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June 27, 2002

ATTENTION: Ms. Lois Rossi, Director SRRD

SUBJECT: Unresolved Issues Concerning the "Reevaluation of the HED Risk Assessment for the Endosulfan Reregistration Eligibility Decisions (RED) Document. Chemical No. 079401. Case No. 0014. Barcode D250471. (Locke D., dated May 30, 2002)

Dear Ms. Rossi:

The Endosulfan Task Force (ETF) recently received the above referenced memorandum pursuant to finalization of the Endosulfan RED document. The ETF has noted, with grave disapprobation, that despite repeated meetings with the EPA Health Effects Division (HED), the proposed final Risk Assessment Chapter still contains data errors, misrepresentation, misquotes of ETF scientific positions, and significant bias in the presentation of selected data from the available global database on endosulfan. As a final step, the ETF is providing the following details on these specific issues which it feels must be corrected prior to public release of the Endosulfan RED document to ensure that the data being disseminated meets acceptable quality standards. [Note: all page references made in the following section pertain to above mentioned subject, Locke D., 30 May 2002, Barcode D250471]

A. Data Errors

1. HED has repeatedly incorporated a known error in their scientific rationale regarding susceptibility of young animals to endosulfan. This error resulted from HED's excerption of information from the ATSDR *Toxicology Profile for Endosulfan* (Sept. 2000), which reported the data incorrectly.

   p.20 & 25-26 EPA responded: *The ETF correctly pointed out that rats exposed to 1 mg/kg/day of endosulfan from postnatal days (PND) 1 to35 exhibit an increase ($p < 0.05$) in binding of serotonin in the frontal cortex of the brain and an increase in aggressive behavior. A similar effect was*

> *not seen in adult rats dosed at the 1 mg/kg/day dose level. However, adult rats exposed to a higher dose, 3 mg/kg/ day, for 15 to 30 days did exhibit the increase in serotonin and aggressive behavior. These findings reaffirm the Agency's current determination of enhanced susceptibility on a quantitative basis (e.g., effects at 1 mg/kg/day in neonates versus 3 mg/kg/day in adults)…*

This indirect reference to adult animals is data from Seth et al. (1986). However, a proper data review of this study by the ETF showed that adult animals were **never** tested at 1 mg/kg/day. Therefore, there is no data in this or any other published literature to substantiate HED's statement on susceptibility of young animals based on these endpoints. This error was discussed by the ETF in a meeting with HED on May 15, 2002, and the actual paper was provided to the Agency shortly after the meeting. We request that HED reviews this paper and changes the RED Chapter to accurately reflect that the literature does not indicate enhanced susceptibility.

2. Page 20, the Agency makes for the following statement:

> *In a study by Sinha et al., both three week and three months old rats were treated orally; decreased intratesticular spermatid count and increased percentage of abnormal sperm were seen in three week old rats at doses lower than those eliciting similar effects in three month old rats.*

First, this was not a single study as intimated by the above statement. The comparison of effects in three-week-old versus three-month-old rats is made between two separate studies conducted two years apart. In fact, nowhere in the available published literature were young and mature animals ever tested within the same study, under the same conditions, using the same batch of technical material.

Second, following a thorough review by the ETF, statistical errors were found in both of these papers. In particular, using a one-way ANOVA (Tukey) test to assess the significance in means (±S.E.), between treated and controls, clearly showed that the reported difference of 6% between controls and three-week-old animals treated at 2.5 mg/kg/day was not significant. Therefore, there was no dose-related difference in effects between three-week-old and three-month-old rats as reported in these studies. Again, this error was identified by the ETF in the meeting with HED on May 15, 2002. The ETF requests that HED reviews these studies for data quality, and remove all statements regarding changes in percentage sperm abnormality since there is no evidence of increase susceptibility for this effect.

