**ENDOSULFAN**

**Toxicological Profile**

## Reviewer #1

**CHAPTER 1. PUBLIC HEALTH STATEMENT** FOR ENDOSULFAN

The chapter does present the essential and important information in a fairly non-technical style. The information is quite technical and needs to be that way to provide the information that is relevant. For instance the section on "Where is endosulfan found" there is a discussion on α-Endosulfan and β-endosulfan, this is somewhat technical, at the same time I think it is presented in a clear and concise manner and should be understood by most readers.

I think the major heading questions are answered completely and adequately. The summary information is consistent and reasonable.

There are a number of scientific terms in this document such as moth larvae, parts per million, picograms per cubic meter, and reduced pup weight during lactation; but these are all in the proper context, and while they are fairly technical, by definition, they are still appropriate for this document and are necessary to properly cover the topical areas in the section.

**CHAPTER 2. RELEVANCE TO PUBLIC HEALTH.**

The document addresses the known human health effects in sufficient detail with a very concise overview of the current and historical literature.

I agree with the authors on their description of the known human health effects.

The rat studies referenced for the MRL derivation are well described. There is a thoughtful and detailed discussion of the studies. Caveats are stated clearly in Section 2.3 to alert the reader of the limitations and "ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs".

Exposure scenarios for endosulfan have been adequately described.

**CHAPTER 3. HEALTH EFFECTS.**

It was interesting, given how long this compound has been registered that there were so few studies found on various organ systems, such as renal, dermal, ocular, endocrine, etc. The authors did an extensive assessment and Of course could not locate studies that are no in existence. Neurotoxicity is the primary effect observed in humans and that was described adequately.

The appropriate (highest) NOAEL values and reliable LOAEL values were reported for neurological effects.

It appears that when appropriate statistical methods were applied to the data. There are descriptions of the OR and CI in the Development Effects Section (3.2.2.6).

I am not aware of additional studies that were not referenced by the authors. Because this is a compound that has been registered for decades there is considerable animal data and in vitro model data. These are described in reasonable detail in Table 3.4 and 3.5.
Exposure scenarios, toxicokinetic data and description of studies for dermal and oral exposure were described; there is no inhalation reference point as these studies do not exist.
As previously mentioned the Genotoxicity section is complete. The PBPK rat model by Chan (2006) is helpful and provides a very good description of the processes although these are no species extrapolation and no risk assessment tools applied to this model.
Mechanism of Toxicity (Section 3.5) is adequately described.
Children's susceptibly (Section 3.7) is well written and concise and provides important information.
The biomarkers of exposure and effect section (Section 3.8) is well written, significant number of citations, and provides a strong platform for understating the exposure scenarios. The biomarkers discussed are for the parent compound and the metabolites.
Interactions with other chemicals (Section 3.9). I think there is adequate discussion with good illustrations such as Phenobarbital, carbon tetrachloride, diazepam, etc. The concentrations where the activity did or did not occur are listed. To the extent known and when appropriate the mechanisms are discussed.
Populations that are unusually susceptible (Section 3.10). The section references the most susceptible population, children which are covered in Section 3.7. Included are current as well as some early references from more that forty years ago. The coverage is adequate.
Methods for Reducing Toxic Effects (Section 3.11), the information for this particular compound is very limited in this regard, it is covered as best it can for this AI. Basic information on treatment is generic, and is similar to most pesticide products, i.e. removal from the contaminated area, removal of contaminated clothing, information on how to wash the clothing, eye irrigation, etc. It is adequate. There is no controversy and this is and has been the standard treatment for this type of exposure for more than fifty years. Limited information on treating susceptible populations other than the dose rate for activated charcoal.
This section 3.11.3 is brief, limited discussion on Vitamin A and Vitamin E use regarding mitigation of toxic effects. There is little available beyond what is listed in this section and the section is short.
Adequacy of the Database (Section 3.12)
As has been stated throughout this review while this compound has been in use for a long time, there are considerable limitations to the database. Figure 3.5 provides a useful summary of the human and animal data. The data are reasonably complete for the animal data, particularly via the oral route of administration. For humans there are considerable gaps for chronic effects on all routes of exposure and for a number of endpoint s for the dermal and oral exposure routes. This is an extensive section that is well written, comprehensive and provides a sufficient assessment of the data needs.

**CHAPTER 4. CHEMICAL AND PHYSICAL INFORMATION.**

The eight tables in this section provide the appropriate information for the reader. The tables are succinct and fairly easy to read and understand.

**CHAPTER 5. PRODUCTION, IMPORT/EXPORT, USE AND DISPOSAL.**

As required this is a good overview and the level of detail is appropriate. Table 5.1 and Figures 5.1 and 5.2 are useful illustrations.

**CHAPTER 6. POTENTIAL FOR HUMAN EXPOSURE.**

The chapter is written well, the NPL site occurrence data is included, the air, water and soil information is limited given the fact that the releases were not required to be reported and this is referenced (EPA 1998).

Figure 6.2 provides a conceptual model of the potential effects of the compound on ecological receptors. There is adequate discussion on bioaccumulation and biomagnifications potential of endosulfan with the appropriate references. Degradation in air, water, soil and sediment, and other media are covered adequately (Section 6.3.2.1 to 6.3.2.4).

Figure 6.3 is an interesting and descriptive graphic regarding α- and β-endosulfan concentrations sampled in North America and is very illustrative. There is considerable data for air, water and soil as well as crop/food residue data in a market basket survey as well as concentrations in bivalves. There are even descriptions of studies with monitoring in wine corks, lichens, frogs and other animal species. This is a complete section with considerable environmental monitoring data that is described clearly for the reader.

The California data and the US EPA data do provide a reasonable description of exposure levels within the population. There are data from several studies with cord blood, breast milk and other matrices that provide good exposure data for children. Applicator exposure data from California and from the US EPA provide exposure scenarios for the individuals most likely to be highly exposed.

Section 6.8.1 provides the information regarding data needs in an adequate and complete form.

For this compound there are no ongoing studies from the US EPA Agency or the registrants (Section 6.8.2).

**CHAPTER 7. ANALYTICAL METHODS.**

Tables 7.1 and 7.2 are very comprehensive regarding analytical methods for endosulfan, there are others, but this is a good and comprehensive summary. The work by Barr et al at CDC is a good example of additional information that could be added.

For example: A Comprehensive Approach for Biological Monitoring of Pesticides in Urine Using HPLC–MS/MS and GC–MS/MS, Dana B. Barr, Anders O. Olsson, Roberto Bravo, and Larry L. Needham in Pesticide protocols / edited by José L. Martínez Vidal, Antonia Garrido Frenich.

