Summary Report of the
U.S. EPA Scientific Peer Review Meeting on the
Toxicological Review and IRIS Summary for Mirex

Cincinnati, Ohio
April 22, 2004

Prepared For:
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July 9, 2004

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1.0 INTRODUCTION

1.1   Meeting Purpose

The U.S. Environmental Protection Agency (US EPA) Scientific Peer Review Meeting on the Toxicological Review and IRIS Summary for Mirex was held on April 22, 2004, at the EPA Andrew W. Breidenbach Environmental Research Center in Cincinnati, OH. This one-day meeting was organized and hosted by Versar, Inc. for the U.S. EPA's Office of Research and Development/National Center for Environmental Assessment (NCEA). The purpose of the meeting was to provide a scientific peer review of the Toxicological Review and IRIS Summary for Mirex.

1.2   Meeting Participants and Agenda

Attendees at the peer review meeting included the five peer reviewers (Appendix A) and about 10 observers (Appendix B). The peer reviewers were selected by Versar with experience in pesticide toxicology, biological statistics, cancer mechanisms, and nongenotoxic cancer mechanisms (tumor promotion). The agenda for the peer review meeting (Appendix C) was developed by Versar and the Chair to include: introductory presentations to provide background and establish the scope for the peer review, discussion sessions among the reviewers on the charge questions, and observer comment periods. In general, the agenda for reviewer discussion followed the organization of the charge questions. Specifically, the meeting began with a welcome and introductions followed by clarifying questions posed by the reviewers to EPA. The meeting continued with reviewer discussion on the toxicological review and IRIS summary, addressing the charge questions. Over the course of the meeting, two observer comment periods were scheduled, to allow the observers to provide input.

1.3 Organization of Meeting Summary Report

This report summarizes the meeting discussions, focusing on the final recommendations and suggestions provided by the peer reviewers.

- $ Section 2 of this report summarizes the welcoming remarks and introductions.

- $ Section 3 presents the Chair's summary of the major discussion, comments, and recommendations provided by the reviewers on the IRIS toxicological review for mirex.

- $ The appendices to this report present a list of reviewers, a list of observers, the agenda, and the reviewers' written comments (prepared before the meeting, and in some cases, revised after the meeting).

2.0 SUMMARY OF OPENING REMARKS/PRESENTATIONS

Opening remarks were provided by several people responsible for organizing and hosting the peer review meeting, including staff from Versar, Inc. and EPA=s National Center for Environmental Assessment, as well as the meeting Chair.

2.1   Welcome from Meeting Organizer/Facilitator

David Bottimore, Project Manager from Versar, Inc., opened the meeting by welcoming the peer reviewers and observers. He provided an overview of the meeting agenda, including a statement of the goals and intended outcome of the peer review. The objective of the meeting was to obtain technical input on EPA's document related to the four charge questions, which address overall document quality, reference dose (RfD) derivation, reference concentration (RfC) derivation, and cancer weight of evidence (WOE) characterization and quantitative assessment. Included in these opening remarks were ground rules and procedures for conducting the meeting. One issue that was emphasized was that consensus would not be sought, rather the goal would be to seek the individual comments and suggestions from the peer reviewers. He also clarified the roles of observers, including EPA staff, and highlighted the time periods set aside to obtain observer comments. These opening remarks concluded with an overview of the agenda and introductions of the five peer reviewers.

2.2   Welcome and Introductions

Harlal Choudhury, of NCEA-Cincinnati, welcomed the reviewers and provided an overview of the document and of issues associated with risk assessment for mirex. Mirex is an environmentally persistent chlorinated compound and is structurally similar to other chlorinated compounds, including endocrine distruptors. Use of mirex in the United States was banned in the late 1970's and not much new information is available since there has not been much research into its toxicity or mode of action (MOA). Mirex has a long half-life in humans and there are

concerns for cancer and non-cancer health effects. The data used for the assessment are from the National Toxicology Program (NTP) (1990), which indicate that mirex is a hepatotoxicant and at lower doses produces target organ toxicity, especially to the liver. The RfD proposed in the revised assessment is 0.0005 mg/kg-day, which was derived using benchmark analysis. The RfD is similar to that from the previous assessment (0.0002 mg/kg-day), which did not use a benchmark dose approach. For the cancer assessment, a 3/4 body weight factor was applied, with a slope factor of 0.5 per mg/kg-day. There is no evidence of mutagenicity and consistent liver effects have been observed, mainly liver tumors, but there is not adequate understanding of the mode of action. As a result, a linear approach was used for the cancer assessment, consistent with the 1999 draft cancer assessment guidelines. No RfC has been derived because of a lack of data.

Michael Dourson chaired the peer review meeting and served as facilitator. He began his introduction by describing the peer review process and setting the ground rules. He reiterated that technical input was sought from each participant, noting that there would be no attempt to achieve consensus. Rather, the discussion should bring out the diverse perspectives of individual experts in the group. He also described the procedure for the observer comment periods and encouraged observers to provide technical information that might be of assistance to the panel and to EPA in revising the assessment. Observers were further requested to answer clarifying questions from either reviewers or authors at the time of their presentation. He also permitted reviewers to approach either authors or observers with technical questions during breaks, but not the reverse. Reviewers were asked to report significant information from such conversations back to the entire group during the discussion sessions. Authors were permitted to respond to reviewers= questions, and were also permitted to ask clarifying questions of panel members, but not to participate in the discussion. He concluded his opening remarks by reviewing the agenda for the meeting.

2.3   Peer Reviewer Clarifying Questions and Initial Discussion

The peer reviewers asked clarifying questions to aid in the discussion and review of the toxicological review and IRIS summary for mirex. Some of these questions were raised prior to the meeting by reviewers in their individual comments (Appendix D).

A question was raised by one reviewer as to why mirex was being re-reviewed. Dr. Choudhury responded by noting that mirex continues to be a contaminant of concern at contaminated sites and had recently been detected in cattle beef and milk at sites with high residues (e.g., Salem, OH). Concern over cattle ingesting contaminated grass and the milk containing high levels of mirex led to the site being listed as a Superfund site. EPA=s Superfund office requested that the IRIS program conduct a full assessment, to update the RfD and also to conduct a cancer assessment.

Another question was posed by a reviewer that dealt with the reproductive study (Gaines and Kimbrough, 1970; Chu et al. 1981), which indicate a possible endpoint of cataracts in rat offspring, though it was found not to be the critical effect. Dr. Choudhury responded by stating that when a special review of mirex was performed in 1978, EPA did not consider the effect relevant to human exposure. In addition, the developmental effect occurred at a dose that was 10-fold higher than that used in the liver study. Karl Rozman noted that the daily dose is not really informative with a chemical such as mirex, because it has a long half-life and that the cumulative dose is more relevant and informative. After further discussion about the reproductive/developmental effects, Michael Dourson suggested that the cataract studies be reviewed in more detail and that a benchmark dose be estimated for comparison with other non-cancer endpoints.

A reviewer pointed out that a typographical error appeared in the document on p. 12, line 31: the sperm count was incorrectly written as 0.05 mg/kg instead of 0.5 mg/kg. Another typographical error was identified on p. 30, in the discussion of the gestation period. The period was listed as 7-162 months, rather than 7-16 months. Another reviewer suggested that the toxicological review document needs to introduce the RfD methods, referencing existing guidance documents on the process used to derive the RfDs.

3.0 CHAIR'S SUMMARY OF PEER REVIEWER COMMENTS

This section presents the Chair's summary of the major recommendations and suggestions provided by the reviewers during the peer review meeting.

1) Overall document quality

Please prepare a statement that addresses the overall quality of the document(s) and provide advice on approaches to improve the assessment from both technical and communication standpoint; and provide suggestions on the integration of data into an overall characterization of hazard. Questions to consider include:

a) Is the document logical, clear and concise? Are the arguments presented in an understandable manner?

b) Are you aware of any other data/studies that are relevant (i.e. useful for the hazard identification or dose-response assessment) for the assessment of the adverse health effects, both cancer and noncancer, of this chemical?

The document is well written, with good descriptions of the available data and their use in deriving reference values for mirex. The presentation is clear and thorough, with tables that were found to be helpful for the reader to see the various data considered in the assessment. One reviewer suggested that the document present summaries at the end of each section to encapsulate the discussion in that section, which would help the reader to identify the key studies, data, and issues to be carried forward in the assessment. As it is written, one must wait until the latter sections to see how information is synthesized (e.g., toxicological significance) in the assessment.

Reviewers noted that this is a very traditional IRIS document, but mirex warrants a different type of assessment because it is a persistent chemical with a very long half-life in humans. Daily dose is not as meaningful as cumulative dose and tissue levels (body burden), which will provide better representation of exposure. An improved presentation of toxicokinetics is needed, not only for humans but also for the animals tested (several additional papers were suggested in individual reviewers comments). This improvement is important since understanding

toxicokinetic differences between humans and animal systems (e.g., mice, rats, rhesus monkeys) is paramount to credible extrapolation from animal data to humans. EPA should reexamine this assessment and consider approaches to account for body burden, such as those used in assessments of other persistent chemicals (e.g., perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), cadmium, boron, and others) to ensure that the approaches are consistent. Overall, reviewers felt that the dose-response assessment should be revised to reflect the persistence of mirex and the resulting cumulative doses in humans. In addition, it should be recognized that mirex toxicokinetics in humans are considerably different than in animals. This difference might be accounted for through the use of an animal to human extrapolation adjustment factor.

Discussion on the mechanism of action raised many issues, particularly for the cancer assessment. There was general agreement that mirex may have several modes of action, is non-genotoxic and acts as a promoter. While some reviewers felt that the MOA was clear, others felt that it did not meet EPA's criteria from the revised cancer guidelines for non-linear modeling for the cancer assessment. After further discussion, reviewers suggested that EPA perform a non-linear cancer assessment in addition to the linear approach used in the draft assessment (both assessments should be presented in the toxicological review). This issue was discussed in more detail under charge question number 4.