B. Misrepresentation of Facts and Data Availability Leading to FQPA Reevaluation

1. Page 6 & 21: HED states that the FQPA Safety Factor Committee recommended retention of the 10x safety factor based on residual uncertainties resulting from "…2) *additional* evidence for endocrine disruption…"

   This statement is inaccurate and misleading. In February 2002, when this recommendation was made, all of the data cited by HED had been available to the Agency since 1998. Summaries of the available published literature and references (dating from 1983-1997) were provided in Appendix A (Liem D., 11/24/98) to the draft Toxicology Chapter dated November 22, 1999. This data was also provided to HED in several ETF response documents (MRDI 44939102, submitted 10/04/99; MRID 45300203, submitted 01/05/01; MRID 45619001, dated 02/28/02), as well as the ATSDR document (dated September 2000).

   The ETF has also noted that the initial toxicology report submitted to the FQPA Safety Factor Committee in October 1998 by Dr. Liem stated that there were no additional acceptable studies available in the published literature that would influence a FQPA decision. This information shows that the Agency had performed a literature search in 1998 and was aware of the available data. Even if, as the Agency stated on May 15th 2002, an "internal policy change" was made in 1999 concerning evaluation of endocrine disruption data that affected the weight-of-evidence procedure, the Agency had ample time to determine that a re-evaluation was needed. [Note: This policy has never been released for public comment and has never been made accessible to the ETF]. In addition, the Agency was actively working on Endosulfan between 1998 and 2002, releasing the draft HED Chapter (dated 01/31/01) to the public in September 2001, and again in January 2002 with the public release of the Response to Comments report, but never determined that a re-evaluation was necessary until after the risk assessment changed due to the HIARC change in the dermal toxicity endpoint.

   > "*On February 11th, 2002 the FQPA Safety Factor Committee convened to determine the impact these changes [reconsideration by HIARC of the appropriate dermal endpoint and safety factor for occupational exposure assessments] might have on the FQPA safety factor determination …" (contained on p. 25)*

   When the FQPA Safety Factor Committee meet in February 2002, the background data they were provided included: a toxicology report and an endocrine report by Dr. David Liem from 1998; a HIARC report from 1998; the FQPA Safety Factor Committee report from 1998; a memorandum from E. Mendez on possible endocrine effects from December 2000; and a summary table of data extracted from the ATSDR Toxicology Profile for endosulfan, 2000.[1] The only "additional" data currently available are two studies (Dalsenter et al., 1999; and Sinha et al., 2001) identified and reviewed by the ETF and provided to the Agency in previously referenced documents and meetings. Since these studies

[1] **Mendez E.,** ***ENDOSULFAN – Supporting documentatiob for findings of the FQPA Safety Factor Committee on February 11, 2002.*** **TXR#: 0050704, dated May 9, 2002. DP Barcode: D282896**

were not in the ATSDR document, the primary source of information provided to the FQPA Safety Factor Committee in February 2002, the Committee made its decision in the absence of any "recent", "additional" or "new" data. Therefore, HED's intimation that "additional" data existed that justified a re-evaluation of endosulfan, after the closing of the public comment phase of the RED process, is misleading. The conclusion is that The FQPA Safety Factor Committee rescinded their 1998 decision to remove the 10x safety factor based on a new interpretation of previously reviewed literature under a policy that has not been released to the public. Clarification of the policy change and how it has affected the endosulfan assessment must be provided in the HED Chapter. Additionally HED should clarify the impetus for the re-evaluation which, as contained on p. 25 was:

> "*On February 11th, 2002 the FQPA Safety Factor Committee convened to determine the impact these changes [reconsideration by HIARC of the appropriate dermal endpoint and safety factor for occupational exposure assessments] might have on the FQPA safety factor determination as well as reconsider* ***recent*** *data regarding effects on the endocrine/neuroendocrine system pursuant to the Agency guidance on the evaluation and consideration of these endpoints for FQPA safety factor determination purposes"*.

It is inappropriate that the rationale be based on: i) an improvement in occupational exposure; and ii) data quoted as "recent" that had been available throughout the RED process.

C. Misquoting of ETF's Scientific Positions

1. Page 23: HED states "*The ETF stated that the Agency has hypothesized about the possible mechanism of endocrine disruption of endosulfan. That is not accurate. Since the registrants had themselves initially limited their criteria for endocrine disruption to receptor binding, the Agency provided a number of examples of potential mechanisms of endocrine disruption (ENDOSULFAN: Evaluation of Registrant Submission "Endosulfan: Evaluation of Possible Endocrine Effects in Mammalian Species." Elizabeth Mendez. December 11, 2000). These are examples, not hypothesis, theories or determination of mechanism of endocrine disruption*."