(Methods in biotechnology; 19) ISBN 1-58829-410-2 (alk. paper) -- ISBN 1-59259-929-X (eISBN)
1. Pesticides--Analysis--Laboratory manuals. I. Vidal, José L. Martínez.
II. Frenich, Antonia Garrido. III. Series. 2006
Methods for the parent compound and metabolites have been indentified, discussed and described where appropriate. The methods and data needs are address appropriately and also a referral back to Chapter 3 in the text. Data needs are addressed in 7.3.1 and there are no active ongoing studies (previously discussed) by the registrants or the US EPA 7.3.2.

**CHAPTER 8. REGULATIONS AND ADVISORIES.**

Table 8.1 Is a complete and comprehensive table, I am not aware of additional regulations or guidelines.

**CHAPTER 9. REFERENCES.**

The reference section is extensive with citations from the early 1970s through the present.

## Endosulfan

## Reviewer #2

Overall I find this a useful comprehensive well written review. As a clinical toxicologist who has seen many patients with endosulfan self-poisoning, my review focuses on clinical aspects of endosulfan and to a lesser extent the animal studies that have provided clinically relevant information. My comments therefore cover Sections 1 to 4. I have also commented on the supplementary file entitled Supdoc.

My comments are either inserted into the Word file's text or added as comments (true for Supdoc as well)

**Overall:**

The report appears to put more weight on individual case reports, confounded in some cases by possible pre-existing disease, rather than larger case series. The latter are generally more reliable in providing valid conclusions.

Human cases are frequently cited throughout the report. However, the cases selected seem rather random - they differ throughout the report when the same facts are being cited. I would prefer the useful human cases to be summarised within the body of the report in a single simple table reporting demographics, estimated dose, time to hospital presentation, features, management and outcome. This table could then be referred to throughout the report, rather than citing papers that might not be immediately available to the reader. Such a table wld be different from the detailed summary of cases presented in the Supdoc file.

Not being familiar with these reports, I found it frustrating that terms like 'acute' and 'intermediate' exposure were used in the earlier sections of the report without being defined. Definitions were provided later in the report but were not referred to earlier. I have commented on other similar situation in the report.

There should also be an earlier discussion of what is meant by the term endosulfan in section 2 - or at least refer to a discussion. Endosulfan can be the technical grade mixture or single $\alpha$ or $\beta$ form, or it can be an agricultural formulation containing solvents and surfactants. The effects of different forms are likely to be

different. Section 2 simply talks about ‘endosulfan’ without discussing these important aspects - the issue is discussed more fully later in the report.(see Comment ME22)

**Specific questions:**

-Are there any data relevant to child health and developmental effects that have not been discussed in the profile and should be?

Not to my knowledge

-Are there any general issues relevant to child health that have not been discussed in the profile and should be?

Not to my knowledge

**CHAPTER 1. PUBLIC HEALTH STATEMENT**

-The tone of the chapter should be factual rather than judgmental. Does the chapter present the important information in a non-technical style suitable for the average citizen? If not, suggest alternate wording.

I believe so. I am probably not the best person to judge this. I find it well written

-Major headings are stated as a question. In your opinion, do the answers to the questions adequately address the concerns of the lay public? Are these summary statements consistent, and are they supported by the technical discussion in the remainder of the text? Please note sections that are weak and suggest ways to improve them.

Some of the references cited are weak. Better options are provided

-Are scientific terms used that are too technical or that require additional explanation? Please note such terms and suggest alternate wording.

Yes - I have raised concerns at a few points. I have clarified one section for accuracy. However, I find “high levels” is too vague - see comment ME4

**CHAPTER 2. RELEVANCE TO PUBLIC HEALTH**

-Do you agree with those effects known to occur in humans as reported in the text? If not, provide a copy of additional references you would cite and indicate where (in the text) these references should be included.

I do not agree how the clinical history of the poisoning is laid out. I have suggested an alternative Comment ME11. I find there is too much reliance on just a few case reports.

Brain damage is not uncommon after poor control of seizures. Therefore brain damage after endosulfan poisoning is not surprising Comment ME13

One reference is missing for thyroid effects Comment ME16

-Are the effects only observed in animals likely to be of concern to humans? Why or why not? If you do not agree, please explain.

-Have exposure conditions been adequately described? If you do not agree, please explain.

Yes

## CHAPTER 3. HEALTH EFFECTS

### Toxicity - Quality of Human Studies

-Were adequately designed human studies identified in the text (i.e., good exposure data, sufficiently long period of exposure to account for observed health effects, adequate control for confounding factors)? If not, were the major limitations of the studies sufficiently described in the text without providing detailed discussions. If study limitations were not adequately addressed, please suggest appropriate changes.

No good quality studies are available in humans. The authors have identified the studies that do exist and critiqued them adequately. However there is an apparent preference for case reports (which are a weak form of data) over case series

-Were the conclusions drawn by the authors of the studies appropriate and accurately reflected in the profile? If not, did the text provide adequate justification for including the study (e.g., citing study limitations)? Please suggest appropriate changes.

Yes. However, needs some discussion of aspiration since many deaths will occur from this non-specific of coma and seizures. This may have been the cause of death in some of the case reports used to report the human toxicity of endosulfan and confounded the interpretation. The co-formulants of agricultural endosulfan are likely to be important here

-Were all appropriate NOAELs and/or LOAELs identified for each study? If not, did the text provide adequate justification for excludingNOAELs/LOAELs including, but not limited to, citing study limitations? Please suggest appropriate changes.

No such data available

-Were the appropriate statistical tests used in the studies? Would other statistical tests have been more appropriate? Were statistical test results of study data evaluated properly? NOTE: As a rule, statistical values are not reported in the text, but proper statistical analyses contribute to the reliability of the data.

Outside of my expertise

-Are you aware of other studies which may be important in evaluating the toxicity of the substance? Please provide a copy of each study and indicatewhere in the text each study should be included.

Yes - relevant studies not included. I have annotated appropriately

### Toxicity - Quality of Animal Studies

-Were adequately designed animal studies identified in the text (i.e., adequate number of animals, good animal care, accounting for competing causes of death, sufficient number of dose groups, and sufficient magnitude of dose levels)? If not, does the inadequate design negate the utility of the study? Please explain.

Adequately discussed in the report

-Were the animal species appropriate for the most significant toxicological endpoint of the study? If not, which animal species would be more appropriate and why?