During the initial discussion of non-cancer effects, reviewers raised issues about the critical effects (hepatitis) and suggested that other endpoints be examined in more detail. Particularly, reviewers felt that reproductive/developmental effects (cataracts in pups) be fully evaluated because they might be a more sensitive endpoint than the liver effects. It was unclear from the assessment whether the cataracts resulted from exposure *in utero* or via lactation, and what the concentrations were in the pups. Papers that present these data, such as Gaines and Kimbrough (1970) and Chu et al. (1981), should be reviewed in more detail to determine if the developmental/reproductive effects might be critical. Furthermore, these papers should be examined for data on body burden of the pups with cataracts and for those that died. Benchmark

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doses (BMD) should be calculated and compared to the BMD from the liver effects to determine the most protective endpoint/reference dose. These topics were discussed in more detail under charge question number 2.

2) RfD derivation

a) Principal Study, Section 5.1.1: The RfD is based on a chronic rodent study in which rats were exposed to Mirex in the diet for 104 weeks (NTP 1990). The critical effect observed was toxic hepatitis. The principal study should present the critical effect in the clearest dose-response relationship. Is use of the NTP (1990) study as the principal study justified and is the rationale for this study adequately explained in the Toxicological Review?

b) Critical Effect, Section 5.1.1: The critical effect is identified as toxic hepatitis. Has the most appropriate critical effect for Mirex been chosen (i.e. that adverse effect appearing first in a dose-response continuum)? Has the critical effect been adequately described? Is this critical effect biologically significant? Finally, does the information presented from animal studies mirror what is known about the toxicity in humans and is this information adequately described?
c) Is the RfD determined for Mirex protective of adverse health effects in the general population and in sensitive sub-populations such as children and pregnant women?

c) Is the RfD determined for Mirex protective of adverse health effects in the general population and in sensitive sub-populations such as children and pregnant women?

d) Methods of Analysis, Section 5.1.2: Benchmark Dose Modeling (BMD) was applied to the chronic study for Mirex. Was the point of departure determined appropriately for this approach? Is the 10% response level appropriate and is the use of this response level supported adequately?

e) Uncertainty Factors, Section 5.1.3: Are the appropriate uncertainty factors used to develop the RfD? Are there other data which should be considered in developing the uncertainty factors? Is the explanation for the selection of each of the uncertainty factors transparent?

The critical effect identified in EPA's assessment, toxic hepatitis, should be described in more detail. Is it an accepted diagnosis (hyperplasia, necrosis, lymphocytic infiltration)? The assessment should also focus on the end point and whether the concern is for toxic hepatitis itself, or its role as a precursor for other liver effects, including cancer. A similar presentation of liver effects was included in the IRIS assessment for vinyl chloride, where the critical effect was liver cell polymorphism.

Considerable discussion focused on other potential critical effects, such as developmental effects (cataracts in pups) that might be more sensitive than the liver endpoint. Reviewers suggested that EPA review in more detail the data from the Gaines and Kimbrough (1970) and Chu et al. (1981) papers. It was suggested that benchmark doses be calculated for these other effects to determine if they occur at doses lower than the liver effects. (Reviewers estimated that the LOAEL for cataracts would be 0.4 vs. 0.7 mg/kg-day for liver effects, which might suggest that this would be a more protective basis for the RfD.) Reviewers also did a rough calculation based on cumulative dose and concluded that the developmental effects (cataracts) were occurring at doses lower than the liver effects. While maternal toxicity was not observed at these doses, developmental effects were detected in pups. This developmental/reproductive endpoint might be of concern for pregnant women and nursing infants, which might be the most sensitive subpopulation.

Reviewers also suggested that EPA conduct these assessments based on cumulative dose/tissue concentrations, rather than according to daily doses, because of the longer half-life of mirex in humans compared to mice, rats, and rhesus monkeys. As a result, an adjustment factor is needed when extrapolating from animal test results to humans. EPA has done this in the past for chemicals with longer half lives, such as PFOA and cadmium. The human equivalent dose adjustment is needed and should be incorporated into the BMD calculation. Reviewers provided several approaches for estimating this adjustment factor, including using data on half lives in the different species. Toxicokinetic studies should be examined (such as Ohio EPA data) to better characterize half-lives in humans relative to animals used in toxicity tests. Later during this discussion, reviewers found that the Chu (1981) and Gaines and Kimbrough (1970) papers had data on tissue levels in pups, which would be helpful in deriving this adjustment factor.

Other non-cancer effects data should be evaluated and BMDs calculated, such as for endocrine effects (e.g., sperm count data from Chu et al. (1981) or Khera (1976) papers) as well as decreased pup survival. The discussion of endocrine effects (male reproductive effects as well as thyroid, liver, and adenoid effects) could be incorporated into page 38 under the "Other Relevant

Studies" subsection. Reviewers also suggested that secondary references from those papers (e.g., Villenueve, Sudram, Chernoff) be reviewed for additional data on endpoints other than the liver effects assessed in EPA's draft toxicological review. One reviewer noted that nested BMD modeling may be needed for the analysis of the sperm count data.

Discussion of the critical effect and critical studies, as described above, raised many issues and concerns that other endpoints should be evaluated and carried through to estimate BMDs and even RfDs to see which would be most sensitive/protective. It was also noted by one reviewer that human sensitivity to liver effects may be lower than rodents, as indicated by the higher doses needed to produce the same effects in rhesus monkey studies. Reviewers concluded the discussion of the RfD derivation by reiterating that EPA examine other endpoints to determine if they are more sensitive than liver hepatitis. It was suggested that the assessment incorporate cumulative dose information as well as adjustments for toxicokinetic differences between humans and the animal systems. Overall, the process should include: BMD analyses conducted for all studies using body burden or cumulative dose, adjust for kinetic differences between animals and comparable human subgroups, determine the human equivalent daily dose, and compare the different endpoints to determine which is most sensitive.

Reviewers considered the uncertainty factors for the toxic hepatitis endpoint and supported some of EPA's values, while suggesting other factors for the assessment. Overall, reviewers recommended that the presentation of these factors be made clearer for the reader, weaving a story on the basis for the factors (e.g., data base UF). It was noted that the uncertainty factors for the assessment, if revised as suggested to incorporate cumulative dose and developmental effects, will need to be revised. For the current assessment, a total uncertainty factor of 30 or 100 was estimated by all of the reviewers during this discussion, which is composed of:

UF_H = 10 (or use a 3-fold factor if the toxicokinetics is already addressed; the remaining 3-fold would be for dynamics)

$UF_A = 3$ for dynamics (assuming appropriate cross-species adjustment for pharmacokinetics is applied and that the dynamic response in toxic hepatitis between species is different at the same internal dose)

$UF_D = 3$

$UF_L = 1$

$UF_S = 1$

Reviewers wrapped up the discussion with suggestions on the types of studies that would be helpful for providing data that might improve this assessment, such as a two-generation reproductive study.

3) RfC derivation

a) No RfC has been developed in this assessment due to lack of adequate toxicity data for the inhalation route of exposure. Does the assessment appropriately addresses toxicity of mirex via the inhalation route of exposure?

b) Does the assessment appropriately addresses toxicity of Mirex via the inhalation route of exposure?

All reviewers agreed that no RfC should be derived because of inadequate data and because there is little concern for the inhalation exposure pathway. Mirex has a low vapor pressure and minimal potential for dust inhalation exposure (based on dioxin). One reviewer raised a question about EPA's presentation of the solubility of mirex (0.6 mg/l) and suggested that EPA reexamine the value for accuracy.

4) Cancer Weight-of-Evidence Characterization and Quantitative Assessment

The weight of evidence characterization and quantitative estimation (oral route) have been discussed in Chapters 4 and 5 of the Toxicological Review document and to a limited extent in the IRIS summary document.

a) Have the appropriate criteria from the U.S. EPA 1999 draft revised Guidelines for Carcinogenic Risk Assessment document been applied?

The available information on toxicity of mirex is discussed in the Toxicological Review and in the IRIS summary documents. The 1999 draft revised Guidelines for Carcinogen Risk Assessment state that a linear dose-response approach should be taken when the mode of action information is supportive of linearity or mode of action is not understood. In the absence of adequate data in support of mode of action for carcinogenesis at dosages higher than the non-cancer events this assessment presents a non-linear Dose-Response model for carcinogenic assessment.

b) Are the tumors observed biologically significant? Are the tumors observed relevant to human health? Points relevant to this determination include whether or not the choice follows from the dose-response assessment, whether the effect is considered adverse, and if the effect (including tumors observed in the cancer assessment) and the species in which it is observed is a valid model for humans.

c) Was the mode of action section presented clearly and logically? Are there any additional studies that would enhance the mode of action information presented in the Toxicologic Review? Based on the mode of action information in the Toxicological Review and IRIS summary as well as the 1999 draft revised Guidelines for Carcinogen Risk Assessment, is linear dose-response modeling for cancer assessment appropriate for Mirex?

Reviewers suggested that the weight of evidence (WOE) statement (page 47-48) be revised as follows:

> "Following U.S. EPA (1999a) Draft Revised Guidelines for Carcinogen Risk Assessment, the hazard descriptor, *Likely to be Carcinogenic to Humans* at high bioaccumulative doses, but highly unlikely at environmentally-relevant levels (ng/kg-day range), is appropriate for mirex based on consistent (toxicologically significant) findings of hepatic carcinogenic responses in several studies of rats and mice chronically exposed to mirex in the diet. The human relevance of the animal evidence of carcinogenicity is assumed in the absence of adequate human data or mechanistic data to indicate that the mode of carcinogenic action in animals is not relevant to humans."

Reviewers suggested that this split descriptor be added because mirex is not thought to be a potent carcinogen (see MOA discussion below) and effects would only result from high exposures, not at environmentally-relevant levels. Also, there were concerns about the tumors found in animal studies; while reviewers agreed that liver tumors were of concern, tumors in other sites were not found consistently. One reviewer voiced concerns about high background liver tumor levels in controls for F344 mice. It was suggested that EPA examine in more detail the background liver tumor data from the NTP study.

