty factor has been adequately justified and transparently described. As indicated in response to charge question B.5, an additional issue has to do with the Agency's characterization of the cancer bioassay, and limitations in the database for understanding the dose dependent impacts of chlordecone on noncancer endpoints. The findings of liver carcinogenicity are being discounted mostly because of study design and reporting issues. If this study characterization persists into the final document, the cancer bioassay is ultimately being treated as substandard. The database uncertainty factor should therefore be increased to 10 to account for the strong need, in the presence of multiple findings of hepatocarcinoma, for an adequate cancer bioassay. Additionally, the lack of understanding of no effect levels for noncancer endpoints due to database limitations is sufficient for increasing the factor to 10.

C. Carcinogenicity of Chlordecone

1. Under the EPA's 2005 Guidelines for carcinogen risk assessment (http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=116283), there is suggestive evidence of the human carcinogenic potential of chlordecone. This characterization lies at the high end of the continuum for this weight of evidence descriptor. Please comment on the scientific justification for the cancer weight of the evidence characterization. Has the scientific justification for the weight of evidence characterization been transparently and objectively described? A quantitative cancer assessment has not been derived for chlordecone. Do the data support an estimation of a cancer slope factor for chlordecone? Please comment on the scientific justification for deriving a quantitative cancer assessment considering the uncertainty in the data and the suggestive nature of the weight of evidence of carcinogenic potential.

Harvey J. Clewell

Although technically the bioassay results may be consistent with a designation of suggestive evidence of carcinogenicity, I would not consider it to be at the high end of the continuum for this descriptor given the other available data regarding the likely mode of action and the flaws in the bioassays. I agree with the decision not to perform a quantitative cancer risk assessment for chlordecone. The available studies are not adequate to support a quantitative analysis with any confidence. Moreover, given the lack of genotoxicity and the evidence for a purely promotional mode of action, I believe that the RfD will be protective against a significantly increased risk of cancer from this compound. It would be informative to perform benchmark analysis on the induction of tumors to determine whether the RfD is likely to be protective against the promotional effects of this compound.

George P. Daston

I agree with the characterization of carcinogenic potential, and I believe that the descriptions of the weight-of-evidence characterization are appropriate. I also agree with the decision not to carry out a quantitative cancer assessment. The data to carry out such an assessment would need to come from the NCI study, and this study had lots of shortcomings. Furthermore, chlordecone appears to not be genotoxic; therefore, a slope-factor-based assessment may not be appropriate for chlordecone.

Gary L. Ginsberg

Kepone has more than suggestive evidence for human carcinogenic potential. This is a professional judgment call based upon the criteria laid out in the updated 2005 cancer guidelines. Kepone has produced liver tumors in rats and mice along clear dose response relationships that are consistent with the liver tumorigenic effect of the closely related mirex (in fact mirex breaks down to Kepone in the environment). As summarized in the ATSDR Toxicology Profile for Mirex and Kepone (ATSDR, 1995), mirex and Kepone have a very similar profile of hepatic changes along a similar dose response: both

compounds induce an adaptive response involving the induction of hepatic enzymes and increase in liver weight; and both target biliary function and induce a variety of histopathologic effects including fatty infiltration and hepatic necrosis at sufficiently high doses. Also, as summarized in the ATSDR Tox Profile, both compounds decrease male rat fertility with evidence of testicular toxicity and decreased sperm counts. Both compounds have caused impaired reproductive success in the limited rat reproduction studies that have been conducted. While Kepone is well documented to be an estrogen receptor agonist, less is known about mirex's mechanism of reproductive toxicity. These similarities in structure and toxicologic profile for mirex and Kepone lend support to the finding of carcinogenic effect for Kepone, as mirex also caused liver proliferative and carcinogenic response along a similar dose response as seen in the NCI Kepone study. This increases confidence not only in the qualitative finding of likely carcinogen for Kepone, but also for the dose response seen in the Kepone NCI study, using the time-weighted average doses estimated by NCI.

