UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

8/2/1991

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: Endosulfan - Submission of Supplemental Data for the 30-Day Rat Study: Reconsideration of the RfD.

TO: Karen Samok
Product Manager (50)
SRRD (H7508C)

FROM: Linda L. Taylor, Ph.D. 8/7/91
Toxicology Branch II, Section II
Health Effects Division (H7509C)

Thru: K. Clark Swentzel. 8/7/91
Toxicology Branch II, Head Section II
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and

Marcia van Gemert, Ph.D. 8/12/91
Chief, Health Effects Division (H7509C)

Registrant: Hoechst Celanese Corporation
Chemical: 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9methano-2,4,3-benzodioxathiepin-3-oxide

Synonyms: Endosulfan; Thiodan; Benzoepin; Endocide
Project No.: 1-1060
MRID No.: 41715201 and 41799301
Record No.: not provided; Case #819236 , Submission: S393468

Action Requested: Please review Registrant's comments to Agency's toxicological evaluation/setting of RfD and subsequent denial of requested import tolerance on dried and spent hops; determine if RfD should be reevaluated.

Comment: The Registrant has submitted an amendment (MRID #417752001) to the 30-Day Rat Oral Toxicity Study (MRID # 40767601), which was designed as a follow-up study to the subchronic rat (MRID # 257727) and two-generation reproduction (Accession No. 256127) studies in which discoloration was observed in the proximal convoluted tubules of the kidney. In addition, a summary report regarding the yellowish discoloration of the renal proximal convoluted tubules

was submitted.

1) MRID # 41775201 - Study Title - Supplement to: Endosulfan - Active Ingredient Technical (Code: HOE 002671 OI ZC97 0003) 30-Day Feeding Study in Adult Male Wistar Rats (MRID # 40767601, Hoechst Doc. No. A37112) Results of the Macroscopic and Light and Electron Microscopic Examinations Following Administration of HOE 002671 (30 Days, Oral, Rat, Documentation No. 746/Path. No. 1778, see pages 22-31 of Original Report); author: KH Langer, dated February 1, 1991.

2) MRID # 41799301 - Study Title: Endosulfan Toxicology Summary and Risk Assessment; The Registrant's Response to EPA's Evaluation of the Existing Data Base dated November 20, 1990; authors: AC Katz, FJ Hawk, and K-H Leist, dated January 22, 1991.

Discussion: Endosulfan is an insecticide registered for use on a variety of crops. A Registration Standard was written in 1982, which identified several data gaps. Several studies have been performed on Endosulfan since 1982, and there are no data gaps. The RfD (0.0005 mg/kg/day) for Endosulfan is based on a two-generation reproduction study. This value was established using the lowest effect level from that study and a 300-fold safety factor. The effect of concern was an increased incidence of discoloration in cells of the proximal convoluted tubules of the kidney observed at all dose levels (see Table below). This effect was also observed in the subchronic oral (90-day) study in rats, and the RfD Committee concluded that this discoloration was indicative of a possible destructive effect of Endosulfan on erythrocytes.

Study dose ppm	Proximal tubule discolor	↓ HGB	↓ RBC	Enlarged/ prolifera lysosomes	↑ kidney weight
30-day 360/720	yes	ND*	ND	yes	HD M
90-day 10/30/60/360	yes	yes	yes	ND	both sexes
2-gen. repro. 3/15/75	yes	ND	ND	ND	both sexes
chronic/carc. 3/7.5/15/75	no	no	no	no	no
1-year dog 3/10/30	no	no	no	no	no
mouse carc. 2/6/18	no	ND	ND	no	no

* not determined

In the 13-week rat study, which included a 4-week recovery period, depressed RBC parameters were observed in the 60 and 360 ppm groups (6 & 13 weeks, both sexes) and in males at 30 ppm (6 weeks). There were several other statistically significant decreases from control, but these were not dose-related. In general, the magnitude of the differences observed at each time point is small (<10%). Kidney weight (relative) was increased in both sexes at the high dose (360 ppm) and in males at 60 ppm. The histopathological findings in the kidney in this study are summarized in Table 2 (current submission). It is stated that two types of findings were noted at 13 weeks: (1) occasional cells of proximal convoluted tubules showing yellowish discoloration of the cytoplasm (all dose levels) , and (2) darker and more particulate granular &/or clumped pigmentation, predominantly in cells of the straight portions, and to a lesser extent, the proximal convoluted tubules ((both sexes at 360 ppm/males at 60 ppm). From the data following the 4-week recovery period, the discoloration &/or pigmentation was not persistent after withdrawal of treatment, which the Registrant states is indicative of the ongoing process of excretion of test material via the kidneys. The Registrant concludes that, although this process can be observed (due to the coloration of the test material itself, a brown solid coinciding with the discoloration of the kidney tissues and the observation of "dark urine"), it is not indicative of an adverse effect on the kidney, as confirmed by the histopathological findings.