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It was suggested that the MOA discussion be improved and presented earlier in the document before the WOE statement (earlier on page 47 or on page 41) because many of the issues impact the WOE statement. Reviewers had divided opinions on the strength of the MOA evidence. While most experts agreed that mirex is not genotoxic and acts as a promotor, it could have several different modes of action, including endocrine disruption activity. The MOAs for mirex include endocrine mechanisms (many different hormonal effects resulting from altered signal pathways), weak tumor promotion, and other mechanisms that are not understood. Several reviewers noted that mirex does not have DNA binding properties, but at least one reviewer noted that most of this evidence is from in vitro tests. Several reviewers agreed that mirex has nonlinear dose response, but acknowledged that the MOA evidence is not sufficient under EPA's cancer guidelines for nonlinear modeling. It was mentioned that having a bone marrow test (an in vivo test, such as the micronucleus test) would be helpful to increase the level of confidence that mirex is not genotoxic; however, it was noted that it is not likely that anyone will undertake future tests on this chemical. Reviewers generally suggested that the MOA evidence was strong enough to suggest that nonlinear dose response modeling be conducted and presented in the toxicological review, along with the linear modeling results. While reviewers generally agreed that the nonlinear approach is more appropriate, they recognized that EPA would have to make a policy decision on which approach to use to derive the slope factor.

Specific wording changes in the MOA descriptions were provided:

> "The modes of action whereby mirex induces liver tumors in animals are not yet completely understood, but available data suggest that a nongenotoxic mode involving mirex induced hepatic cell proliferation is plausible **and consistent with evidence**."

> "Short-term and repeated oral exposure to mirex are known to cause hepatic cytomegaly, vacuolization, fatty metamorphosis, and necrosis, which may be precursor events to the eventual development of liver tumors, but this hypothesis **has not been fully tested**."

Based on the MOA and WOE discussions, reviewers provided recommendations on different approaches and options for EPA's revision of the carcinogenic dose-response modeling for mirex. The linear approach, as presented in the existing document, could be used. Alternatively, two different non-linear modeling approaches could be considered, based on cumulative dose.

The first of these cumulative dose approaches would take the existing LED10 and divide it by a factor of 70 for animal to human kinetic differences. This approach was mentioned on page 20 of the premeeting comments (from Karl Rozman) and is based on the differences in half-lives in rats and humans. With this approach, it is mandatory that the assessment have a table with available half-life data for mice, rat, rhesus monkeys, and humans. The half-life values should be referenced and clearly explained in the text, especially if they are not directly derived or evident.

In the second cumulative dose approach, the point of departure is calculated as the existing LED10 multiplied by the length of the experimental animal bioassay and then divided by the lifespan of humans. For either approach EPA would need to consider margins of exposure of either 10, 30 or 100, depending on overall uncertainties remaining after the extrapolations. For example, it may be that the only remaining uncertainty is interindividual variability which warrants a MOE of 10.

Reviewers wrapped up this discussion by reiterating that these are options that EPA might consider as they revise the cancer assessment, noting that EPA's guidelines allow flexibility to consider non-linear or MOE approaches. Most reviewers felt that a non-linear approach is more appropriate but recognized that EPA would have to make a policy decision. It was recognized that the very low number would be inconsistent with the WOE discussion earlier, where mirex is not believed to be carcinogenic at environmentally-relevant levels. This discrepancy should be noted by EPA in the assessment and in the discussion of WOE and MOA.

Reviewers concluded the discussion by congratulating EPA on a well-done assessment and suggesting that the Agency consider these recommendations as they revise the report. It was reiterated that in the case of mirex, toxicokinetics issues need to be described in more detail and EPA needs to make sure that all the available and relevant literature has been cited.

APPENDIX A

Peer Reviewers

Mirex Peer Reviewers

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APPENDIX B

Observers

Scientific Peer Review of Toxicological Review and IRIS Summary for Mirex

April 22, 2004

Observers	
Name	**Organization**
Harlal Choudhury	EPA/NCEA
Bernard Gadagbui	TERA
Andrew Gillespie	EPA/NCEA
Lisa Jackson	SRC
Eric Hack	TERA
Chandrika Moudgal	EPA/NCEA
Debdas Mukerjee	EPA/NCEA
W. Bruce Peirano	EPA/NRMRL
Jon Reid	EPA/NCEA
Glenn Suter	EPA/NCEA
Mike Troyer	EPA/NCEA
Raghuraman Venkatapathy	ORISE/EPA/NCEA

APPENDIX C

Agenda

United States
Environmental Protection Agency
Office of Research and Development

Scientific Peer Review of Toxicological Review and IRIS Summary for Mirex

Room 230
U.S Environmental Protection Agency
Andrew W. Breidenbach Environmental Research Center
26 W. Martin Luther King Boulevard
Cincinnati, OH

Agenda

THURSDAY, APRIL 22, 2004

8:15AM   **Registration Begins**

8:30AM   **Welcome, Introductions, and Goals of Meeting**
                David Bottimore, Versar, Inc.

8:45AM   **Welcome**
                Harlal Choudhury, EPA, National Center for Environmental Assessment

9:00AM   **Chair's Introduction**
                Michael Dourson, TERA, Workshop Chair

9:10AM   **Clarifying Questions from Panel**

9:30AM   **Discussion Session (with break as appropriate)**

1)   Overall document quality

        a) Is the document logical, clear and concise? Are the arguments presented in an
            understandable manner?

        b) Are you aware of any other data/studies that are relevant (i.e. useful for the hazard
            identification or dose-response assessment) for the assessment of the adverse health
            effects, both cancer and noncancer, of this chemical?

2)	RfD derivation

a) Principal Study, Section 5.1.1: The RfD is based on a chronic rodent study in which rats were exposed to Mirex in the diet for 104 weeks (NTP 1990). The critical effect observed was toxic hepatitis. The principal study should present the critical effect in the clearest dose-response relationship. Is use of the NTP (1990) study as the principal study justified and is the rationale for this study adequately explained in the Toxicological Review?

b) Critical Effect, Section 5.1.1: The critical effect is identified as toxic hepatitis. Has the most appropriate critical effect for Mirex been chosen (i.e. that adverse effect appearing first in a dose-response continuum)? Has the critical effect been adequately described? Is this critical effect biologically significant? Finally, does the information presented from animal studies mirror what is known about the toxicity in humans and is this information adequately described?

c) Is the RfD determined for Mirex protective of adverse health effects in the general population and in sensitive sub-populations such as children and pregnant women?

d) Methods of Analysis, Section 5.1.2: Benchmark Dose Modeling (BMD) was applied to the chronic study for Mirex. Was the point of departure determined appropriately for this approach? Is the 10% response level appropriate and is the use of this response level supported adequately?

e) Uncertainty Factors, Section 5.1.3: Are the appropriate uncertainty factors used to develop the RfD? Are there other data which should be considered in developing the uncertainty factors? Is the explanation for the selection of each of the uncertainty factors transparent?

11:45AM	**Observer Comment Period #1**

12:00PM	**Lunch**

1:00PM	**Discussion Session (continues, w/ breaks as appropriate)**

3)	RfC derivation

a) No RfC has been developed in this assessment due to lack of adequate toxicity data for the inhalation route of exposure. Does the assessment appropriately addresses toxicity of mirex via the inhalation route of exposure?

b) Does the assessment appropriately addresses toxicity of Mirex via the inhalation route of exposure?

4)   Cancer Weight-of-Evidence Characterization and Quantitative Assessment

        The weight of evidence characterization and quantitative estimation (oral route) have
        been discussed in Chapters 4 and 5 of the Toxicological Review document and to a
        limited extent in the IRIS summary document.

        a) Have the appropriate criteria from the U.S. EPA 1999 draft revised Guidelines for
        Carcinogenic Risk Assessment document been applied?

        The available information on toxicity of mirex is discussed in the Toxicological Review
        and in the IRIS summary documents. The 1999 draft revised Guidelines for Carcinogen
        Risk Assessment state that a linear dose-response approach should be taken when the
        mode of action information is supportive of linearity or mode of action is not understood.
        In the absence of adequate data in support of mode of action for carcinogenesis at
        dosages higher than the non-cancer events this assessment presents a non-linear Dose-
        Response model for carcinogenic assessment.

        b) Are the tumors observed biologically significant? Are the tumors observed relevant to
        human health? Points relevant to this determination include whether or not the choice
        follows from the dose-response assessment, whether the effect is considered adverse, and
        if the effect (including tumors observed in the cancer assessment) and the species in
        which it is observed is a valid model for humans.

        c) Was the mode of action section presented clearly and logically? Are there any
        additional studies that would enhance the mode of action information presented in the
        Toxicologic Review? Based on the mode of action information in the Toxicological
        Review and IRIS summary as well as the 1999 draft revised Guidelines for Carcinogen
        Risk Assessment, is linear dose-response modeling for cancer assessment appropriate for
        Mirex?

3:30PM   **Observer Comment Period #2**

3:45PM   **Recap of Comments and Recommendations**

4:30PM   **Closing Remarks and Adjourn**

APPENDIX D

Peer Reviewer Comments

**Appendix D
Peer Reviewer Comments on Mirex
Organized by Charge Question**

General Comments

Harvey Clewell

The overall document quality is very good, and the discussions of the toxicological studies appear to be thorough, balanced, and informative. My only major concern is the cavalier way in which the developmental endpoints were dismissed. I do not believe that liver toxicity is necessarily the critical noncancer effect for mirex. Increased incidence of cataracts in nursing pups and failure to thrive at weaning occurred at a dose of 0.4 mg/kg/day in two different studies that dosed throughout the entire period of lactation. Other developmental studies that showed these effects only at higher doses involved shorter-term exposures during only a portion of the lactational period. These affects are clearly associated with exposure through lactation, and as such should be of special concern for children's health. Benchmark dose modeling should have been performed for cataracts in the Chu study for comparison with the benchmark doses for liver toxicity.

Other concerns are listed below.

1. It is hard to believe there is no data on the clearance half-life of mirex in the human. This information is critical for assessing correct cross-species dosimetry. It appears that the half-life in the rat and monkey could be characterized from studies cited in section 3.4.

2. The benchmark modeling of the liver toxicity should have evaluated the impact of throwing out the highest dose (or even the highest two doses). The saturation of response appears to confound the ability of the dose-response models to adequately fit the low dose region of concern for the BMDL.

3. The cross-species scaling used in the cancer dose-response assessment should be specifically described. It is not on p.53 or in Section 5.1.2 (as suggested on p.53). It appears from the IRIS summary that body weight to the three quarters scaling was used. This should be stated on p.53 as well.