Yes

-Were the conclusions drawn by the authors of the studies appropriate and accurately reflected in the text? If not, did the text provide adequate justification for including the study (e.g., citing study limitations)?

Yes

-Were all appropriate NOAELs and LOAELs identified for each study? Were all appropriate toxicological effects identified for the studies? If not, please explain.

Yes

-If appropriate, is there a discussion of the toxicities of the various forms of the substance? If not, please give examples of toxicological effects that might be important for forms of the substance.

Yes - this is discussed in 3.1. However there is a lack of data addressing the different forms of technical grade endosulfan and particularly formulated agricultural endosulfan to which most humans will be exposed.

-Were the appropriate statistical tests used in the interpretation of the studies? If not, which statistical tests would have been more appropriate? Were statistical test results of study data evaluated properly? NOTE: As a rule, statistical values are not reported in the text, but proper statistical analyses contribute to the reliability of the data.

Outside of my expertise

-Are you aware of other studies that may be important in evaluating the toxicity of the substance? If you are citing a new reference, please provide a copy and indicate where (in the text) it should be included.

No

**Levels of Significant Exposure (LSE) Tables and Figures**

-Are the LSE tables and figures complete and self-explanatory? Does the "Users Guide" explain clearly how to use them? Are exposure levels (units, dose) accurately presented for the route of exposure? Please offer suggestions to improve the effectiveness of the LSE tables and figures and the "User's Guide."

I would prefer Figure 3.2 to show human data as well since Moon & Chun provides some indication on the lethal dose of endosulfan to humans

-Do you agree with the categorization of "less serious" or "serious" for the effects cited in the LSE tables?

Yes

-If MRLs have been derived, are the values justifiable? If no MRLs have been derived, do you agree that the data do not support such a derivation?

Yes

**Evaluation of Text**

-Have the major limitations of the studies been adequately and accurately discussed? How might discussions be changed to improve or more accurately reflect the proper interpretation of the studies?

Fine

-Has the effect, or key endpoint, been critically evaluated for its relevance in both humans and animals?

Yes

-Have "bottom-line" statements been made regarding the relevance of the endpoint for human health?

Yes

-Are the conclusions appropriate given the overall database? If not, please discuss your own conclusions based on the data provided and other data provided to you but not presented in the text.

Yes

-Has adequate attention been paid to dose-response relationships for both human and animal data? Please explain.

Yes

-Has the animal data been used to draw support for any known human effects? If so, critique the validity of the support.

**Section 3.3 GENOTOXICITY**

**Section 3.4 TOXICOKINETICS**

-Is there adequate discussion of absorption, distribution, metabolism, and excretion of the substance? If not, suggest ways to improve the text.

No use of clinical information to indicate speed of effective absorption - see Comment ME44

-Have the major organs, tissues, etc. in which the substance is stored been identified? If not, suggest ways to improve the text.

Yes

-Have all applicable metabolic parameters been presented? Have all available pharmacokinetic/pharmacodynamic models and supporting data been presented? If not, please explain.

Yes to my knowledge

-Is there adequate discussion of the differences in toxicokinetics between humans and animals? What other observations should be made?

Very weak data are available from humans so difficult to compare with animal data

-Is there an adequate discussion of the relevance of animal toxicokinetic information for humans? If not, please explain.

Yes

-If applicable, is there a discussion of the toxicokinetics of different forms of the substance (e.g., inorganic vs. organic mercury)?

Not applicable

## Section 3.5 MECHANISMS OF ACTION

Have all possible mechanisms of action been discussed? If not, please explain.

No mention of endosulfan's role as substrate and inhibitor of p-glycoprotein  is given

The order of possible mechanisms is poor - the likely mechanism with good experimental data shld be given first, with less likely mechanisms given at the end. Referencing could be improved.

## Section 3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

## Section 3.7 CHILDREN'S SUSCEPTIBILITY

## Section 3.8 BIOMARKERS OF EXPOSURE AND EFFECT

-Are the biomarkers of exposure specific for the substance or are they for a class of substances? If they are not specific, how would you change the text?

No specific biomarkers. Just endosulfan and its metabolites.)

-Are there valid tests to measure the biomarker of exposure? Is this consistent with statements made in other sections of the text? If not, please indicate where inconsistencies exist.

-Are the biomarkers of effect specific for the substance or are they for a class of substances? If they are not specific, how would you change the text?

No specific biomarker of effect. However, non-specific biomarkers of clinical severity after poisoning exist - creatine kinase activity and lactate concentration (seizure induced rhabdomyolysis and metabolic acidosis respectively. These are not discussed.

-Are there valid tests to measure the biomarker of effect? Is this consistent with statements made in other sections of the text? If not, please indicate where inconsistencies exist.

## Section 3.9 INTERACTIONS WITH OTHER CHEMICALS

-Is there adequate discussion of the interactive effects with other substances? Does the discussion concentrate on those effects that might occur at hazardous waste sites? If not, please clarify and add additional references.

Weak section. Little data available to support proposed interactions. Alternative approach, from other pesticides, is presented

-If interactive effects with other substances are known, does the text discuss the mechanisms of these interactions? If not, please clarify and provide any appropriate references.

## Section 3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

-Is there a discussion of populations at higher risk because of biological differences which make them more susceptible? Do you agree with the choices of populations? Why or why not? Are you aware of additional studies in this area?

No other studies. Association with renal failure patients is remarkably weak, being based on lesions in rodents

## Section 3.11 METHODS FOR REDUCING TOXIC EFFECTS

### *Reduced absorption*

-Is the management and treatment specific for the substance, or is it general for a class of substances?

General

-Is there any controversy associated with the treatment? Is it a "well accepted" treatment?

The test here is too positive. There is no data for any benefit from charcoal and gastric lavage might only be useful if administered early. The hazards of treatment are not discussed.

-Are there any hazards associated with the treatment of populations that are unusually susceptible to the substance (e.g., infants, children)?

Yes, gastric lavage will be hazardous in these populations unless done very carefully

### *Elimination*

-Are treatments available to prevent the specific substance from reaching the target organ(s), or are the actions general for a class of substances?

No effective treatment

-Is there any controversy associated with the treatment? Is it a "well-accepted" treatment? If the discussion concerns an experimental method, do you agree with the conceptual approach of the method?

-Are there any hazards associated with the treatment of populations that are unusually susceptible to the substance (e.g., infants, children)?

-Are there treatments to prevent adverse effects as the substance is being eliminated from the major organs/tissues where it has been stored (e.g., as a substance is eliminated from adipose tissue, can we prevent adverse effects from occurring in the target organ[s])?

