

Disposition of Peer Review Comments for Endosulfan

Prepared by:

SRC, Inc.
Chemical, Biological and Environmental Center

SRC No. 549

Prepared for:

Agency for Toxic Substances and Disease Registry
U.S. Public Health Service
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October 2012

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ATSDR would like to thank these scientists for their review of the document.

Comments provided by Reviewer #1.

No responses were necessary to comments provided by Reviewer #1

Comments provided by Reviewer #2.

Comment: The reviewer states that the profile appears to put more weight on individual case reports than on larger case series. The reviewer notes that larger case series are generally more reliable in providing valid conclusions.

Response: ATSDR does not agree that the profile puts more weight on individual cases reports. It may appear that way because there were more individual case reports than publications with multiple cases. ATSDR thanks the reviewer for pointing out additional information from the larger case reports that had been left out from the profile.

Comment: The reviewer suggests summarizing the cases reports 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.

Response: ATSDR will consider the reviewer's suggestion for the post-public version of the profile.

Comment: The comment refers to text on Section 2.2 that states the stages involved following acute intoxication with endosulfan according to Blanco-Coronado et al. (1992). The reviewer finds that there is too much reliance on just a few case reports. The reviewer provided alternative text.

Response: Blanco-Coronado et al. (1992) described 6 cases, one of which resulted in death. There is no reason to believe that the text provided by the reviewer is better or more accurate than the observations and laboratory findings reported by Blanco-Coronado et al. (1992). In the latter report, 5 of the 6 cases were family members who had eaten the same food item contaminated with endosulfan; therefore, they all ingested the same endosulfan formulation and the time-to-hospital presentation was similar, thus removing some variability involved when comparing unrelated cases. No changes were made.

Comment: The reviewer would prefer Figure 3-2 to show human data. In a related comment in the annotated pages, the reviewer wonders why human reports that estimated ingested doses could not be considered for MRL derivation. The reviewer specifically mentions the retrospective study of 52 cases by Moon and Chun (2009)

Response: The reviewer probably refers to Table 3-2 (Levels of Significant Exposure, oral); Figure 3-2 is generated from Table 3-2. Moon and Chun (2009) conducted a multivariate step-wise logistic regression analysis to identify predictive factors from variables expected to be related to health outcomes. The results of the analysis showed that the amount of endosulfan ingested being >35 grams (500 mg/kg assuming 70 kg body weight) was the variable that best predicted mortality. Moon and Chun (2009) stated the following: "We suggested that ingestion of more than 35 g of endosulfan may be able to predict fatality." While this information is valuable, it is not reliable enough to be listed in Table 3-2. ATSDR also would like to point out that MRLs are not derived based on serious effects such as seizures or lethality. Moon and Chun (2009) as well as other human studies are presented in the Supplemental Document.

Comment: In reference to the Mechanism of Action section, the reviewer states that there is no mention of endosulfan's role as substrate and inhibitor of p-glycoprotein. The reviewer suggested including data from a study by Bain and Leblanc (1996).

Response: ATSDR reviewed the study by Bain and Leblanc (1996) and considered the information to be of too limited scope for inclusion in the profile. The study was conducted in B16/F10 murine melanoma cells transfected with the human MDR1 gene, which codes for P-gp. P-gp is a membrane-bound glycoprotein shown to efflux a variety of structurally-diverse anticancer drugs from neoplastic cells. Endosulfan was one of thirty-eight pesticides tested and showed slight though significant transport mediated by P-gp. It is unclear how this information relates to the well-known action of endosulfan on the GABA receptor that results in hyperactivity of the nervous system manifested as tremors and seizures. No change was made.

Comment: The comment refers to a paragraph in Section 3.9, INTERACTIONS WITH OTHER CHEMICALS, stating that phenobarbital has a mitigating effect on endosulfan toxicity in rats (Hoechst 1984c). The comment refers to the following sentence: "*It is possible that phenobarbital-induced microsomal enzymes increased the metabolism of endosulfan.*" The reviewer states that this is a pretty unlikely and an unnecessary hypothesis.

Response: Speculation on plausible mechanisms of interaction is allowed in this section. The reviewer states that the mitigating effect is due to phenobarbital's effect on the GABA A receptor. Unfortunately, no reference was provided. No change was made.

Comments provided by Reviewer #3.

Comment: The reviewer suggest that an intermediate-duration inhalation study in rats, cited as Hoechst (1984c) in the profile, could have been used to derive an intermediate-duration inhalation MRL for endosulfan. The reviewer states that a concentration of 0.002 mg/L (2 mg/m³) endosulfan was the study LOAEL based on emaciation, pale skin, squatting position and high-legged position, decreased body weight gain and food consumption, increased water consumption, and changes in clinical parameters. The NOAEL was 0.001 mg/L (1 mg/m³).

Response: The Hoechst (1984c) study, which was the only intermediate-duration inhalation study available. ATSDR reviewed the Hoechst (1984c) study and, in agreement with the study authors, concluded that there no significant adverse effects even at the highest concentration of endosulfan tested (0.002 mg/L). Since a LOAEL was not identified, in accordance with ATSDR's policy, the study was not appropriate for MRL derivation. Emaciation, pale skin, squatting position and high-legged position occurred in one male (out of 15) exposed to 0.002 mg/L. Males in this exposure group showed reduced weight on day 20 of the study and their body weight was lower than other groups until the end of the study (29 days after a 21 day exposure period), but the difference was not statistically significant. Food consumption was markedly reduced on day 20 in males from the 0.002 mg/L group. Some hematology and clinical chemistry parameters differed significantly from controls; however, they were within the normal range for the strain of rat used and in many cases were not exposure concentration-related. Organ weights were not significantly affected by exposure to endosulfan and gross and microscopic evaluation of tissues and organs did not reveal exposure-related changes. Based on these results, ATSDR considered the high concentration tested, 0.002 mg/L, the study NOAEL.