Michael Dourson (and TERA Staff)

1. The authors state several times that the possible modes of action are not sufficient to warrant a move away from the linear approach. This is an easy statement to make, but one that is not satisfying scientifically, since criteria are never discussed that would show what additional data are needed to support the choice of a nonlinear MOA. Another approach is to make a judgment as to the likeliness of the linear response of the tumor endpoint given the data in hand. This seems to be much easier, since none of the

available data would suggest a linear response; in fact the data strongly suggest a nonlinear response. Dr. Gadagbui comments on various MOAs more extensively in an attachment.

2. The data base uncertainty factor of 3 is not well supported with the available write up. I suggest that the authors investigate the available data from the point of view of whether the rat "children" are sufficiently tested for the likely critical effect, and afterwards make a judgment whether this factor is needed. In addition, further clarification could have been used for some of the cancer and noncancer modeling descriptions, as Mr. Hack further explains in an attachment

Anna Fan

The document is well prepared. It covers the pertinent aspects generally included in a toxicological review document, and follows U.S. EPA guidelines in specific sections as appropriate.

The information presented seems accurate, including data on toxicity, metabolism, and exposure. A separate section on endocrine effects would be good. It would also help to have the mode of action discussions shown all in one place.

The approach used in developing the RfD and the methodologies used for carcinogenicity data assessment seems appropriate, including interpretation of carcinogenicity data and mode of action (or lacking thereof of a known mode of action).

The scope and level of detail are generally appropriate. The tables summarizing the pertinent data are very informative and helpful.   The discussion in the text and major conclusions are generally supported by data presented in the tables. Tables 6 and 7 need clarification. In particular, the reproductive/developmental effects need additional review and evaluation regarding two specific studies (see #3 below, under "Specific Observations"), those by Gaines and Kimbrough (1970) and Chu et al. (1981a) and relevant studies as appropriate.

For data evaluation and interpretation, the data set and supporting information in the dose-response assessment seems appropriate, based on the data provided in the document, and the data support the conclusions. For identification of key studies, the final determination would have to depend on the outcome of the additional review and evaluation of the studies of Gaines and Kimbrough (1970) and Chu et al. (1981a), and relevant studies, as noted above.

The overall synthesis and evaluation sections are very helpful. The titles of the sections on overall synthesis can be slightly simplified.

Bonnie Stern

This is a well-written Toxicological Review, with study summaries clearly and comprehensively described and data well integrated. The rationale for decision-making in terms of study selection, inadequate data to derive a RfC, and cancer classification is reasonably well explained.

It would be helpful to the reader if, at the conclusion of key study summaries, a brief statement regarding the study authors' conclusions, with accompanying NOAELs and LOAELs be given in the hazard characterization section. Similarly, I suggest that the findings presented in each of the key hazard identification sections (e.g., Toxicokinetics, Prechronic and Chronic Studies and Cancer Bioassays, and Reproductive/Developmental Studies) be summarized in one or two short paragraphs at the end of each of these sections. A lot of data are presented, and brief summaries are useful in describing and integrating the range of adverse effects within a major section in a succinct and comprehensive manner.

Response to Charge Questions

1) Overall document quality

Please prepare a statement that addresses the overall quality of the document(s) and provide advice on approaches to improve the assessment from both technical and communication standpoint; and provide suggestions on the integration of data into an overall characterization of hazard. Questions to consider include:

a) Is the document logical, clear and concise? Are the arguments presented in an understandable manner?

Anna Fan

a. The document provides a logical review and presentation of relevant information. The information is clear and concise and the argument generally presented in an understandable manner. There is a need for an additional review and evaluation of the reproductive/developmental toxicity data. See above and # 3 below, under "Specific Observations".

Karl Rozman

ad.1 The document reads well, its structuring is similar to other documents dealing with hazard identification and dose-response assessments of different chemicals. This is at the same time the "problem" with this document because mirex is very different from most chemicals because of its long half-life (lack of biotransformation) and hence its kinetics

represent the rate-determining step in its toxicity. This is not at all clear from the way this document has been written.

Bonnie Stern

(a) The toxicological review for Mirex provides a comprehensive and well-written summary of the range of effects of mirex. Overall, the document is logically, clearly, and concisely written, sufficient information is provided on each of the studies to provide a complete toxicological "profile" of the study. However, as noted above, a summary at the end of each of the key sections would be useful in pulling together major findings. For example, in the toxicokinetics section, one or two sentences on absorption, distribution, metabolism (or lack thereof), excretion, and placental/lactational transfer of mirex would present a short and concise overview of major toxicokinetic characteristics.

Although studies and statistical significance are well described, for many studies, the toxicological significance of the findings of some key studies are not discussed until Section 4.5. The toxicological significance, and possible reasons for inconsistencies in results (e.g., in the developmental toxicity studies), should be discussed in the context of each key study or group of studies.

Specific Comments:

Chronic Toxicity and Cancer Bioassays:

In studies of chronic toxicity and cancer, a range of tumors were observed in the NTP study (1990, p. 17), including increased incidences of pheochromocytomas of the adrenal glands and of mononuclear cell leukemia. However, results are internally inconsistent, for example, in female rats, increased adrenal gland tumors were observed at 3.9 mg/kg/day in the first study, but not at 3.9 mg or 7.7 mg/kg/day in the second study (p. 17, lines 36-39). Similarly, inconsistencies were found with mononuclear cell leukemia (incidences were significantly elevated in male rats at 1.9 mg/kg/day but not at 3.9 mg/kg/day, and in females at 1.9 and 3.9 mg/kg/day in the first study but only at 7.7 mg/kg/day in the second study (i.e., not at 3.9 mg/kg/day)). Table 2 reports a statistically significant increase in leukemia also at 0.7 mg/kg/day, and this should be noted in the text. When the results of both studies are combined, statistically significant elevations of mononuclear cell leukemia are reported to occur at 0.7 mg/kg/day and above (p. 18, lines 1-3). However, the toxicological significance and possible reasons for these inconsistencies are not discussed. Adrenal tumors and mononuclear cell leukemia are commonly occurring cancers in aging F344 rats and statistical significance was only evaluated relative to concurrent controls. NTP has a large data base of historical control tumor rates. These data should be discussed in the context of historical, as well as concurrent, control rates. If the increased incidences of these cancers are within historical control rates, then they would not be considered toxicologically significant.

With respect to non-neoplastic lesions in the NTP (1990) study, the following findings are reported: parathyroid hyperplasia in male rats, nephropathy in female rats, epithelial

hyperplasia of the renal pelvis in male rats, and splenic fibrosis in male rats. Nephropathy, a common occurrence in aging F344 rats is discussed following the listing of the nonneoplastic effects (p. 21, lines 1-6), and although the severity scores are somewhat higher in treated animals relative to controls, examination of these scores does not suggest treatment-related statistical significance. On p. 44 (lines 27-28), this interpretation is noted in the report; it should be repeated on p. 21. Similarly, with regard to parathyroid hyperplasia, it is not until p. 44 (lines 30-33) that the report mentions that the NTP study authors concluded that "parathyroid hyperplasia is likely a secondary physiological response to nephropathy", and thus appears to be unrelated to exposure. This information should also be presented immediately following the listing of nonneoplastic effects. Otherwise, the reader is given the impression that these findings are treatment-related and toxicologically significant, when in fact they are not.

The discussion of liver neoplastic nodules, and independent pathology evaluation as well as U.S. EPA-sponsored reviews and recommendations is well described (p. 21-23), and provides the basis for the quantitative cancer assessment. The paragraph on beginning on p. 21 (line 35) and ending on p. 23 (line 31) needs a conclusion. Although some animals with adenomas did not exhibit nonneoplastic lesions, this finding in no way invalidates a cytotoxicity "mode of action" for the development of liver tumors in Mirex-treated animals. Other subcellular changes (e.g., increased cell proliferation, increased DNA synthesis) not measured in the study may have been occurring in these animals.

On p. 27, the rhesus monkey study summary (Fulfs et al., 1977) also needs a conclusion. Doses administered to the monkeys were lower than those at which neoplastic effects were observed in rodent studies. Further, the exposure duration (26 months) was quite short relative to the normal lifespan of these monkeys. It is possible that exposure duration was not long enough for tumors to develop. These are important points; otherwise the reader is left with the impression that nonhuman primates appear to be less sensitive to the tumorigenic effects of Mirex than rodents and that results from rodent studies may overestimate potential human cancer risks. This line of reasoning would call into question the human relevance of the rodent studies.

Reproductive/Developmental Effects

These studies are in general well reported and well described. However, on p. 28 (line 17), it is stated that a 2-generation reproductive toxicity study was conducted by Gaines and Kimbrough(1970). Based on the description of this study, it was not a 2-generation study, according to standard test guidelines, but rather a series of single-generation studies in which timing of treatment with respect to gestation and/or lactation was varied to determine the critical period of cataract formation. Further, in the development of the RfD, an uncertainty factor of 3 is applied to the LED10 to account for data base deficiencies, including "lack of a 2-generation reproductive toxicity study". Therefore, this statement is erroneous.

Cataract formation in offspring of mothers exposed to Mirex during gestation and lactation is not comprehensively addressed. There are inconsistencies in the studies

performed that evaluated this endpoint in terms of LOAELs and NOAELs; however, possible reasons for these inconsistencies are not well discussed. These may include, differences in study design/protocol, rat strain, percent of offspring with cataracts, dosing regime (in studies with the lowest NOAELs for cataract formation, (Gaines and Kimbrough, 1970, and Chu et al., 1981b), dams were dosed prior to mating as well as during gestation and lactation, whereas in the other studies, dams were dosed either during gestation and/or lactation, and/or pups were cross-fostered following birth. When dosing during gestation only is compared with dosing during lactation only, cataract formation is higher in the lactation-only groups. However, when dosing occurred during premating, gestation, and lactation, there was also a high incidence of cataract formation in the offspring (p.28). Although the cross-fostering experiment suggested that the critical window for cataract development was during lactation, the administered dose of Mirex was 5 ppm, which was the NOAEL for cataract formation in the long-term exposure study (premating, gestation, lactation).These findings suggest that although lactation may be a critical window, there may be other periods of development that also increase the incidence of cataract formation. Further, it raises the question as to why cataract formation was not seen in the longer-exposure study at 5 ppm. A more thorough discussion of these inconsistencies is needed, both in Section 4.3.1 and in Section 4.5. These findings are also important in highlighting the need for a multi-generation reproductive toxicity study.
P. 35, beginning line33, - p.36. Studies by Yarborough et al. (1981) and Chu et al. (1981b) show effects on sperm counts and testicular damage. Some discussion interpreting these findings is needed, particularly with regard to the relationship between decreased sperm count and fertility..

b) Are you aware of any other data/studies that are relevant (i.e. useful for the hazard identification or dose-response assessment) for the assessment of the adverse health effects, both cancer and noncancer, of this chemical?