### *Treatment*

-Are treatments available to prevent the specific substance from reaching the target organ(s), or are the treatment's actions general for a class of substances?

General for poisoned patients

-Is there any controversy associated with the treatment? Is it a "well accepted" treatment? If the discussion concerns an experimental method, do you agree with the conceptual approach of the method?

Yes - phenytoin shld not be given for poisoned patients. See Shah & Eddleston 2010. Reference listed

-Are there any hazards associated with the treatment of populations that are unusually susceptible to the substance (e.g., infants, children)?

No

## Section 3.12 ADEQUACY OF THE DATABASE

### Existing Information on Health Effects of [Substance X]

-Do you know of other studies that may fill a data gap? If so, please provide the reference.

No

### Identification of Data Needs

-Are the data needs presented in a neutral, non-judgmental fashion? Please note where the text shows bias.

Yes

-Do you agree with the identified data needs? If not, please explain your response and support your conclusions with appropriate references.

Yes

-Does the text indicate whether any information on the data need exists?

-Does the text adequately justify why further development of the data need would be desirable; or, conversely, justify the "inappropriateness" of developing the data need at present? If not, how can this justification be improved.

Fine

## CHAPTER 4-8

Outside of my expertise.

PEER REVIEW OF ENDOSULFAN TOXICOLOGICAL PROFILE

Reviewer #3

In general I thought the presentation was exceptionally clear, concise and readable.

***Inhalation MRLs (page 16)***

The risk assessment for endosulfan in California derived a NOEL for inhalation and in California endosulfan is listed as a Toxic Air Contaminant. All references cited below can be found in:

Beauvais S. 2008. Endosulfan Risk Characterization Document, Volume II: Exposure Assessment. Worker Health and Safety Branch, Department of Pesticide Regulation, California Environmental Protection Agency, Sacramento, CA. http://www.cdpr.ca.gov/docs/whs/pdf/hs1647.pdf.

Silva MH. 2008. Endosulfan: Risk Characterization Document. Medical Toxicology and Worker Health and Safety Branches, Department of Pesticide Regulation, California Environmental Protection Agency, Sacramento, CA. http://www.cdpr.ca.gov/docs/emon/pubs/tac/finaleval/endosulfan.htm:

Generally a margin of exposure (MOE) of at least 100 is considered sufficiently protective of human health when the NOEL for an adverse systemic effect is derived from an animal study. This MOE allows for the possibility of humans being 10 times more sensitive than animals and for a 10-fold variation in sensitivity between the lower range of the normal distribution in the overall population and the sensitive subgroup (Dourson et al., 2002). The MOEs should be a 1000-fold or greater for the general public exposed to endosulfan via inhalation. MOEs of less than 1000 for public exposure scenarios result in the consideration of listing of pesticides as toxic air contaminants (California Food and Agricultural Code: 14021-14027).

In California, endosulfan has been monitored and detected in 34/39 or 23/39 samples by 8 hours after application for the α- and β-isomers, respectively (Beauvais, 2008). Endosulfan can drift after aerial application and can be transported long distances before being removed by wet or dry deposition (NRCC, 1975). A model for environmental distribution of organic chemicals in air was characterized, in terms of persistence and spatial range, by Scheringer (1997). This model shows that endosulfan has a limited spatial range (15% of the earth's perimeter) and a persistence of less than 10 days. The spatial range will increase with increased sorption of the compound to particulate matter, a condition hypothesized to preclude a fast reaction of semivolatile compounds with OH radicals. This suggests that bystander populations (non-occupational) could potentially be exposed to endosulfan. Endosulfan, due to these factors and the low inhalation NOELs, has been listed as a toxic air contaminant (California Food and Agricultural Code: 14021-14027).

Evaluation of Acute, Subchronic and Chronic Inhalation NOELs:

An acute inhalation ($LC_{50}$) study was performed (Hollander and Weigand, 1983), however, a NOEL was not achieved (LOEL = 0.61 mg/kg). Therefore, an acceptable subchronic rat inhalation study (based on a subchronic rangefinding study with a LOEL of 0.44 mg/kg reported within Hollander et al., 1984) with a NOEL of 0.0010 mg/L (0.194 mg/kg/day; LOEL = 0.387 mg/kg/day) was used to calculate the potential for acute single-day inhalation exposure to workers, and for exposure to endosulfan in bystander air (Hollander et al., 1984). The rationale for the use of the subchronic inhalation study for the Acute NOEL is that LOELs from all three studies were similar (0.61, 0.44 and 0.387 mg/kg/day), more animals treated in the subchronic (15/sex/dose subchronic versus 5/sex/dose in the acute), and the subchronic study used a 29 day recovery with 5 per sex per dose (acute 14d observation). The NOEL of 0.194 mg/kg/day is a reasonable selection based on the LOELs from the 3 studies. It is also a conservative estimate for an acute NOEL, since acute NOELs are usually higher than subchronic or chronic NOELs. It is also noted that all three studies were performed at the same laboratory and in the same timeframe (12/7/83—Acute; 8/15/83--Subchronics).

In this study, endosulfan was administered by aerosol (nose-only) for 21 days at 6 hours per day, followed by a 29-day recovery. The NOEL for inhalation was based on emaciation, pale skin, squatting position and high-legged position, decreased bodyweight gain (not statistically significant) and food consumption, increased water consumption occurring in one high dose male, and clinical chemistry parameters (reversed during recovery). The advantages to using the subchronic study for the critical inhalation NOEL instead of the $LC_{50}$ are: A) a NOEL was achieved (0.194 mg/kg/day); B) there were 15 animals per sex per dose treated instead of 5 per sex per dose; C) the LOEL achieved in the subchronic study (0.387 mg/kg/day) was 2/3rds the dose of the LOEL in the $LC_{50}$ (0.61 mg/kg) and D) the NOEL in the subchronic study was, in general, more conservative, because the acute NOEL is usually higher than the subchronic NOEL. Both studies were acceptable according to FIFRA Guidelines. The subchronic inhalation NOEL was also selected instead of the oral NOEL of 0.7 mg/kg/day from the rabbit developmental study because not only is it lower, but more importantly, it is route-specific. Therefore the rat subchronic NOEL was used to estimate the MOE for acute inhalation (occupational and (non-occupational) bystander air exposure.

The definitive study for subchronic inhalation exposure the same as that described above for the acute inhalation value (critical subchronic inhalation NOEL of 0.0010 mg/L [0.194 mg/kg/day] and a LOEL of 0.0020 mg/L [0.3873 mg/kg/day]).