As stated in an earlier question, IARC (2B), NTP (likely) and CalEPA (low dose linear slope used in RA) all have more definitive cancer statements for Kepone. Therefore, this document should reconsider the "suggestive" descriptor. Further, CalEPA has used the NCI studies for cancer risk assessment while this draft IRIS document does not on the grounds that the study irregularities are too great for quantitative dose response characterization. While the study irregularities are significant, it is still possible to use the calculated time-weighted average exposure in dose response modeling. The argument against using this quantitative approach is not compelling or well supported. It is based upon the possibility that the high rate of exposure during early stages of the NCI studies may have led to a disproportionately greater risk due to high dose toxicity; this damage and accumulation of cancer risk would theoretically not occur from doses that are calculated from the smoothed over time-weighted averages provided by NCI for the lifetime exposure. However, this assumes a non-linearity in the dose response based upon a toxic mechanism in the liver which has not been demonstrated. The toxicity that precluded continued use of the high dose in rats and mice was primarily neurotoxicity, an effect which is not known to be a risk factor for liver cancer. In fact, the NCI studies did not find evidence of hepatotoxicity in rats or mice and carcinogenic effects were observed in mice at doses that were not overtly toxic in terms of increased mortality or hepatotoxicity. The Mirex NTP (1990) cancer bioassays did not have protocol irregularities associated with the Kepone bioassays – while mirex shares many toxic effects with Kepone, it is not neurotoxic in the manner that Kepone is. Therefore, it is not surprising that at comparable doses, the chronic mirex study did not exceed the maximum tolerated dose (MTD) and did not require adjustment of doses. The similarity in liver cancer dose response between the Kepone NCI and mirex NTP studies provides key support for the conclusion of likely carcinogen for Kepone and for using the NCI dose response information to derive a cancer potency factor on IRIS for Kepone. Another important piece of evidence is the finding of Kepone-induced promotion in a 2 stage initiation-promotion study at very low s.c. doses (the low dose, 0.05 mg/kg/d was promotional; Sirica, et al., 1989), an effect level for promotion that is below the doses associated with tumorigenesis in the NCI studies. At this low dose, it is very unlikely that frank toxicity had anything to do with the promotional effect of Kepone.

It is speculative to assume that the cumulative long-term average daily dose is not a reasonable dose metric for Kepone carcinogenicity in this study. This, in fact, is the default assumption in cancer risk assessment (long-term average dose is most relevant for predicting cancer risk). The promotional effects of Kepone are not known to have a threshold and, in fact, the lowest dose tested in an initiation/promotion study was effective (Sirica et al., 1989). Therefore, without further evidence on toxicity-mediated enhancement of the tumorigenic process in the early stages of the NCI bioassay, the rationale for discounting the NCI study for quantitative analysis is weak and speculative. EPA should also recognize that the irregular NCI protocol in some ways decreased the sensitivity of these bioassays to detect Kepone carcinogenicity in that exposure was only for 80 weeks, near the end of the study, exposure in some groups was not continuous (high dose male rats received compound on alternative weeks only), and excessive mortality decreased the numbers of animals available to detect the effect.

A lack of quantitative cancer assessment in this IRIS file leaves the risk assessor with no option for quantitative evaluation of cancer risk other than to use the slope factor derived by CalEPA. U.S. EPA could support the use of the NCI Kepone study for quantitative cancer risk assessment by referring to the mirex studies which found a similar dose response in studies which did not have the same methodological issues, and by referring to the evidence of low dose Kepone promotional effects in the Sirica, et al. 1989 study. U.S. EPA could also theoretically use the Sirica promotional dose response as part of the array for non-cancer (RfD) assessment. However, this is an unorthodox approach that would not represent a thoughtful attempt to harmonize cancer and cancer assessment and would require more mechanistic information to treat the cancer endpoint as a non-cancer finding.

Michael I. Luster

I believe the NCI bioassay, even with its flaws, provides good evidence for carcinogenicity. I agree, however, that because of the described issues in conducting the NCI bioassay, the data are not amenable for quantitative risk assessment and development of an RfD. In addition to study design and conduct problems, a large number of early deaths occurred in treated animals independent of tumors and except for the dose used in female mice, many of the original doses appeared to exceed the MTD. In addition, use of the data in quantitative risk assessment would be further complicated by the fact that other studies indicated that chlordecone is not a complete carcinogen, but rather acts only as a promoter, and is not genotoxic.