Anna Fan

See #3. Under "Specific Observations".

Bonnie Stern

No.

2. RfD derivation

a) Principal Study, Section 5.1.1: The RfD is based on a chronic rodent study in which rats were exposed to Mirex in the diet for 104 weeks (NTP 1990). The critical effect observed was toxic hepatitis. The principal study should present the critical effect in the clearest dose-response relationship. Is use of the NTP (1990) study as the principal study justified and is the rationale for this study adequately explained in the Toxicological Review?

Anna Fan

The principal study chosen (i.e., the NTP study, 1990) for the RfD derivation may or may not be justified, depending on the outcome of the additional review and evaluation of the studies of Gaines and Kimbrough (1970) and Chu et al. (1981a), and relevant studies, as noted above, and below, under "Specific Observations, #3". The NTP study was well designed, conducted and reported and adequately explained in the Toxicological Review document. The Toxicological Review document indicated that the NTP study (with the liver histopathological slides) was further re-evaluated by a working group (Pathology Working Group, PWG) convened by Pathco, Inc. (1992), and provided the summary results of the PWG. The PWG report itself was reviewed by another USEPA sponsored reviewer (USEPA 1999) who concluded that the re-evaluation was appropriate and the re-evaluated rat liver tumor incidence data were valid for risk assessment. Non-neoplastic changes were also reclassified. The current review is based on the data from the Toxicological Review document.

Other studies on various endpoints and of varying exposure and study duration were reviewed and discussed. To more adequately address the issue of justification, addressing #3 under "Specific Observations" is needed.

Karl Rozman

2 a) The choice of the NTP(1990) study as the principal study to base the RfD on is a correct one as is the endpoint of toxicity (toxic hepatitis) if cancer drives the risk assessment.

Bonnie Stern

(a) and (b) Yes. The principal study and critical effects are appropriate. However, Section 5.1.3 would benefit from (1) a more thorough discussion of the rationale for selection of this study; including a brief paragraph summarizing the study protocol and findings, including LOAEL and NOAEL; and (2) an expanded discussion of other studies demonstrating toxicity in other target organs, particularly those showing developmental toxicity (decreased pup survival and cataract formation) and effects on male reproductive parameters, integrating findings. These are end points of concern. A weight-of-evidence approach should be more clearly organized articulated.

As noted in the previous paragraph, more detail should be presented on the critical effect, and its toxicological significance. The paragraph on human relevance is well written and covers the major points regarding human relevance.

b) Critical Effect, Section 5.1.1: The critical effect is identified as toxic hepatitis. Has the most appropriate critical effect for Mirex been chosen (i.e. that adverse effect appearing first in a dose-response continuum)? Has the critical effect been adequately described? Is this critical effect biologically significant? Finally, does the information

presented from animal studies mirror what is known about the toxicity in humans and is this information adequately described?

Anna Fan

The most critical effect (liver toxicity, further described as dose-dependent hepatic changes, and toxic hepatitis) may or may not be appropriately chosen and adequately described, depending on the outcome of the additional review and evaluation of the studies of Gaines and Kimbrough (1970) and Chu et al. (1981a), and related studies, as noted above, and below, under "Specific Observations, #3". As it currently stands, the reproductive/developmental effects (e.g., cataracts) seem to be a more sensitive indicator and the most critical effect, but inadequate details were provided in the draft Toxicological Review document to permit a more detailed review and a more definitive determination. It is necessary for the author(s) to take the data and go through the procedure and general assumptions used to derive a RfD for reproductive/developmental effects, and compare it with the RfD derived based on toxic hepatitis. In the re-evaluation process, pharmacokinetics consideration needs to be incorporated, explained and justified.

On the basis of the existing review as written in the draft Toxicological Review document, liver toxicity reported in the study chosen (NTP 1990) (and further re-evaluated) is biologically significant, and further supported by numerous other studies reported in the literature. The hepatic changes included both adaptive and toxic effects both of which have been well characterized. Liver toxicity was observed after acute, intermediate and chronic oral exposures in the experimental animals. The specific effects might be different depending on the duration of exposure (e.g., effects on hepatobiliary function after acute exposure vs. histopathological changes such as fatty degeneration, necrosis, hyperplasia, periportal fibrosis, hepatocytomegaly, and sinusoid dilation after chronic exposure). Quantitatively, a dose level of 0.7 mg/kg-d and higher produced statistically significant increased incidences of non-neoplastic lesions in the liver following oral chronic exposure. The text discussion was consistent with the data provided in the table (based on PWG evaluation). Following the BMD analysis, a LED10 value was obtained, and the use of 300 for a total uncertainty factor was appropriate.

There is limited information on the toxicity of mirex in humans. Mirex is no longer manufactured or used in the U.S. The most likely way for exposure in the general population is from food intake, including fish, wild game, and meat, but particularly from fish taken from contaminated areas. In the absence of more human data and of other evidence indicating otherwise, it is reasonable to assume that the effects seen in animals may also occur in humans.

Karl Rozman

(b). Toxic hepatitis is the most sensitive chronic endpoint of an effect for mirex. Whether or not this is an adverse effect or not will remain controversial for some time (see Borman et al.'s Society of Toxicologic Pathology Position Paper, Assessment of

Hyperplastic Lesions in Rodent Carcinogenicity Studies, Toxicol. Pathol. 31:709-910, 2003). The effect is adequately described. I am not aware of human data on intoxication with mirex. Environmental and occupational exposure did not result in toxic hepatitis. The study by Fulf et al. (1977) indicates that primates may be less sensitive to mirex than rodents because female rhesus monkeys dosed with 0.25 or 1 mg/kg of mirex 6 times a week for 36 or 26 months appeared to have normal livers except for occasional focal lymphocytic infiltration although their body burden was at least as high if not higher as that of rats fed a diet corresponding to a dose of 0.7 mg/kg for 104 weeks.

c) Is the RfD determined for Mirex protective of adverse health effects in the general population and in sensitive sub-populations such as children and pregnant women?

Anna Fan

Information from the literature did not identify any human population known to be especially sensitive to mirex. However, based on data especially on animals, some sub-populations can be identified that may be potentially more sensitive to mirex exposure and health effects. These include the following:

> Ingestion of parental milk is a source of exposure. Limited data showed that mirex was found in the milk of women who live in certain contaminated areas, so associated nursing infants could be exposed. But most of the studies were directed towards determining body burden of chlorinated pesticides and not associated health effects. Mirex was excreted via milk in nursing rats and was found in maternal milk, and milk sample taken from pups' stomachs, in pup brain and liver tissues. Mirex was also found to be absorbed transplacentally as it was detected in maternal plasma, liver, and kidneys, and in fetal placenta, brain, heart, and kidneys following administration to pregnant or nursing animals. It was found in the milk of cows, rats and goats after oral dosing. But none have been reported to be at a harmful level.

> Young animals or juvenile rats immediately after birth were reported to be more sensitive to the effects on the nervous system (trembling, tiredness and weakness after short-term, high level mirex exposure).

> The very young represent a population especially susceptible to cataracts (diffuse anterior corneal opacities, lens with increased water and sodium content relative to potassium content). Exposure to mirex before or soon after birth in animals may cause cataracts in animals. Exposure during the first few days in life appears to be critical to the development of cataracts.

> Short-term low-level exposure to mirex may affect reproduction and development in animals. The lowest LOAEL was 0.4 mg/kg-d (e.g., for cataracts), but no NOAEL was established in the Toxicological Review document.

> High-level exposure to mirex may cause miscarriage in animals.

Pregnant rats appeared to be somewhat more sensitive to the lethal effects of mirex.

Based on quantitative assessment of available and relevant data, the RfD derived based on liver toxicity (toxic hepatitis) reported in the NTP (1990) study (and re-evaluated) is anticipated to be adequately protective of these potentially sensitive sub-populations. But as it currently stands, the reproductive/developmental effects (e.g., cataracts) seem to be a more sensitive indicator and the most critical effect, yet inadequate details were provided in the draft Toxicological Review document to permit a more detailed review and a more definitive determination. To ensure protection of the sensitive sub-populations, addressing #3 under "Specific Observations" is needed. It is necessary for the author(s) to take the data and go through the procedure and general assumptions used to derive a RfD for reproductive/developmental effects, and compare it with the RfD derived based on toxic hepatitis. In the re-evaluation process, pharmacokinetics consideration needs to be incorporated, explained and justified.

Karl Rozman

(c) Toxic hepatitis was present in 75% of rats dosed with heptachlorodibenzo-p-dioxin, which lived two months longer than controls without increased cancer incidence (Rozman et al. 2004). Therefore, and because of lower sensitivity of primates the RfD will be protective for even the most vulnerable population, if based on the NTP (1990) study.

Bonnie Stern

c) Given that "toxic hepatitis" occurs at lower doses than other effects, the RfD derived from this end point would be considered to be protective of adverse health effects in the general population and in sensitive sub-populations such as children and pregnant women. However, there is a discrepancy between the LOAEL reported for decreased sperm counts in male rats in Section 5.1.1 (0.5 mg/kg/day) versus that reported in Section 4.2.1 (p. 12, line 31, where it is reported as 0.05 mg/kg/day (Yarborough et a l., 1981). In Section 4.5.1 (p.46, line 12), the LOAEL is also reported as 0.5 mg/kg/day. This value needs to checked and appropriate correction made. Yarborough et al. (1981) was a 28-day study and so would not be considered of sufficient exposure duration for derivation of an RfD. However, if the correct value is 0.05 mg/kg/day, then additional discussion is needed regarding this endpoint.

d) Methods of Analysis, Section 5.1.2: Benchmark Dose Modeling (BMD) was applied to the chronic study for Mirex. Was the point of departure determined appropriately for this approach? Is the 10% response level appropriate and is the use of this response level supported adequately?