A chronic inhalation study was not performed to obtain a NOEL for scenarios involving long-term occupational inhalation exposure or long-term exposure to the public for bystanders. There were several chronic inhalation-specific scenarios both occupationally and to the public, so in the absence of an acceptable chronic inhalation study, the NOEL for the subchronic rat inhalation study was used. To adjust from subchronic to chronic, a 10x uncertainty factor was added (0.194 mg/kg/day )10 =

0.0194), resulting in a chronic inhalation ENEL of 0.0194 mg/kg/day for exposure and MOE estimates.

***Oral MRLs (page 18)***

***I don't agree that Banerjee and Hussain, 1986 should be used as the definitive study for the determination of an intermediate or chronic MRLs when they (Banerjee and Hussain) state that the work is preliminary. Their 1987 article provides a somewhat higher NOEL but the study is their definitive work. I think the fact that the 1986 work is preliminary should be indicated in the ATSDR. In my opinion, the B & H, 1986 study is weak and should be used only as supplemental information. There are many other much stronger studies showing a lack of effect in blood chemistry, clinical chemistry, spleen or blood organ effects at higher doses for intermediate durations (see below, following my reviews of B & H, 1986 and 1987).***

Pubertal male Wistar rats (85-90g; 10-12/dose/sacrifice time) were fed endosulfan (technical grade; 98%) in the diet at nominal doses of 0 (ground nut oil), 5, 10 or 20 ppm for 8, 12, 18 and 22 weeks to evaluate subchronic treatment on humoral and cell-mediated immune responses in albino rats (Banerjee and Hussain, 1986). Rats were immunized with tetanus toxoid (TT- stimulated group) in Freund's complete adjuvant subcutaneously 20 days before terminating the exposure with an equal number of animals (NI - unstimulated group) not immunized (10-12 rats/dose/sacrifice time). The humoral immune response was characterized by serum globulin (SG) level, immunoglobulin (IgM & IgG) concentration and antibody titre against tetanus toxoid. The cell-mediated immune (CMI) response was measured by lymphocyte migration inhibition (LMI) and macrophage migration inhibition (MMI) factors. At 22 weeks, spleen/body weight ratio was statistically significantly decreased at 20 ppm in TT groups. Albumin/ globulin ratio was statistically significantly increased weeks 12-22 at 10 ppm and at 22 weeks at 10 ppm in TT groups. Antigen-induced increases (TT) in SG (8-22 weeks), IgG (12-22 weeks), LMI (8-22 weeks) and MMI (8-22 weeks) were observed at 20 ppm. It was concluded that endosulfan exerts a marked suppression of the humoral and CMI responses in rats at 5 ppm (0.5 mg/kg/day). Both IgG and CMI were decreased in a dose-time related manner. It was concluded that endosulfan treatment disrupts the immune system in male rats. **Clinical effects were not described in this study; only "no overt toxicity signs and symptoms." This comment is open to interpretation and since there are no data presented any effects it is unknown if animals experienced subtle neurotoxicity after treatment. The authors considered their report to be preliminary.**

Pubertal male Wistar rats (85-90g; 16/dose) were fed endosulfan technical (98%) in the diet at nominal doses of 0, 10, 30 or 50 ppm (equivalent achieved doses based on a 150 g rat for the duration: 0, 1.5, 4.5 or 7.5 mg/kg/day, respectively) for 6 weeks (Banerjee and Hussain, 1987). The study was designed to evaluate the effects of subchronic doses of endosulfan on humoral and cell-mediated immune responses. After 25 days of exposure, the animals were immunized subcutaneously with tetanus toxoid. Serum antibodies to the toxin, IgG, IgM, LMI (lymphocyte migration inhibition) and MMI were measured. There were no

"overt signs of toxicity", however, the schedules for and extent of observations was not described. At termination, relative liver weights were significantly increased by 15% at 50 ppm. The immune system showed signs of suppression, compared to the control, by a dose-related decrease in serum antibody to tetanus toxoid. Serum IgG (28%) and IgM (25%) and γ-globulin (33%) were significantly decreased at 50 ppm, compared to the control. Group hemagglutination was significantly decreased by 14% at 30 and 43% at 50 ppm. Cell mediated immunity was decreased in a dose-related manner as indicated by the suppression of LMI by 24% and 40% at 30 and 50 ppm, respectively. MMI was significantly decreased by 20% and 44% at 30 and 50 ppm, respectively. The NOEL was 1.0 mg/kg/day based on increased relative liver weights and a decreased serum antibody response to tetanus toxoid. Effects observed in the 1986 report were observed only at doses of $\geq$ 30 ppm (20 ppm was not tested in this study). The fact that 20 ppm was not used in the follow-up study leads me to believe the authors had little faith that a true effect was occurring at that dose.

***I appreciate that you noted in your report that these studies should be repeated but they form a very weak basis for the definitive oral MRL determinations for both chronic and subchronic durations. FIFRA Guideline subchronic and chronic studies have been performed on rats, mice and dogs where full blood panels and clinical chemistry have been performed at low doses. No results of well performed studies have indicated that at very low doses the immune system is a target. Although animals in these studies weren't specifically challenged for immune effects, there appeared to be no long term effects to adults, fetuses or offspring after exposure in diet for 2 generations (NOEL = 1.18 mg/kg/day; Edwards et al., 1984). There were no effects on spleen in the studies performed over 1 or 2 years in rats and dogs. All studies listed below were performed in the same time frame.***

***Intermediate Term Studies that are FIFRA Guideline and of much better quality than B & H, 1986.***

Barnard, A.V., Jones, D.R., Powell, L.A.J., Heywood, R., Street, A.E., Gibson, W.A., Gopenath, C., Majeed, S.K. and Almond, R. (Huntingdon Research Centre, England), 1984. 13-Week toxicity study in mice. DPR Vol. 182-042 #0472

Barnard, A.V., Jones, D.R., Powell, L.A.J., Heywood, R., Street, A.E., Gibson, W.A., Gopenath, C., Majeed, S.K. and Almond, R. (Huntingdon Research Centre, England), 1985. 13-Week toxicity study in rats followed by a 4-week withdrawal period (final report). DPR Vol. 182-032 #035803.

Donaubauer, H.H., 1988. Carcinogenicity study in mice, 24 month feeding study. DPR Vol. 182-064 #075035.