First, the ETF never, in any of the four written responses to the Agency, "limited their criteria for endocrine disruption to receptor binding." In all cases, the ETF relied on the current OECD and EDSTAC definitions, which expressly state that the more reliable indicator of endocrine disruption are endpoints derived from *in vivo* test systems. This is clearly shown in statements by the ETF that HED quotes on p. 22 of the referenced memo.

Second, the Agency has indeed "hypothesized" about the mechanism of action of endosulfan as it relates to hormone levels in rats. HED has repeatedly stated that "…*observed decreased testicular testosterone in conjunction with increased*

*serum testosterone which **suggests** sex-hormone binding globulin (SHBG) may be affected" and "These decreases in LH may lead to decreases in the activity of Steroidogenic Acute Regulatory Protein…."* (p.22-23) These statements, which the Agency has incorporated into a number of documents, are not examples. An example is "a particular single item, **fact**, incident or aspect that is representative of all of a group or type; a parallel or closely similar case especially when serving as a precedent or model." (Merriam-Webster, 1987) No facts or other scientific evidence has been provided by currently available data on endosulfan that leads to these suppositions by HED. To ***suggest***, "to offer for consideration or as a **hypothesis** to seek to influence, to mention or imply as a possibility" (Merriam-Webster, 1987), hypothesize or even provide examples that cannot be substantiated by valid scientific data when making an assessment of safety is completely inappropriate. The remit to EPA provided under FQPA is to make expert judgements based on reliable data, not suggest or otherwise lead the public to speculate upon mechanisms of actions in the absence of factual data.

The ETF request that these comments be removed from the HED Chapter prior to public release of the endosulfan RED.

2. HED states in point 4 on page 26, "*The ETF disagreed with the Agency's use of open literature studies in its evaluation claiming the literature studies have not been thoroughly reviewed. However, all of these studies were peer reviewed.***"** They also stated in point 9 on page 27, *"…the ETF stated that the Agency should rely on the results of the guideline studies only, which did not detect the effects discussed in the literature studies*."

   These statements are completely inaccurate. The ETF has repeatedly stated that the entire database should be evaluated in order to make a sound scientific weight-of-evidence determination. In every response submitted by the ETF, all of the available data, including published literature not identified or investigated by Agency, were presented. The ETF has made every effort to present the entirety of available data on endosulfan and maintain the scientific integrity of the weight-of-evidence process, as should be expected of any body of experts making a sound-science determination.

   The ETF's concern regarding the degree of scientific *review* of the published literature is valid. The peer review process for publication in journals varies widely and does not abdicate the Agency from making appropriate data quality determinations before using this type of information for regulatory purposes. Recent history has shown that erroneous data can and does get published (e.g. Arnold et al. 1996). A basic review of the endosulfan-related papers by the ETF also showed errors, as was discussed earlier. Therefore, the ETF's intent was to emphasize the need for rigorous and unbiased scientific evaluations of all the available data including the public literature data. HED's implication that the ETF requested that open literature not be used at all is incorrect and must be removed from the HED Chapter.

3. Point 5, page 26: HED states "*The ETF argues that since similar results were not observed in another study in which Wistar rats were dosed from gestation day (GD) 15_PND 21, the effects seen in the Druckrey rats are invalid*."

   Again this statement and its implied message regarding the ETF's technical arguments are inaccurate and misleading. The ETF discussed in detail both of these studies in our response documents, as well as in our meetings with the Agency in April and May 2002. The ETF's position was that these were both important studies and needed to be assessed in the overall weight-of-evidence. The ETF's primary concern, as with most of HED's assessment of the available data, is that HED was very selective in its presentation of results. In every case, HED discussed only positive effects that supported their position, completely disregarding other key aspects of the studies, with absolutely no mention or discussion of endpoints and results that were in opposition to their point of view. Since these are public documents, EPA has an obligation to provide the public with all of the available information, and not just selected parts. This type of biased reporting of data is unprincipled and needs to be corrected.