Michael Dourson (and TERA Staff)

Comments by Mr. Eric Hack on dose-response for non-cancer effects.

Using the data in appendix B, I reproduced the estimates of the ED10 and LED10 and I agree that the log-logistic model is the appropriate selection from the BMDS suite of models. The definition of the ED10 should say that it is the dose associated with a 10% **increase** in incidence. Use parenthesis in the definition of extra risk to make the order of operations clear. That is, (incidence – background) / (1- background) and $(P_{(d)} - P_{(0)}) / (1 - P_{(0)})$.

Anna Fan

If the NTP data were to be used in the final determination, then use of the BMD method of analysis is appropriate. The points of departure were determined based on male and female rat data, whereas the text presented data for males and females and then used averaged doses for males and females to discuss the findings. The LED10 values obtained for the males and females were similar and they were then averaged and taken as the point of departure for the derivation of the RfD. The 10% response level (for toxic hepatitis) seems appropriate. The document can discuss more regarding how adequately this is supported.

Karl Rozman

(d) I do not have any problem with BMD as a curve fitting tool although a NOAEL or LOAEL is just as good. I disagree with the use of cookbook type uncertainty factors although they seem to yield a similar number for mirex as kinetic calculations, except they are unscientific in contrast to kinetics.

Bonnie Stern

(d) Yes, the POD was determined appropriately. A 10% response level for derivation of the LED is appropriate. However, the rationale for selection of a 10% increased response level relative to background, could be expanded.

e) Uncertainty Factors, Section 5.1.3: Are the appropriate uncertainty factors used to develop the RfD? Are there other data which should be considered in developing the uncertainty factors? Is the explanation for the selection of each of the uncertainty factors transparent?

Bonnie Stern

(e) The uncertainty factors are appropriate but insufficient rationale to support their application is presented. In particular, UFs extrapolating from rats to humans and accounting for within human variability should be discussed in terms of toxicokinetics

and toxicodynamics. Further, data base deficiencies should be more comprehensively addressed. Lack of a 2-generation reproductive toxicity study is a major data base deficiency, because of concern for adverse effects in the F2 generation, given findings in the single-generation studies. Additional data base deficiencies include a lack of information on possible mode(s) of action of Mirex-induced toxicity, and very limited data on potential neurotoxicity. However, a data base deficiency UF of 3 is judged to be appropriate.

3) RfC derivation

(a) No RfC has been developed in this assessment due to lack of adequate toxicity data for the inhalation route of exposure. Does the assessment appropriately addresses toxicity of mirex via the inhalation route of exposure?

b) Does the assessment appropriately addresses toxicity of Mirex via the inhalation route of exposure?

Anna Fan

Mirex can enter the body via inhalation, ingestion and via the skin. There is very limited information on exposure and health effects of mirex in humans following inhalation and this is stated in the document. There is no data from other routes that could be used for extrapolation to inhalation exposure.

Mirex does not dissolve easily in water or evaporate easily in the air, so people are not likely to be exposed to the chemical by drinking water (including showering) or by inhaling air. The pertinent issues are addressed and it is appropriate not to derive an RfC.

Karl Rozman

ad. 3   For a compound with a vapor pressure of 3×10^{-7} mm Hg it would be meaningless to derive a RfC. Inhalation exposure could only occur if mirex-containing dusts were inhaled. I am not aware of data to support the notion of exposure by inhalation. For dioxin, it was assumed that only 1% of body burden was derived from inhalation of dusts.

Bonnie Stern

Yes. The rationale given for considering the data inadequate for derivation of an RfC is well presented.

4) Cancer Weight-of-Evidence Characterization and Quantitative Assessment

Anna Fan

The data for evaluation of carcinogenicity and risk assessment are based on the re-evaluation by the PWG (1992) of the NTP (1990) study. It was noted that the PWG used more current criteria and terminology than those used in the original NTP pathology report. For neoplastic nodules, these were reclassified as hepatocellular adenomas, esosinophilic foci of cellular alteration, or regenerative hyperplasia. This reevaluation reported statistically significant increases in the incidence of hepatocellular adenomas in male rats treated with mirex at 1.9 and 3.9 mg/kg-d and in female rats in the second study at 3.9 and 7.7 mg/kg-d. Thus 1.9 mg/kg-d was indicated as the lowest dose level that induced a significant tumor response in the reevaluation, whereas the original NTP report showed 0.7 mg/kg-d as the level that resulted in a significant increased incidence of "neoplastic nodules". Hepatocellular carcinoma incidence was not shown to be affected by mirex treatment regardless of criteria and terminology used in the pathological examination.

Several other studies were considered for carcinogenicity evaluation. The study of Innes et al. (1969) administered mirex to mice in capsule by gavage (7-28 days) and then in diet at an estimated dietary dose of 7 mg/kg-d for the rest of their lifetime. The incidences of hepatomas were increased by treatment and this was at a higher level (and a maximum tolerated dose in the study) than the lowest effect level in the NTP study. No data were given on the incidence of non-neoplastic lesions by the authors. In the study of Ulland et al. (1977), rats were given mirex in the diet at timed weighted doses of 4 or 7 mg/kg-d for males and 4 or 8 mg/kg-d for females for 18 months, and further observed for 8 months. Statistically significant increased incidence of hepatic neoplastic nodules occurred only in the high dose male group. Non-neoplastic lesions occurred in all groups. Both studies produced findings that corroborated with the findings of effects in the livers of animals treated with mirex, but these are inadequate for use in risk assessment compared with the NTP study. Two other chronic studies were considered but were determined not useful for risk assessment because of the limited focus on the liver and a lack of reporting on experimental animals and incidence data (Fulfs et al., 1997), or the small number of animals used plus a lack of reporting on incidence data and statistical significance (Abraham et al., 1983).

Karl Rozman

ad. 4   I strongly disagree with the weight of evidence and quantitative risk assessment characterization. Mirex is not genotoxic, it does not have reactive metabolites, it has no strong binding affinity to any protein or any other biological structure other than limited solubility in triglycerides. Mirex has been shown to be a promoter and also that it perturbs signaling (hormonal) pathways, which is the hallmark of promoters.

a) Have the appropriate criteria from the U.S. EPA 1999 draft revised Guidelines for Carcinogenic Risk Assessment document been applied?

Anna Fan

a. Overall, the available data provided evidence of carcinogenic response in the livers following oral lifetime exposure in rats and mice. Mirex is determined to be Likely to be Carcinogenic to Humans. The criteria from U.S. EPA 1999 draft revised guidelines for carcinogenic risk assessment has been applied.

Karl Rozman

a. The U.S. EPA 1999 draft revised Guidelines have been misapplied.

Bonnie Stern

(a). Yes. Please note though that U.S EPA's 1999 Cancer Risk Assessment Guidelines are no longer considered "draft" guidelines. It is also my understanding that the 1986 guideline are no longer reported in Toxicological Reviews and IRIS summaries.

b) Are the tumors observed biologically significant? Are the tumors observed relevant to human health? Points relevant to this determination include whether or not the choice follows from the dose-response assessment, whether the effect is considered adverse, and if the effect (including tumors observed in the cancer assessment) and the species in which it is observed is a valid model for humans.

Anna Fan

b. In the absence of data that indicate otherwise, the tumors observed can reasonably be assumed to be biologically significant and relevant to human health.

Karl Rozman

b. Tumors are always biologically significant although sometimes they are not relevant for human health. Rhesus monkeys are probably a better model for dose-response assessment than are rodents but it is my opinion that given high enough accumulation of body burden humans would also respond similarly to primates and rodents. The only reason for species differences resides in differential kinetics of mirex.

Bonnie Stern

(b) In the absence of human data, human relevance is assumed, according to cancer guideline default assumptions. The consideration of these tumors as both biologically significant and relevant to human health has been evaluated by an independent pathology working group, and EPA-sponsored reviews, both external and internal. Therefore, interpretation of these findings as biologically significant and of human relevance is consistent with current EPA science policy with regard to cancer hazard characterization and risk assessment.

c) Was the mode of action section presented clearly and logically? Are there any additional studies that would enhance the mode of action information presented in the Toxicologic Review? Based on the mode of action information in the Toxicological Review and IRIS summary as well as the 1999 draft revised Guidelines for Carcinogen Risk Assessment, is linear dose-response modeling for cancer assessment appropriate for Mirex?

Michael Dourson (and TERA Staff)

TERA Comments by Mr. Eric Hack

Comments on dose-response for cancer effects.

An appendix such as the one shown for the non-cancer dose-response would have been very helpful.

Linear is the way to describe the extrapolation from the point of departure to the origin. The way it is worded, it might be inferred that the models used were linear. These are nonlinear dose-response models down to the point of departure, and then a linear extrapolation to the origin is performed.

Do you mean the Multistage model when you say an exponential polynomial function of dose? $P(d) = 1 - \exp(-q0 - q1{*}d - q2{*}d^2 - \ldots - qn{*}d^n)$.

The conversion to a human equivalent dose is not discussed in Section 5.1.2.
I could not reproduce the LED10 of 0.193 mg/kg/day or the q1* of 0.53 per mg/kg/day with the 3-stage model, though it says a three-degree polynomial form of the dose function provided the best fit to the data. Was it computed using the time-to-tumor model? This should say 0.2 mg/kg/day because your doses contain only 1 significant figure at most levels.

After scaling the doses by $bw^{(3/4)}$, I was able to reproduce the ED10, LED10, and risks associated with 1e-4, 1e-5, and 1e-6 using BMDS and a probit model. However, it is not clear that this is the most appropriate model from the BMDS suite. The overall fit is best if you look at the p-value and the AIC. But the Gamma Multi-Hit model, for example, appears to fit better in the range of the BMD, and the AIC is very similar (207 -vs- 206). The human equivalent BMDL from the Gamma model, as well as most of the others, is 0.3 mg/kg/day, which is lower than the BMDL from the probit model.

I did not reproduce the time-to-tumor modeling results.

TERA Comments by Dr. Bernard Gadagbui

The EPA classified mirex as "likely to be carcinogenic to humans" by the oral route based on increased incidence of liver tumors in two strains of each of two species, rat and mice, and less consistent findings of tumors in other tissues of both rats and mice.