Edwards, J.A., Reid, Y.J., Offu, J.M., Almond, R.H. and Gibson, W.A., 1984. Effect of endosulfan-technical (Code: HOE 02671 0 I AT209) on reproductive function of multiple generations in the rat. DPR Vol. 182-022 #035789

***The following studies of 1 year duration (dog), 2 years (rat) and 18 months (mouse) show that there is no effect to hematology, clinical chemistry, spleen or other blood organ at levels higher than B & H, 1986. There are aneurysms in blood vessels in the rat study but***

***only at the highest dose. These are well-performed FIFRA-Guideline studies where all individual data are accessible.***

***The chronic dog study by Brunk, R., 1989. Testing for toxicity by repeated oral administration (1-year feeding study) to Beagle dogs. DPR Vol. 182-065 #074850 is much stronger and offers a comparable NOEL of 0.57 mg/kg/day.***

Endosulfan was fed to Beagle dogs (6/sex/dose) at 0, 3, 10, 30 or 30/45/60 ppm or measured dosages of 0, 0.22, 0.57, 2.09, and 2.2/3.08/3.7 mg/kg/day for males and 0.19, 0.65, 1.98, and 1.95/2.78/3.57 mg/kg/day for females for 1 year (Brunk, 1989). In the high dose group, dogs were treated for 54 days at 2.2 mg/kg/day in males and 1.95 mg/kg/day in females; for 52 days at 3.08 mg/kg/day in males and 2.78 mg/kg/day in females and 19 - 40 days at 3.7 mg/kg/day in males and 3.57 mg/kg/day in females. One male at 2.09 mg/kg/day was killed *in extremis* on day 126, after 125 treatments. All high dose dogs were sacrificed on days 146 to 147, due to an onset of extreme sensitivity to noise, frightened reactions to optical stimuli and jerky or tonic contractions of the muscles in the chaps (temporal muscles), extremities and face, after the dose was increased to 3.7 mg/kg/day in males and 3.57 mg/kg/day for females. One male at 2.09 mg/kg/day and one male at 3.7 mg/kg/day were terminated on days 276 and 126, respectively, due to poor condition (see Table 7 for major effects). Both sexes showed neurotoxicity (impairment of the reflex excitability and postural reactions), which developed with increasing doses at the high dose level. On the morning of the 136th day, after 135 applications, one female, at the high dose, was found with its fur wet and smeared with excrement. Since the clinical reactions occurred during the time between 3 p.m. and 7 a.m., the dogs in all groups were subsequently treated on a number of days at an earlier hour. It was then possible to observe at various intervals a sudden and violent contraction of the abdominal muscles with contraction of the upper abdomen, and also convulsive movement of the chaps, though not followed by vomiting. These reactions occurred starting 2.5 to 6 hours after treatment. Neurological symptoms, having to do with reflexes, were noted only at termination. Decreased body weights were observed (not significant) in males at 2.09 mg/kg/day (-5%) and 3.7 mg/kg/day (-7%), beginning at week 44 (44th weighing). Both sexes showed a temporary decrease in percent of food consumed at 2.09 mg/kg/day (and greater) for males, and 1.99 mg/kg/day (and greater) for females. The decreases were not statistically significant when compared to controls. The NOEL was 0.57 mg/kg/day for males and 0.65 mg/kg/day for females, based on violent contractions of the upper abdomen and convulsive movement in males at 2.09 mg/kg/day and greater, beginning at 2.5 to 6 hours post-feeding. Body weights for males and food consumption for both genders were decreased at doses of 1.98 mg/kg/day or greater.

***Also consider the chronic studies performed on rat with a similar NOEL to that of dog:***

Crl:CD (SD) BR rats (70/sex/dose) were fed endosulfan in the diet for 104 weeks at 0, 3.0, 7.5, 15 or 75 ppm (Ruckman et al., 1989). This main group was intended primarily for tumorigenic evaluation. Also treated for 104 weeks was a satellite group of 20 rats/sex/dose, intended for blood sampling at intervals and for sacrifice after 104 weeks of treatment. There

were no interim sacrifices in this study. The intakes of endosulfan were 0.1, 0.3, 0.6 or 2.9 mg/kg/day in males and 0.1, 0.4, 0.7 or 3.8 mg/kg/day in females, based on food consumption. Bodyweight gain was decreased 8% to 18%, compared to controls in both sexes at 2.9 mg/kg/day in males and 3.8 mg/kg/day in females (statistically significant in both sexes). Absolute testes weights appeared to decrease (non dose-related) at the high dose, however these weights were within historical control range. There were no differences in relative testes weights at any dose (body weights within historical control range). These observations were therefore not considered to be of toxicological significance. Kidney enlargement occurred in females at 3.8 mg/kg/day. Progressive glomerulonephrosis was increased in both sexes at the high dose (statistically significant in females at 3.8 mg/kg/day) and was stated in the report to be a common, age-related, spontaneously occurring renal disease associated with proteinuria (especially in males). There was a non-dose related increase in glomerulonephritis in males at 0.3 mg/kg/day and greater. The chronic NOEL was 0.6 mg/kg/day in males, based on an increased incidence of aneurysms in blood vessels at 2.9 mg/kg/day, which primarily affected the pancreas, mesentery and/or liver after week 80. There was a slight increase in the incidence of pituitary adenomas in males at 75 ppm but there was no dose-related trend. Incidences were, control through high dose (n = 50), 23 (control), 18, 16, 21 and 27 for males and 31, 31, 39, 34 and 32 for females. In females, the incidences for mammary fibroadenomas were 34, 34, 36, 29 and 31. Incidences of adenoma, fibroadenomas with atypia and adenocarcinomas also showed no trend with dose. The conclusion is the study did not identify any tumor types with exposure to endosulfan. In females the NOEL was 0.7 mg/kg/day, primarily based on the increased incidence in enlarged kidneys and progressive glomerulonephritis at 3.8 mg/kg/day. The study was acceptable. See Table below for observations

**Non-neoplastic Pathological effects in a 104-Week Dietary Rat Oncogenicity Study[a]**

| **Observations[b]** | **Males - Doses (mg/kg/day)** | | | | | **Females - Doses (mg/kg/day)** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 0.1 | 0.3 | 0.6 | 2.9 | 0 | 0.1 | 0.4 | 0.7 | 3.8 |
| **Kidneys** | | | | | | | | | | |
| Enlargement | 38 | 32 | 39 | 34 | 39 | 10 | 18 | 19 | 17 | 26** |
| Percent of animals with enlargement | 54 | 45 | 55 | 48 | 55 | 14 | 26 | 27 | 24 | 37 |
| Marked Progressive Glomerulonephrosis[c] | 20 | 18 | 22 | 24 | 30 | 1 | 6 | 6 | 5 | 8** |
| Percent with glomerulonephrosis | 29 | 26 | 31 | 34 | 43 | 1 | 8 | 8 | 7 | 11 |
| Number with glomerulonephrosis/total | 20/70 | 18/70 | 22/70 | 24/70 | 30/70 | -- | -- | -- | -- | -- |
| **Blood Vessels[d]** | | | | | | | | | | |
| Aneurysms[e] | 10 | 6 | 14 | 10 | 19* | 0 | 1 | 1 | 0 | 0 |
| Percent of animals with aneurysms | 14 | 8 | 20 | 14 | 27 | 0 | 1 | 1 | 0 | 0 |

a - Ruckman, et al., 1989

b - The incidence = # of lesion bearing animals per animals at risk (70/sex/group). This includes the satellite animals.