Lauren Zeise

As summarized on pages 68-70, the evidence for the carcinogenicity of chlordecone is:

- The compound caused hepatocellular carcinoma in male and female mice and rats.
- The compound bears close structural similarity to mirex, which also causes liver tumors in rodents.
- There are no adequate epidemiology studies of cancer effects for evaluation.

- Human occupational data demonstrate that the liver is a target site of toxicity and of compound accumulation.
- The mode of action of liver cancer in rodents is unknown.

No evidence is provided to indicate that these findings are irrelevant to humans. Some weaknesses in the NCI studies reporting these findings are discussed. These weaknesses cannot explain these multi-species, multi-sex findings.

The available evidence is consistent with a finding "likely to be carcinogenic to humans," and not, as indicated in the draft, a finding of "suggestive evidence of carcinogenic potential."

According to the EPA 2005 *Guidelines for Carcinogen Risk Assessment* a descriptor of "likely to be carcinogenic to humans" fits when:

- "An agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans."

Kepone tested positive in more than one species and in more than one sex, and the label "likely to be carcinogenic to humans" is appropriate.

In contrast "suggestive evidence of carcinogenic potential" applies when:

- "a small, and possibly not statistically significant, increase in tumor incidence observed in a single animal or human study" or
- "a small increase in a tumor with a high background rate in that sex and strain" or
- "evidence of a positive response in a study whose power, design, or conduct limits the ability to draw a confident conclusion" or
- "a statistically significant increase at one dose only, but no significant response at the other doses and no overall trend."

Clearly, with positive findings of liver cancer in both rats and mice, and large increases in incidence in both dose groups in both male and female mice, none of the above cases applies. The descriptor "suggestive evidence of carcinogenic potential" does not fit. The Toxicological Review document does present some information pertinent to the third bullet, but in no case presents evidence with enough weight to rule out the positive findings of liver cancer.

The Toxicological Review calls into question the use of the term hepatocellular carcinoma in the NCI studies, indicating that they were likely benign lesions. The NCI clarified what it meant by this characterization in its studies for Kepone:

"The term "hepatocellular carcinoma" was used to diagnose proliferative lesions of the livers in mice which, in the judgment of the pathologists, had the potential or the capacity for progressive growth, invasion, metastasis and for causing death of the host. This

judgment was based upon the cytologic and histologic features of the neoplasms and the knowledge that lesions with the same morphologic characteristics as those observed have exhibited malignant biologic behavior. The terms "neoplastic nodule" and "hepatocellular carcinoma" used to diagnose proliferative liver lesions in rats were based upon the morphologic criteria and nomenclature recently reported from a workshop on the classification of specific hepatocellular lesions in rats (6).”

The statement is also made in the Toxicological Review that doses used in the study were excessively high and compromised the study. However, the NCI adjusted doses to be better tolerated by study animals, and no evidence is presented in the Toxicological Review that toxicity confounded the consistent finding of liver cancer. It might be speculated that liver toxicity caused the cancer, but there is no substantial evidence to support this.

Other institutions, on the basis of the same evidence, have made weight of evidence calls consistent with the EPA’s category of “likely to be carcinogenic to humans:”

- The National Toxicology Program classifies this compound on the basis of this evidence as reasonably anticipated to be a human carcinogen.
- The International Agency for Research on Cancer (IARC) found the evidence of carcinogenicity sufficient in animals and categorized the chemical as a 2B carcinogen.
- The National Institute for Occupational Safety and Health classifies Kepone as a potential human carcinogen and notes that in its Recommended Standard for Occupational Exposure to Kepone.
- The Agency for Toxic Substances and Disease Registry indicates in its public information FAQs that “The Department of Health and Human Services (DHHS) has determined that mirex and chlordecone may reasonably be anticipated to be carcinogens.”
- International Programme for Chemical Safety concluded “There is sufficient evidence of its carcinogenicity for mice and rats.”

While acknowledging that the mode of action for liver carcinogenicity is unknown, xenoestrogenicity as a possible mode of action could be better explored in the document. Xenoestrogens such as ethinyl estradiol are liver carcinogens in the rat. Findings of liver cancer from xenoestrogens are of increased relevance for humans since, according to IARC, there is sufficient evidence of human liver cancer from use of combined contraceptive therapy. Experience epidemiologically and in the laboratory with these compounds indicates timing is important, with different sites being affected depending on when the xenoestrogen is applied.