On page 21, paragraph 3, re-evaluation of the principal NTP (1990) study established 1.9 mg/kg/day as the lowest dose level that induced significant tumor response in male rats. Chronic exposure to mirex at 1.9 mg/kg/day resulted in tumor responses including hepatocellular adenomas in male rats, pheochromocytoma or malignant pheochromocytoma in male rats, and mononuclear cell leukemia in male and female rats (Tables 2 and 4). Rats of both sexes exposed to 3.9 mg/kg/day had significantly elevated incidences of liver adenomas, benign or malignant pheochromocytomas, and combined liver adenomas and carcinomas. However, there was no statistically significant incidence of liver carcinomas in any animal at 1.9 mg/kg/day or higher. There is a strong association only between the liver adenomas and non-neoplastic liver lesions (toxic hepatitis – centrilobular hepatocytomegaly, centrilobular fatty change, apoptosis, centrilobular necrosis, and bile duct proliferation) at 1.9 mg/kg/day and above. However, some animals with adenomas did not exhibit non-neoplastic liver lesions. [As stated on page 47, the liver tumors were mostly benign in F344/N rats at 1.9 mg/kg/day and above (Table 4). On the other hand, out of 26 CD rats one male, 4 males and 1 female at 4 mg/kg/day, 7 mg/kg/day, and 8 mg/kg/day, respectively, had liver carcinoma that was concurrent with foci or areas of cellular alteration (characterized by hepatocyte enlargement and cytoplastic vacuolation with a finely granular eosinophilic material; occasional degeneration was also observed). Therefore, the statement (page 47) that the tumors are of equal mix of benign and malignant in CD rats may be more applicable to the higher doses (> 4 mg/kg/day). Given that the lowest dose to which the CD rats were exposed was 4 mg/kg/day, it is impossible to predict whether liver carcinoma occurred in CD rats below this dose level.

Other statistically significant neoplastic responses that were not strongly associated with mirex exposure and therefore not exposure-related included incidence of transitional cell papillomas of the renal pelvis in male rats.

Based on the incidences of the liver adenomas at 1.9 mg/kg/day and above, the EPA took a linear low-dose extrapolation approach for the quantification of human cancer risk for mirex, an approach that is in line with EPA guidance for treating DNA non-reactive chemicals whose mode of action is not established.

While mirex appears to be a hepatocarcinogen in rats and mice, sufficient data have been presented and accepted by the EPA that it is not mutagenic in both in vitro and in vivo assays. This suggests that mirex may produce carcinogenic responses via a non-genotoxic mode of action that may involve mirex-induced hepatic cell proliferation. Several hypotheses have been tested and advanced for the mirex-induced tumorigenic responses. One hypothesis is that mirex may cause liver tumors via promotion of previously initiated cells. A 2-stage mouse skin model provided an indirect support for this hypothesis (Kim and Smart, 1995; Kim et al., 1997; Morse et al., 1992, 1993). Another hypothesis is that the mirex-induced liver growth is composed of hypertrophic (enhanced growth of existing cells) and hyperplastic (enhanced cell division) components that may involve interaction with endocrine systems (Yarbrough et al., 1984). Studies on adrenalectomized male rats given corticosterone supplemented and thyroidectomized rats given thyroxine

(T4) supplement appeared to support this hypothesis. A third hypothesis involves mirex induction of ornithine decarboxylase (OD). This enzyme is the first in the biosynthetic pathway for polyamines that play roles in regulation of various cell functions and metabolism. Oral exposure to mirex induced the hepatic enzyme whereas it failed to induce the epidermal enzyme at a dermal dose level that strongly promoted mouse skin tumor in previously initiated cells; a phorbol ester at tumor promoting dose levels induced the epidermal enzyme. A fourth hypothesis is that mirex is preferentially cytotoxic to tetraploid and octaploid hepatocytes, which is the key phenomenon in the induction of mirex hepatotoxicity and carcinogenicity (Abraham et al., 1983). Although tests in rats showed a dramatic change in ploidy pattern in liver tissue adjacent to carcinomas and in carcinomas, this hypothesis is not relevant to humans in that hepatocytes in primates and humans are predominately diploid (>99%). In addition, a 3-year dietary study in monkeys failed to produce histopathological changes in liver sections, thereby suggesting that mirex may be less hepatotoxic to primate and humans than to rodents. Although these hypotheses are plausible, there exists a data gap on the actual mechanism or mode of action of mirex.

Mirex also causes cataracts in offspring of female rats, produces developmental effects at higher exposure levels during gestation, and impairs reproductive functions in male rats or female rats. However, the mechanisms or modes of action for these effects are also not understood.

The evidence for genotoxic mode of action involving non-neoplastic liver cell changes as precursor events, as acknowledged also by the EPA, is stronger than the evidence of a genotoxic mode of action without a threshold (see page 48).

Toxicokinetic studies may contribute to mode of action analysis if an active form of an agent is identified that is central to the mode of action. Metabolic studies in animals failed to identify potentially reactive mirex metabolic intermediates that may be genotoxic. This provides further evidence that mirex is likely to cause liver tumor via a non-genotoxic mode of action with a threshold. However, a structurally related compound, chordecone (i.e. kepone) produces positive results in at least one of the mutagenicity assays (induces sister chromatid exchanges in CHO - Chinese hamster ovary – cells) and also induces liver tumors (hepatocellular carcinomas) in rats and mice (NCI, 1976). This is one of the criteria that EPA uses to 'support' a linear-dose response modeling of a DNA non-reactive chemical whose mode of action is not well known.

It appears that EPA is convinced that some of the hepatocellular adenomas in mirex-exposed rats in the NTP (1990) bioassay were diagnosed without non-neoplastic liver lesions. On pages 23 and 52, the document indicated that at 1.9 mg/kg/day, 4/6 male rats exhibited both adenomas and toxic hepatitis or eosinophilic foci but 2 did not, while at 3.9 mg/kg/day, 8 had adenomas with non-neoplastic liver lesions while 2 did not; 52 rats were exposed to each dose level. I wonder whether this low frequency of the incidence of liver adenoma without non-neoplastic liver lesions (i.e., 2/52 animals each exposed to 1.9 mg/kg/day and 3.9 mg/kg/day) should trigger the use of a linear approach to model the cancer risk in these animals. Although statistically significant increase in the incidence of

liver adenomas was observed over controls, there is no comparison to historical controls to show that the incidence is actually very low in control animals.

In Table 4, the frequency of the incidence of liver carcinoma is the same in males exposed to 1.9 mg/kg/day and controls. It appears that more emphasis is being placed on the incidence of liver adenoma than the carcinoma, and the use of data from liver adenomas and carcinomas combined in predicting doses associated with extra risk need further discussion.

Anna Fan

c. The mode of action for mirex's ability to induce tumors is not known. The brief discussion presented is reasonably clear and logical relating to the plausibility of a non-genotoxic mode (but not definitive), no existing knowledge of known reactive metabolites or intermediates, hypothesis of being a promoter of previously initiated cells, and the hypothesis of liver pathological changes preceding tumor development, with the acknowledgement that the mode of action whereby mirex induces liver tumors in animals is not yet completely understood. Understanding of the modes of action by which mirex induced other non-carcinogenic effects is also not known and this is also clearly indicated in the Toxicological Review document. It is to be noted that the gentotoxicty data are mainly obtained in *in vitro* systems. Tumors did not occur in all animals with non-neoplastic liver response. Considering all of he above, the use of a linear approach to does-response assessment is appropriate for mirex for which the mode of action is not established, in accordance to U.S. EPA guideline.

Karl Rozman

c. The mode of action is not presented clearly because it actually obfuscates the only really important issue--that it is the cumulative dose only that matters in mirex's toxicity. Because of this simple fundamental mistake the daily dose rate is depicted as a dose which it is not. This resulted in identifying a very high dose effect as the most sensitive endpoint of toxicity. The cumulative dose in the NTP study (0.7 mg x 104 weeks x 7 days = 509.6 mg/kg) is much higher than doses causing developmental and reproductive effects (40-60 mg/kg). A dose of 58.2 mg/kg of mirex (dose rate: 0.08 mg/kg/day) was not only devoid of carcinogenicity but did not result in any elevation in presumed precursor lesions (toxic hepatitis). Therefore, basing mirex's risk assessment on the most sensitive endpoints of toxicity (developmental or reproductive) will protect individuals against any type of toxicity including cancer. This implies that a nonlinear risk assessment is mandatory for mirex.

Bonnie Stern

c) Although it seems clear, given lack of mutagenicity/genotoxicity, and findings of nonneoplastic effects in association with liver tumors, that the mode of action of Mirex-induced hepatocarcinogenesis is likely to be nonlinear, insufficient information on possible or probable mode(s) of action precludes the application of a nonlinear approach

to cancer dose-response assessment. However, the mode of action section (Section 4.6) should be expanded to incorporate information presented in Section 4.4.3 on mechanistic studies.

Specific Observations

Anna Fan

1. Statistically significant decreases in sperm counts at doses as low as 0.05 mg/kg/d were noted on page 12 of the Toxicological Review document. This is a dose level that is lower than that of 0.07 mg/kg-d for liver toxicity observed in the 1990 NTP study. Further examination showed that on page 14 it was described that such decreases were found at 0.05, 0.5 and 5 mg/kg-d, but not at 7.1 mg/kg-d (Yarbrough et al., 1981.) This information on 7.1 mg/kg-d which indicated a lack of does-response should be shown on page 12 also.

2. On page 17, second paragraph, under the description of the NTP (1990) study, it was stated, "… Thus, 0.007 and 0.08 mg/kg-d (0.1 and 1 ppm in diet) were no effect levels for non-neoplastic liver responses in this study." It is not clear from the discussion immediately before this, how it led to this conclusive statement. Which endpoint led to which of these two levels (averaged for males and females)? Particularly, how was the 0.007 mg/kg-d arrived at since increases in hepatomegaly were reported to be seen at 0.0007 but not at 0.08 mg/kg-d, and other effects were observed at 0.7 mg/kg-d and above?