c - *Marked* progressive glomerulonephrosis Historical Controls from 6 studies that were performed in male Sprague-
Dawley rats (50/study: Incidence = 11, 19, 8, 13, 5 and 14; mean = 11.6). No historical control data were presented
for females. Glomerulonephrosis was considered a direct cause of death and treatment-related. It was not observed
in satellite animals that were terminated at one year.

d - Includes main and satellite rats found dead, killed *in extremis* or at scheduled sacrifice. Aneurysms were not observed in rats that died on study at 1 year and later. Aneurysms, that affected pancreas, mesentery and/or liver,
were observed after week 80. The effects were considered to be treatment-related.

e - Aneurysm Historical Controls (6 studies) were performed in male Sprague-Dawley rats (50/study: Incidence = 6,
9, 2, 4, 7, and 2; 50, 50, 45, 55, 50 and 50 kidneys examined, respectively; mean = 5). No historical control

data
were presented for females.
*, ** - P ≤ 0.05 and 0.01, respectively (1- tailed test) by Fisher Exact Test.

Endosulfan technical was fed in the diet to NMRI Hoe:NMRKf (SPF71) mice (80/sex/dose) at 0, 2, 6 or 18 ppm (Males: 0.28, 0.84 or 2.48 mg/kg/day; Females: 0.32, 0.98 or 2.8 mg/kg/day) for 24 months (Donaubauer, 1988; Hack et al., 1995). Interim sacrifices of 10/sex/group were performed at 12 and 18 months. Males at 2.48 mg/kg/day showed a 17% decrease in body weights. Results in females showed that mortality was increased at 2.8 mg/kg/day (43/60, 72%) when compared with controls (33/60, 55%). Deaths began to occur in males at 45 weeks and in females at 15 weeks. Mortality occurred primarily between weeks 27 and 52 at 2.48 mg/kg/day for males and 2.8 mg/kg/day in females. From weeks 79 through 104, there was no difference among groups for mortality. There was no specific target organ toxicity. There were no clinical signs of neurotoxicity. Bodyweight gain was statistically significantly decreased in males at 2.48 mg/kg/day, however the reduction was only 5% and therefore not considered to be a noteworthy effect. At termination (104 weeks), there was no treatment-related oncogenicity. The most common neoplasm was multicentric lymphosarcoma in both sexes. With an n of 60, that included all those that died or were terminated in the main group, the incidences were 11, 13, 18, and 16 for males and 22, 25, 21 and 15 for females, control group through high dose. In animals that died sporadically in the main group, the incidence of multicentric lymphosarcomas that were the final contributors to death (FCTD), compared to the overall incidence of these tumors was, 11/11, 8/13, 14/18 and 11/16 for males and 11/22, 18/25, 15/21 and 12/15 for females, control group through high dose. Therefore, these tumors did not appear to be associated with treatment throughout the study. Multicentric lymphosarcomas occurred initially at 12 months (FCTD) at 2/10 and 2/10 in control and low dose males and in no other groups for either gender. At 18 months, these tumors occurred at 0, 1, 0, 0, of 10 males and 1, 1, 1, and 0 of 10 females in the controls through high dose groups. No tumor type showed a positive trend with increasing dose in either sex. Therefore, endosulfan was not considered to induce tumors in mice after 18 months of dietary treatment. This interpretation was supported by the USEPA review of the same study (USEPA, 2001b), which stated that there were no increases in incidence of any neoplastic lesion that was observed in either sex at any dose. These results were later published in the open literature (Hack et al., 1995). The chronic NOEL was 0.84 (males) and 0.98 (females) mg/kg/day, based on increased mortality in the main group of females at 2.8 mg/kg/day. This study was acceptable.

***In my opinion publications by the Cabaleiro/Caride laboratory are poorly performed and the results are often not interpretable. They show lots of asterisks for significant effects but often they appear to be within background range (range on vertical axis is very small and "effects" appear large), show no pattern and often no dose response. The number of animals treated is small and the results of this laboratory and of Caride are nothing but preliminary. Cabaleiro T, Caride A, Romero A, Lafuente A. 2008. Effects of in utero and lactational exposure to endosulfan in prefrontal cortex of male rats. Toxicol Letts 176:58–67. Below is my review of the above paper.***

Endosulfan was administered by gavage to pregnant Sprague-Dawley rats (4/dose) at 0 (sesame oil), 0.61 and 6.12 mg/kg/d from GD1 through PND 21 (Cabaleiro et al., 2008). These are 100 and 1000x the acceptable daily intake (ADI). The male pups (10/group) were sacrificed at PND 15, 30 and 60. Maternal effects included a dose-dependent reduction in body weight at the end of gestation, along with a reduction in litter size at 6.12 mg/kg/d.