4. Point 10, page 27: "*The ETF acknowledged "indications of potential disruption of reproductive hormones in males" (p. 12 ETF's April 5, 2002 document). This statement by the ETF reaffirms the Agency's position of the potential for endocrine disruption by endosulfan.*"

   This is a direct misquote of the ETF position paper and must be removed. First, this excerption was part of a summary of comments from the ATSDR document. Second, excerption of partial statements, with the express intent to misrepresent or mislead is both inappropriate and unethical. If the Agency wishes to utilize comments made by the ETF it must include the position in its entirety and within its original context.

   The correct ETF comment: "The Agency has relied on a summary of public literature prepared by ATSDR on endosulfan with regard to potential hormonal effects from *in vivo* testing in rats (e.g. serum and testicular testosterone levels, androgen enzyme induction, spermatological endpoints and in vitro binding assays). The citations provided in the FQPA Safety Factor Committee [report] only represents one side of the available data, and are not consistent with a science-based weight-of-evidence evaluation. As was summarized by ATSDR, the evidence from in vitro testing is mixed with equal numbers of positive and negative findings. However, as was addressed in Table 1, in vivo testing has not shown any endocrine disruption potential in females, and limited indications of potential disruption of reproductive hormones in males. The weight of this evidence in males must be interpreted with caution, as recent validation efforts in male endocrine assays has shown sperm and hormone parameters to be highly

variable, and sensitive to exogenous influences (e.g. circadian fluctuations and stress).[2,3] "

D. Biased Representation of Available Data on Endosulfan

As stated previously, HED has consistently included in this public document only data that is supportive of their position. There are no full descriptions of published literature studies with all of the data presented. In addition, studies that provided negative findings are not noted or discussed at all. EPA is held to a standard of professionalism that requires unbiased and sound-science based expert judgements of reliable data for every chemical it assesses. Endosulfan has not received this type of assessment, and the public is being presented with a document, which is full of biased representation of data that is both inappropriate and misleading.

Further examples of HED's bias come from discussions of endpoints from submitted guideline studies:

1) The agency has repeatedly referred to effects in the reproductive toxicity study as potential indications of endocrine disruption. In spite of repeated responses by the ETF providing scientific evidence showing a lack of toxicological significance of these findings, HED has not only ignored this information, but has never presented or addressed these technical issues.

   Other than a brief and incomplete statement regarding the ETF position on this issue (point 8, page 27), HED has demonstrated a complete disregard for sound scientific issues, many of which were provided by HIARC in their review of the study.

   Key technical issues that have been ignored include:
   - Uterine weight
     1) No dose-response
     2) No histopathological evidence of change
     3) Effects occurred in only one mating, in one generation. No effects were noted in the second mating of the same generation or in either mating of the second generation
     4) The control values for this group were unusually low compared to the rest of the controls, the mean uterine weight for the high dose group was well within the range of the controls for the study
     5) Four (4) uterotrophic assays, a validated screening assay for estrogen effects, using endosulfan were all negative.
   - Pituitary weight

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[2] **Andrews et. al.** ***Feasibility and potential gains of enhancing the subacute rat study protocol (OECD test guideline no. 407) by additional parameters selected to determine endocrine modulation. A pre-validation study to determine endocrine-mediated effects of the antiandrogenic drug flutamide. Arch Toxicol (2001) 75:65-73.***

[3] **Ulbrich B. and Palmer A.K.,** ***Detection of Effects on Male Reproduction A Literature Survey.*** **J. American Col. Of Toxicol. Vol. 14, pp.293-327. 1995**

1) No dose-response
2) Effects occurred in only one mating (female only), in one generation. No effects were noted in the second mating of the same generation or in either mating of the first generation
3) No histopathological evidence of cellular changes
4) Statistical significance due to a single high-end animal

Using basic principles of toxicology, organ weight changes in the absence of dose-response or histopathological findings are not considered of toxicological significance, and are typically not considered treatment-related.