After this paragraph, nephropathy was reported in female rats exposed to 0.08 mg/kg-d and above, along with Table 3. This could lead to a no effect level of 0.007 mg/kg-d. But this was not supported by the second study in which females exposed to higher doses (3.9 and 7.7 mg/kg-d) did not show significant increases compared to controls.

Table 4, which gave the re-evaluation of the PWG, showed that the no-effect level for toxic hepatitis was 0.08 mg/kg-d. This is further described in the text (page 23) which supported the data in the table.   Toxic hepatitis was described as consisting of centrilobular hepatomegaly, fatty change, apoptosis, necrosis, bile duct proliferation, parathyroid hyperplasia, nephropathy and splenic fibrosis.

3. Under the discussion of reproductive and developmental effects, there is some confusion relating to Table 6 and Table 7. It is not clear if Table 6 (pages 29-30) intended to include both reproductive effects and developmental effects. It seemed like it did, but the title only showed developmental effects. In the text under the discussion of reproductive effects (page 35), it referred the reader to Table 6. But in the discussion of cataracts development in offspring (page 44), it referred the reader to Table 7. For Table 7 (page 39), the title and data were on tumor incidences. So there needs to be some clarification on the tables.

Using Table 6 as an overview of reproductive and developmental effects, then the lowest LOAEL would seem to be 0.4 mg/kg-d in two studies, for three endpoints (decreased survival of pups, increased incidence of cataracts in offspring, decreased pups/litter). Cataracts were seen at the same dose level in two species of rats. This also seemed to be the only dose level used for one study (Gaines and Kimbrough, 1970) (as shown in the

table, but the actual publication showed more dose levels) and the lowest level tested in another study (Chu et al., 1981b) with no NOAEL identified. The draft Toxicological Review document further discussed these on pages 44-45 but did not explain why these studies showing low effect levels were not used for RfD development. If the studies were judged to be inadequate or to be of insufficient quality, then this should be pointed out and explained. Otherwise it is necessary to take the data and go through the procedure and general assumptions (e.g., uncertainty factors) used to derive a RfD and compare it with the RfD derived based on toxic hepatitis (See above. NTP, 1990). Also, other studies provided more evidence on the reproductive/developmental effects such as pregnancy failure, decreased fetal survival rate and fetal visceral abnormalities (Khera et al., 1976), and cataracts (Chernoff et al., 1976), which are consistent with the findings of Gaines and Kimbrough (1970) and Chu et al. (1981a). So the overall review and evaluation should re-examine in more details the data from these studies (Gaines and Kimbrough, 1970; Chu et al., 1981a) and relevant studies. In the re-evaluation process, pharmacokinetics consideration needs to be incorporated, explained and justified.

Karl Rozman

pg. 5   1st line, delete "in"

pg. 5   How do you get 75.2% when 24.2% were excreted during first day. One or the other number is wrong.

pg. 5   Therefore, gastrointestinal absorption in goats (and not sheep) was approximately 80%.

pg. 9   The "first two half-lives" is improper wording in terms of kinetics. Distribution and redistribution half-lives is better. There is only one true half-life which is derived from the terminal slope.

pg. 9-10 The section on elimination and excretion is not good. The most relevant information for human health assessment is that the half-life of Mirex was longer than 13 years (Rozman et al 1981, citation missing) in rhesus monkeys and possible exceeding the life span of this species (Pittman et al. 1976). The half-life of mirex in rodents and other species is also long but much shorter than in rhesus monkeys. However, it is well-known that the half-life of other highly lipophilic chemicals such as HCB and TCDD is even longer in humans than in rhesus monkeys. Therefore, it is virtually certain that the half-life of mirex in humans would be on the order of decades possibly exceeding human life span.

The half-life of mirex in rats (354 days) must also be identified because of its importance for the chronic toxicity.

pg. 12-15 The section on subchronic toxicity is misleading which is related to the section on elimination. For the duration of a subchronic study (13 weeks) there will be linear accumulation of mirex. Therefore providing daily dose rates (mg/kg/day) is highly

misleading for a compound of such a long half-life. A dose rate of 0.3 mg/kg/day for 13 weeks, for example is identical in terms of AUC to a single dose of 27.3 mg/kg. Therefore, cumulative doses should be given to keep or rather to establish the perspective for chronic toxicity. For example, 0.7 mg/kg/day for 28 days amounting to a dose of 19.6 mg/kg resulted in 100% animals with liver lesions. Adaptation was clearly occurring because at 12 weeks only 50% of the animals had liver lesions (at a time when only about 15% of mirex had been eliminated) and only 11% after 48 weeks even though less than 50% of dose was eliminated during this time resulting in a huge AUC. Even more adaptation is apparent in the chronic bioassay where a dose rate of 0.08 mg/kg/day amounting to a dose of 58.2 mg/kg just started to cause toxic hepatitis.

IRIS Summary

Chlordecone is structually not similar to mirex as claimed because mirex does not have a reactive functional group and chlordecone is a ketone which is actually in the hydrated form which can be glucuronidated. Therefore the half-life of chlordecone is much shorter than that of mirex and can be further shortened by cholestyramine for above reasons, whereas that of mirex cannot be changed by that treatment for lack of a reactive functional group.

Drinking Water Concentrations

0.01 mg/L	10^{-4}
0.001 mg/L	10^{-5}
0.0001 mg/L	10^{-6}

0.1 µg/L = 100 ng/L

0.6 mg/L is claimed as solubility, I do not buy this.

If mirex does not meet the new guidelines to move away from a linear no threshold cancer risk extrapolation, then no compound ever will, in which case the new guidelines are nothing but the old guidelines in disguise.

I agree with the use of a total uncertainty factor of 300 for the derivation of the RfD but not for the reasons stated. Kinetics is driving toxicity for any compound of such a long half-life. As a highly inert compound with no known or expected high affinity binding to any receptor it is highly unlikely that species differences will be due to differential dynamics. Rather, species differences will be entirely due to differential kinetics. The half-life of mirex in rats is one year in humans estimated at 100 years. The two year bioassay's liver lesion, which are the basis of the RfD occurred at a time when rats reached about 75% of steady state which can be estimated at 1.13 µg/ml.

$$\overline{C} = \frac{FX_0}{VK\tau}$$

X_0....Dose rate = 0.15 mg/kg/day

F....Fraction absorbed $\approx$ 1

V....Volume of distribution $\approx$ 50L/kg

K....Elimination rate constant $\approx$ 0.002 d^{-1}

τ....Dose rate interval = 1 day

$$\overline{C} = 1.5 mg/L = 1.5 \mu g/ml$$

Assuming a half-life of 100 years for humans would change the elimination rate constant to $1.9 \cdot 10^{-5}$ d^{-1}.

Thus the concentration of mirex in humans at steady state (after 664 years) would be 157.9 mg/L (= 157.9 µg/ml). After 100 years 50% of steady state would be reached equaling 78.9 µg/ml. Thus a safety factor of 70 (78.9:1.13) is justifiable on grounds of science when extrapolating from rats to humans. This would yield a safe daily dose rate for mirex of 2.0 µg/kg/day (= 0.002 mg/kg/day) which is 10 and 4 times higher than the RfD derived in 1992 and 2003, respectively.

Bonnie Stern

Specific comments have been addressed in preceding sections, mainly in Section 1.

Other comments:

Section 4.7. Susceptible Populations
Possible childhood susceptibility and gender differences have been addressed. However, individuals with pre-existing liver disease should also be considered a potentially susceptible population.

P. 44, line 44: Replace Table 7 with Table 6.

P. 54 line 9: change "is anticipated" to "may"

P. 54 lines 14-17: Separate decreased sperm count from reduced male fertility. That is "(3) decreased sperm counts based on observations of decreased sperm counts in male rats exposed for 28 days to oral doses as low as 0.5 mg/kg/day (5 ppm diet) and (4)

reduced male fertility based on observations of decreased fertility in male rats exposed for 13 weeks to doses of 2.8 mg/kg/day (40 ppm in diet) or for 10 days to gavage doses of 10 mg/kg/day (but not 6 mg/kg/day)." The rationale for this separation is that decreased sperm count does not necessarily lead to a reduction in male fertility in rodents.

P. 54 lines 29-30, delete "but not all rats with adenomas were diagnosed with nonneoplastic liver lesions". Change line 29 from "adenomas in mirex-exposed rats" to "adenomas in most mirex-exposed rats".

P. 54. Line 31. Give EPA cancer classification.

Secondary Data

Anna Fan

The Toxicological Review document provided secondary data for review and evaluation. It is necessary that the original publications/data on reproductive/development effects as discussed in #3 under "Specific Observations" be reviewed and evaluated. These include the studies of Gaines and Kimbrough (1970), Chu et al. (1981a), Khera et al. (1976), Chernoff et al. (1976), and other relevant studies as appropriate. In the re-evaluation process, pharmacokinetics consideration needs to be incorporated, explained and justified.

Bonnie Stern

The use of secondary data i.e., published or unpublished data that are either from secondary sources and/or not published in the primary peer-reviewed literature, is entirely appropriate in the context of this document, with the possible exception of one citation.

Major secondary sources cited in the document are:

(1) 10 references to ATSDR (1995), including one concerning a National Cancer Institute (NCI) study on kepone, structurally similar to Mirex.: ATSDR documents undergo extensive peer review and are therefore considered to be reliable and valid sources of information. Further, studies cited from ATSDR (1995) in this document are not key studies and/or support data from primary literature sources.

(2) 1 references to NCI (1968) study on selected pesticides and industrial chemicals, published as an NTIS document. NCI documents also undergo extensive peer review and in the absence of primary literature studies, are considered to be acceptable secondary citations.

(3) NTP (1990) and PWG (1992): NTP Technical Report on the Toxicology and Carcinogenesis Studies of Mirex. These documents provides details on the principal study and critical effects. As with all NTP reports, this technical report has undergone

external peer review. Further, histopathological findings have been examined by an independent pathology working group, and histopathologic characterization and interpretation has been thoroughly vetted by a qualified panel of experts.

(4) Weinberg Consulting Group (1992). A review of mirex. This is an unpublished report, and no information is given in the document as to who sponsored this report and whether it has undergone external peer review. If the report has undergone external peer review, then I consider it an acceptable citation. If no external review has been conducted, then I would question its inclusion as a secondary data source.