Dam body weight was similar to controls at delivery and weaning. Male pup body weight was also reduced at 6.12 mg/kg/d at PND 15, 21 and 30 but not at PND 60. Prefrontal cortex from pups was obtained PND 15, 30 and 60 and assayed for aspartate, glutamate, glutamine, GABA, taurine, dopamine (DA), 3,4-dihydroxyphenylacetic acid:dopamine (DOPAC:DA) ratio, homovanilic acid/dopamine (HVA:DA) ratio, 5-HT and 5-hydroxyindole acetic acid:serotonin (5-HIAA/5-HT) ratio. For the amino acids, all (4) were elevated by endosulfan at 6.12 mg/kg/d at PND 15 and at PND 30. At PND 60, however, there was a decrease at both doses, for aspartate, glutamate, glutamine and GABA. The meaning of these apparent increases and decreases at PND 30 and PND 60 is unclear and, moreover, it is possible that all of these points, treated and control, are part of a single data set and therefore are not related to endosulfan treatment, i.e. are not biologically relevant. For taurine, dopamine, DOPAC, HVA and 5-HT concentrations, in various brain regions, there were differences between dosed and control rats. However, there was an absence of a clear or consistent dose/response relationship at any of the times considered (PND 15, 30 & 60). Other unusual Dose/Response relationships were found for 5-HT at PND 30 where the increase was greater at 0.61 mg/kg/d than at 6.12 mg/kg/d. At PND 60, the increases were 0.61 (N.S.) and 6.12 mg/kg/d ($p<0.05$), compared with controls. The 5-HIAA/5-HT ratios were also reported: at PND 15, there was a dose-dependent reduction (N.S.), at PND 30, there was a non-dose/dependent reduction and at PND 60, there was a dose/dependent reduction ($p<0.05$ at 6.12 mg/kg/d). A major deficiency in this study is that there were no positive (or historical) control data for any of the measured endpoints; thus the magnitude of the effects have unknown relevance and it is unclear whether endosulfan causes dose/related increases or decreases in transmitter levels in the prefrontal cortex or striatum in the neonatal male rat that are toxicologically relevant, i.e. adverse. Another deficiency that limits the use of this study for risk assessment is the lack of description of clinical signs, primarily at the high dose. Surely endosulfan itself would be expected to cause clinical signs at this dose, based on other studies.

***The article by Caride et al., 2010 has the same problem. There were only 4 dams/dose treated and only 8 male offspring/dose analyzed. These are the same animals used in their previous study. The study and evidence, especially when Cabaliero is also used as a reference are not strong. They use a lot of techniques but their sample is very small and yet they make a lot of sweeping conclusions and claims based on data that should at best be considered very preliminary.***

***For the reproductive, developmental and neurotoxicity section may I recommend my paper by Silva and Gammon: Birth Defects Research (Part B) 86:1–28 (2009). I think that should be used as a reference.***

***Children's Susceptibility (page 117)***

I offer the following analyses for of Zaidi and Seth for Intraperitoneal (i.p.) Neonate/Pup: Neonatal albino rats (1 day old, 4/sex/dose/time point, strain not stated) were treated with endosulfan i.p. at 0 (40%polypropylene glycol), 0.5 and 1.0 mg/kg/d for 3 or 5weeks, followed by an 8-day recovery (Zaidi et al., 1985).There was a statistically

significantly increased 3H-5HTbinding to frontal cortical membrane at 5 weeks (1.0 mg/kg/d). This may have been due to increased maximum binding sites or alterations in the receptoraffinity. At 1.0 mg/kg/d (after 5 weeks of treatment) there was an increase in fighting behavior induced by endosulfan treatment that was reversed when the 5-HTblocker, methysergide, was administered. The NOEL was 0.5 mg/kg/d. Neurotoxicity was not reversed after the 8-day recovery period. Data were of limited use in the enlightenment of the endosulfan mechanism in the CNS because of numerous deficiencies (unnamed rat strain, purity of dosing material not stated, no adult comparison group, no clinical signs of neurotoxicity reported, no clinical sign data, no positive controls) but a NOEL was achieved for rat pups (0.5 mg/kg/d) in this and the following study.

Seth et al. (1986) treated pregnant female rats (ITRC breeding colony) with endosulfan i.p. (purity unknown) in the following groups to examine the effects on dams and pups (in utero and post-natally). Gestational Exposure Dams (5/group) received treatment (3 mg/kg/d) or vehicle (40% propylene glycol) only during gestation as follows: (1) Vehicle dams with natural pups; (2) Treated dams with their treated pups; (3) Treated dams foster nursed with control pups; (4) Control dams foster nursed with treated (in utero) pups. Pups were culled to 8 pups per dam. Gestational- Lactational Dams (5) were treated at 3 mg/kg/d throughout gestation and lactation up to 3 weeks of post-partum (PP) age for their pups (culled to 4/sex/litter). Lactational Exposure Dams (5) were treated with 3mg/ kg/d from PPD for 1 to 2 or 3 weeks. Neonatal Exposure Pups (4/dose; M/F) received endosulfan at 0, 0.5, and 1.0 (i.p.) for 5 days per week up to 2, 3, or 5 weeks old. Adult Exposure males (8/dose; 8wk old) were given endosulfan i.p. at 1 mg/kg/d (1 day) or 3 mg/kg for 15 or 30 days. At termination, brains were excised and examined in high-affinity binding assays with synaptic membrane preparations from several brain regions (corpus striatum, frontal cortex, and cerebellum). Effects of endosulfan treatment on receptor binding in brain were compared in adults and pups (gestation, lactation, and growth) using labeled ligands. Receptor binding for dopamine, acetylcholine (ACh; muscarinic), benzodiazepine, serotonin, and GABA was tested with 3H-spiroperidol, 3H-quinuclidinyl benzilate (QNB), 3H-diazepam, 3H-5HT, and 3H-muscimol, respectively. It was shown that 3H-spiroperidol binding (dopamine) was increased in pups that had received 3 mg/kg/d endosulfan (Gestational Exposure) throughout gestation (po0.05 at 2, 3 and 5 weeks post-partum) and in pups treated in utero (3 mg/kg/day) but foster-nursed to control dams ($p<0.05$ weeks 2 and 3). Gestation-Lactation Exposed pups (4/sex/dose; 3 mg/kg/d) had an increase in 3Hspiroperidol binding at weeks 2, 3, and 5. Neonatal Exposure at 0.5 mg/kg/d showed no effects from day 1 to 5 weeks (4/sex; either sex) but at 1.0 mg/kg/d at 5 weeks there was a slight increase in 5-HT and benzodiazepine and a decrease in dopamine binding. Foot-shock fighting behavior in neonatally exposed pups was examined in 10 control and 10 treated (randomly selected) and was increased in pups treated to 5 weeks of age at 1.0 mg/kg/d. These changes were observed 8 days after cessation of treatment. Adults treated at 3 mg/kg/d for 15–30 days had increased 3H-5-HT binding along with increased footshock fighting (continuing 8 days post-treatment). Developing rats appeared to have increased sensitivity to endosulfan but adults were not tested at 1.0 mg/kg/d for 5 weeks, so it is not known whether or not toxicity would be comparable, especially since toxicity at 1.0 mg/kg/day was only observed at 5 weeks. Adults were tested only at 3.0 mg/kg/d for a shorter period of time (2–4 weeks). There were no effects on ACh in any group. Despite the same deficiencies as the Zaidi et al. (1985) study, a pup NOEL 0.5 mg/kg/d was also achieved in this study.

See Page 136 of Toxicology Profile Draft for comments on use of Abadin et al., 2007 as a reference. The rest of my comments are on the Toxicology Profile Draft.