As a technical, statistical note, U.S. EPA could adjust for early mortality in making pairwise comparisons between treated animals and controls using the poly-3 test. This test is now standard at the National Toxicology Program for evaluating the statistical significance for determining whether exposures should be considered treatment related.

Using individual animal data from the NCI study, the Agency could perform and report these tests in the Toxicological Review.

A quantitative analysis of the available cancer dose response data should be performed using approaches described in the EPA 2005 *Guidelines for Carcinogen Risk Assessment.* While the data are not ideal, a sensitivity analysis can be performed to evaluate the uncertainty in dose characterization on the dose response.

V. SPECIFIC OBSERVATIONS

Harvey J. Clewell

The document is well written and essentially free of errors. My only suggestion is to revise the sentence on p.9: "Maternal tissue levels were 4 to 5 times higher than fetal concentrations, indicating that the placental barrier retards the distribution of chlordecone to the fetus." There is no evidence that the placenta retards the distribution of chlordecone to the fetus. In fact, chlordecone was observed in fetal tissues within 4 hours of dosing. The lower accumulation of chlordecone in the fetus more likely reflects a lower fat content of the fetus compared to the dam and a lower binding of chlordecone in the fetal liver.

George P. Daston

P. 81, second full paragraph, last sentence: The observation of enlarged renal pelvis in a developmental toxicity study is used here as additional support that chlordecone has the potential to be a renal toxicant. Enlarged renal pelvis in rodent fetuses is not related to the renal toxicity observed in chronic studies. Enlarged renal pelvis is considered to be a developmental delay (Woo and Hoar, 1972, Teratology 6: 191-196) that is often observed in conjunction with maternal toxicity and/or other manifestations of developmental delay (e.g., decreased fetal weight) that were observed in the developmental toxicity study on chlordecone. I recommend deleting this sentence.

Gary L. Ginsberg

No suggestions noted.

Michael I. Luster

In a number of places, the report indicates that the NZB and Balb C mice represent the range of genetic association with autoimmune disease and is not representative of humans. I don't think this is correct. It is more likely that these 2 strains represent the extremes in the human population regarding genetic influence on autoimmune disease with of course the vast majority of the population somewhere between the two – one wants to evaluate the most sensitive.

Also requiring some clarification is the description of the kidney results in workers or residents exposed to chlordecone. It was not always clear whether kidney biomarkers were not examined or they were examined and there were no effects.

Pg 83: The statement that 'glomerulosclerosis is believed to be irreversible' should be referenced. It would also be useful to briefly describe the histopathology of the lesion and in what other type of kidney diseases might it be found (it certainly is not a chemical-specific or autoimmune-specific lesion).

Pg 7, par 2: Report indicates that fat to blood concentrations for chlordecone is relatively low compared to other organochlorine pesticides – reference should be provided of what is meant by 'low in comparison to other organochlorines.'

Pg 50, Par 2: Sentence beginning with "the affinity of chlordecone." This sentence seems to be incorrect – The tissue difference in binding to the estrogen receptor is likely related to the difference in receptor concentration and not difference in receptor affinity –please confirm. Also, if available, it would be worth noting the relative potency of chlordecone binding to the estrogen receptor, relative to estradiol.

Pg 52, par 3,2nd from last sentence: The statement 'histopath associated with renal disease was similar between the groups.' Clarify that this is referring to treatment groups and not to controls.

Pg 57, par 2: The sentence beginning with "The mode of action" is repeated twice in the paragraph.

Lauren Zeise

p. 71, top: The fact that liver neoplasia was not seen by Guzelian in 12 workers biopsied within 16 months of initial exposure to Kepone should not be included in the weight of evidence discussion for cancer. Liver cancer in humans has a long latency period and no cancer would be expected by this time, even for strong known human liver carcinogens. This is an irrelevant piece of information that should be deleted from the WOE section.

Page 28, Table 4-4: Footnote "NA" means "not applicable" and not as indicated "not available." No tumors were observed so that "time to 1st tumor" does not apply.