Comment: The reviewer disagrees with ATSDR's decision to derive an intermediate-duration oral MRL based on a study by Banerjee et al. (1986) that reported altered immunocompetence in male rats following

administration of endosulfan in the diet. The reviewer notes that the authors acknowledge that the study is preliminary and that an additional study by the same authors (Banerjee et al. 1987) reported immunological alterations only at higher doses of endosulfan. The reviewer further states that there are several additional studies of much wider scope than the Banerjee et al. (1986) study that found no evidence of gross or microscopic alterations in the lymphoreticular organs from rats, mice, or dogs and that could be considered for MRL derivation (Barnard et al. 1984, 1985; Brunk 1989; Donaubauer 1988; Edwards et al. 1984; Ruckman et al. 1989).

Response: ATSDR acknowledges that the Banerjee et al. (1986) study was considered preliminary by the authors and that a follow-up from the same group of investigators reported similar effects but at higher doses. For these reasons, ATSDR has suggested throughout the text that the study be replicated by other laboratories. All the studies mentioned by the reviewer have been reviewed by ATSDR and are included in the profile, although they are cited differently (Hoechst 1984a, 1984b, 1985a, 1988b, 1989a, 1989c). None of these studies examined immunocompetence and the LOAELs identified were higher than the LOAEL identified in Banerjee et al. (1986). As stated in Appendix A of the profile, proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology and Human Health Sciences (proposed), expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies, including EPA. The general public also gets the opportunity to comment before the final version of the profile is released. ATSDR will reevaluate this issue in the post-public version of the toxicological profile.

Comment: The reviewer notes that the studies by Cabaleiro et al. (2008) and Caride et al. (2010) are poorly performed and the results are often not interpretable. Cabaleiro et al. (2008) appears first in Section 3.2.2.5, Reproductive Effects and both Cabaleiro et al. (2008) and Caride et al. (2010) appear in Section 3.2.2.6, Developmental Effects. The reviewer states that she would not base any regulatory decisions on the results from these studies.

Response: The quality of the studies in question is good enough to be included in the profile. ATSDR finds that their conclusions are in line with the results that are shown in the figures, and the level of speculation regarding the interpretation of the results is acceptable. A sentence was added in the text stating that the toxicological significance of the changes in neurotransmitter levels in the brain of rat pups following perinatal exposure to endosulfan is unknown. A sentence was also added indicating that no information was provided regarding maternal effects. Neither study serves as basis for regulatory decisions.

Comment: The comment refers the subsection Chronic-Duration Exposure and Cancer in Section 3.12.2, Identification of Data Needs. The reviewer suggests that a 1-year oral study in dogs by Brunk (1989) would be a much better choice for derivation of a chronic-duration oral MRL for endosulfan than adopting the intermediate-duration oral MRL based on altered immunocompetence in rats as reported by Banerjee et al. (1986).

Response: The Brunk (1989) study, cited as Hoechst (1989c) in the profile, was considered for MRL derivation (see Section 2.3, Minimal Risk Levels). Among clinical chemistry parameters, dogs in the ≥ 10 ppm diets groups showed a significant increase in mean serum alkaline phosphatase activity relative to controls (up to approximately 2-fold) beginning at 1.5 months. In the absence of significant changes in other serum enzymes and lack of treatment-related histological alterations in the liver, the investigators did not consider the changes in alkaline phosphatase activity toxicologically significant. However, a review of the study by the EPA noted that the importance of the changes in alkaline phosphatase activity could not be judged without laboratory reference data. A better study was a 2-year bioassay in rats cited as Ruckman et al. (1989) by the reviewer and Hoechst (1989a) in the profile. This study was selected for derivation of a chronic-duration oral MRL for endosulfan. However, as discussed in Section 2.3, the

MRL that could have been derived was slightly higher than the intermediate-duration oral MRL. Therefore, ATSDR decided to adopt the intermediate-duration oral MRL as chronic-duration MRL.

Comment: The comment refers to a study by Abadin et al. (2007) that is cited in the immunotoxicity subsection of the Section 3.12.2. The profile cites the study as evidence that immune effects have been the most sensitive effects for a number of chemicals that have been the subject of ATSDR's toxicological profiles. The reviewer notes that the chlorinated compounds referred to in the Abadin et al. (2007) study are not comparable to endosulfan in metabolism, pharmacology, or toxicity. The reviewer adds that compounds like TCDD and DDT are very different from endosulfan and cannot be reasonably compared to endosulfan. The reviewer seems to imply that because endosulfan is not dioxin-like, it is unlikely that immune effects would occur at low doses.

Response: The chemicals for which MRLs are based on immunological and/or lymphoreticular effects discussed in the Abadin et al. (2007) study are: benzene, chlorfenvinphos, endosulfan, heptachlor, gamma-hexachlorocyclohexane (lindane), dibutyltin, tributyltin, PCBs, 2,3,4,7,8-pentachlorodibenzofuran, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and 2,4-dichlorophenol; DDT does not have MRLs based on immunotoxicity. The list of chemicals in the Abadin et al. (2007) study includes a wide variety of chemical structures and mechanisms of action. Interestingly, the intermediate-duration oral MRL for lindane, a chlorinated pesticide also known to interact with the GABA receptor and induce seizures, is based on immunological effects. So is the intermediate-duration oral MRL for chlorfenvinphos, a pesticide better known for its effects on the nervous system due to its anticholinesterase activity.