2) HED has also repeatedly cited effects from a 1978 NCI chronic/carcinogenicity study in rats. HED has never provided the details of this study in their discussions with regard to the FQPA safety factor, nor have they acknowledged that this study was found to be an **unacceptable** guideline study. More importantly, the FQPA Safety Factor Committee has never acknowledged that a guideline **acceptable** chronic/carcinogenicity study in rats exists within the core database for endosulfan, and that this study was negative for the effects of concern.

   The ETF has repeatedly raised issues with the use of the unacceptable NCI study for the following reasons:

   - Both the low and high doses exceeded the MTD
   - The study was terminated prior to completion due to excessive mortality at all dose levels
   - The effects on the parathyroid were considered, by all reviewers, as secondary to renal failure

   The Agency has supported their continued use of effects in this study, stating that existence of systemic toxicity does not negate the potential for endocrine disrupting effects. This is not a case of professional judgement related to some equivocal or marginal signs of systemic toxicity (e.g. minimal weight loss) in concurrence with definitive effects on endocrine-related organs. The animals in this study were mortally intoxicated, with clear signs of life-threatening degradation of the entire system. Selective observations of one or two endpoints, with complete disregard for the remaining evidence of toxicity is completely inappropriate and not within the purview of any current endocrine disruption definitions.

   As stated previously, use of the NCI study, with complete disregard to the value of the current guideline acceptable chronic/carcinogenicity rat study, is inappropriate and a violation of the basic principles of the weight-of-evidence process. As a minimum, HED must provide the public enough details of the NCI study to make appropriate scientific conclusions regarding the weight-of-evidence of the noted effects. Dr. David Liem presented the following statements in his report to HIARC (dated 11/24/98) which was attached as Appendix A to the HED Toxicology Chapter (dated 11/22/99):

"Dose-related depression in the rates of growth and survival were shown in the male rats. At week 54, 52% of the high-dose males died (the Tarone test for a positive dose-related trend in mortality was highly significant). The low- and high-dose male rats were terminated during week 74 and week 82, respectively. No appreciable difference in mean body weight among the females was noted. At termination (week 102), 70% of the control, 62% of the low-dose and 50% of the high-dose groups survived" p.35

"A parathyroid hyperplasia was reported to be associated with renal lesions and occurrred in 21/48 low-dose (20.4 mg/kg/day) and in 18/47 (40.8 mg/kg/day) males. Only 1/49 parathyroid lesion was noted in the low dose female."

"This study was classified as unacceptable guideline study for carcinogenicity study in rats."

The ETF also request that HED acknowledge that a guideline acceptable chronic/carcinogenicity study in rats exist in the current endosulfan database, and that this study showed no effects on any reproductive organs or other endocrine-related systems.

3) With regard to the Agency's updated arguments pertaining to residual uncertainties and other considerations in the 10x Safety Factor assessment (pp. 28-30), HED has misrepresented the data, such that the general public will not be able to make a valid scientific determination of the weight-of-evidence.

   a) Residual Uncertainties (pp. 28-29): HED relies almost exclusively on the findings of the two most recent published literature papers (Dalsenter, 1999 & Sinha, 2001) provided to them by the ETF as a result of our thorough review of the available data. Again, HED presents only positive findings from these studies, with the implication that these two studies derived similar results and were fully supportive of the Agency's position. In fact, these studies were quite divergent in their results and were brought to the attention of the Agency by the ETF as evidence of the need for rigorous scientific review and appropriate weight-of-evidence evaluation

      As a quick comparison, the following table shows the individual results of these studies:

Table 1: Comparison of Results from Dalsenter et al. 1999 & Sinha et al. 2001

	Dalsenter et al. 1999	Sinha et al. 2001
Body weight	↓	↓
Testes weight	↑	↓
Epididymis weight	No change	↓

Prostate weight	No change	No change
Seminal vesicle	No change	↓
Sperm count in cauda epididymis	↑	↓
Sperm production	↓	No data
Spermatid count	No data	↓
Sperm abnormality	No change	No data
Seminiferous tubuli with complete spermatogenesis	No dose-response	No data

Based on this data, HED made the following conclusions:

p. 28 *"…published literature data describe effects on sperm parameters, lactate dehydrogenase and sorbitol dehydrogenase activity, as well as testicular, epididymal, and seminal vesicle weights at a dose level of 1.0 and 1.5 mg/kg/day (the lowest doses tested in the studies, i.e. no NOAEL for these effects has been identified)."*

Incorrect: according to the data summary above the effects noted at 1.0 and 1.5 mg/kg/day in the two studies do not match. In fact, there are less effects noted at 1.5 mg/kg/day than at 1.0 mg/kg/day. HED needs to restate this comment to clearly define the results as shown in the above table.

p. 28 *"…In a 1999 study by Dalsenter et al., exposure to endosulfan from gestation day 15 through post-natal day 21 at the lowest dose tested (1.5 mg/kg/day) elicited a 21% decrease in daily sperm production."*

HED omits mention of the fact that a statistically significant decrease in sperm production was only seen at PND 65 and not at PND 140. Nor is there any attempt to discuss the relevance of this change in effect in juvenile animals that have not completed a full spermatogenic cycle versus adult animals.

p.28 "*…Additionally, histopathological assessments demonstrated that the percentage of seminiferous tubules with complete spermatogenesis was significantly decreased at puberty by 16%."*

HED omits mention that this decrease was not dose-related, and that there was no statistical significance difference at PND140.

p. 28 *"The persistence of these effects is noteworthy since dosing ceased on PND21 yet effects were noted on PND 65 (i.e. puberty) and PND 100 (young adults). Similar results were reported by Sinha et al. in 2001."*

First, the animals were evaluated at PND 65 and PND 140 (mature adults), and the effects noted at PND 65 were not significant at PND 140. Second, there is no

indication that the results are similar between Dalsenter and Sinha, as noted in Table 1.

p.29 *"The 10X FQPA factor would also be applicable to the chronic reference dose (cRfD) since a NOAEL for effects on sperm parameters, testicular histopathology, and reproductive organ weights has not been identified."*

This statement is misleading. A NOAEL for testicular histopathology can be established for Dalsenter et al. 1999, since there was no dose-response and the effect wasn't consistent across time, as well as for the 2-generation reproductive toxicity study, where there were no histopathological changes in any reproductive organs at any dose level. In addition, there was no effect on organ weights in Dalsenter et al., and there is a clear NOAEL for organ weight changes in the 2-generation reproductive toxicity study. Therefore, this statement needs to be clarified to specify the study being referenced and the exact effects as they relate to the study.

The ETF request that HED, at a minimum, correct the above referenced statements that incorrectly summarize or represent the findings of these studies. The ETF also requests that HED provide full details of the studies they rely on for their FQPA rationale in order that the public be given an opportunity to properly assess the scientific evidence.

b) Other Considerations (p. 29-30): Based on the information provided in this letter, if the available data is presented correctly and in its entirety, HED has not established a case for increased susceptibility. In no instance were neonatal or juvenile animals tested in the same study, under identical study design or with the same technical material as adult animals. Nor has an endpoint been identified in which a LOAEL was established for young animals for an effect at a dose level lower than an established NOAEL in adult animals. HIARC has concluded in all three reviews that there is no evidence of increased susceptibility in young animals from the core endosulfan database. Given an unbiased presentation of the published literature, there is no evidence of increased susceptibility. The ETF has stated previously that, when the Agency has finalized the screening criteria for evaluating chemicals for endocrine disruption, the ETF will fulfill those data requires for endosulfan. Regardless, the FQPA established the use of an additional safety factor for the protection of children.

By basing their residual uncertainties on endocrine-related effects, in the absence of evidence for increased susceptibility, HED has made a clear departure from the remit of FQPA and application of an additional safety factor. The ETF request that HED make the identified data corrections, and restate their rationale based on scientific evidence that directly relates to susceptibility of young animals.

Conclusion

The ETF believes that without the requested corrections, the HED RED Chapter does not accurately portray the database associated with endosulfan. The requested changes are consistent with the principles outlined by OMB in "Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity of Information Disseminated by Federal Agencies" [Federal Register: February 22, 2002 (Volume 67, Number 36, Pages 8451-8460]. We appreciate you consideration in this matter. If you have any questions or need further information, please contact me at 610 793 3222.

Sincerely

Bert Volger
Chair
Endosulfan Task Force