In the 30-day study in rats, which was designed to examine the kidney effects observed in the 2-generation reproduction and 90-day feeding studies and available to the RfD Committee, brownish discoloration of the kidney was observed, which was not evident after a 4-week recovery period. Proliferation and enlargement of lysosomes of the cells of the proximal convoluted tubules and granular pigmentation were observed at both dose levels tested (360 & 720 ppm) following 4 weeks of treatment but were not observed following the recovery period. In a follow-up report (not available at the time of the RfD Committee meeting), residue analysis studies indicated that alpha- & beta-endosulfan were temporarily stored in the kidneys in proportion to the administered dose; that only Endosulfan-sulfate and Endosulfan lactone were detected in any appreciable quantities in the kidney; only minimal levels of Endosulfan & metabolites were detected in the blood; only traces were detectable following the recovery period.

In the current submission, the results from the re-examination of the kidneys and liver from the highest dose group (720 ppm) animals of the 30-day study [obtained at study termination and stained with Prussian Blue stain to detect the presence of ferritin in tissues (evidence of hemosiderosis)] are presented. Sections from paraffin blocks of the tissues (after fixation in Carnoy's fluid or formalin) were made (apparently on 5 animals). Histochemical detection of trivalent iron was performed with the Prussian Blue

reaction. It was concluded that since the reaction was negative, it was evident that ferritin and/or hemosiderin were not stored in any of the cells (macrophages) or cytoplasmic compartments (lysosomes).

In the chronic rat study (not available at the time of the RfD Committee meeting), there was no similar effect (discoloration of tubules) noted in the kidneys as was observed in the reproduction and subchronic (30- and 90-day) studies. Hemosiderosis was observed as follows:

	Males	Females
Control	40/70	33/70
3 ppm	25/45	27/44
7.5 ppm	18/48	28/45
15 ppm	22/47	28/45
75 ppm	36/70	47/70

The incidence in all groups was considered to be within the normal range. Other effects noted in the chronic study include an increased incidence of blood vessel aneurysms in males at the highest dose level and, although there was no dose-related increase in the incidence of progressive glomerulonephrosis, an increase in the severity of the finding was noted. The NOEL was set at 15 ppm (0.65 mg/kg), the LEL at 75 ppm (3.45 mg/kg), based on reduced body-weight gain (both sexes), increased incidence of bilaterally enlarged kidneys, marked glomerulonephrosis, and blood vessel aneurysms in males. Tumor incidence was comparable among the groups.

Incidence of progressive glomerulonephrosis

	Males	Females
Control	55/70 (20)*	29/70 (1)
3 ppm	52/70 (18)	37/70 (6)
7.5 ppm	64/70 (22)	42/70 (6)
15 ppm	61/70 (24)	39/70 (5)
75 ppm	44/70 (30)	37/70 (8)

*(# with marked severity)

In order to determine whether residues of Endosulfan or its metabolites could be found in the kidney or livers of the animals of this study, tissues from all animals were removed at necropsy and analyzed for parent and primary metabolites. No residues above the limit of quantitation were observed in the liver or kidneys at 15 ppm or in the kidney at 75 ppm. The only measurable residues observed were Endosulfan-sulfate, an oxidation product, which was found in the liver at 75 ppm (ranging from 0.2-0.4 ppm, which the Registrant stated did not indicate a significant accumulation of test material or metabolites,.

Two additional studies not available at the time of the RfD Committee meeting are summarized below.

1) Chronic dog - Doses tested: 0, 3, 10, and 30 ppm for one year. The NOEL = 10 ppm (0.57 mg/kg M; 0.65 mg/kg F), the LEL = 30 ppm, based on decreased body-weight gain in males, increased incidence of neurologic signs in both sexes (loss or weakening of placing and righting reactions, tonic contractions of abdominal muscle and masticatory muscles a few hours after feeding).

2) Mouse carcinogenicity - Doses tested: 0, 2, 6, and 18 ppm. Systemic NOEL = 6 ppm, LEL = 18 ppm, based on mortality in females, body-weight reduction in males. negative for carcinogenicity, but histologically, the incidence of lymphosarcoma was high in dosed and control animals.

CONCLUSION

The observation of a yellowish discoloration of the kidneys was found in a 30-day feeding study, a 90-day feeding study, and a 2-generation reproduction feeding study, all performed in the rat. This observation was not found in the rat chronic toxicity/carcinogenicity study, the one-year dog study, or the mouse carcinogenicity study. NOTE: These latter three studies were not available at the time of the RfD Committee meeting. Copies of the DER's of these studies will be submitted along with this current review of the Registrant's submission to the RfD Committee for their consideration.

The Registrant argues that data from special and chronic toxicity and metabolism studies indicate that this is not an adverse hematopoietic effect, but is indicative of the physical presence and harmless process of elimination of endosulfan and its metabolites via the kidney. Electron microscopy and tissue residue analysis of the kidneys from the 30-day study indicated that the alpha-Endosulfan and to a lesser extent beta-Endosulfan, Endosulfan sulfate, and Endosulfan lactone were stored temporarily in the kidneys. Additionally, negative results were obtained from the staining of the kidneys with Prussian Blue to detect the presence of ferritin (evidence of hemosiderosis).

The arguments presented appear reasonable, and these will be provided to the RfD Committee for their consideration.

NOTE: The MRID # hand-written on the Supplement appears to be 41775201; the second 5 appears to have been changed to a 2. On the bean sheet, under Data Review Instructions, the number is 41715201. A second bean sheet (HED Project # 1-1184) attached to this same submission material was sent to TB II, and the 2 in the MRID # 41715201 has been changed to a 5 (handwritten) under Data Review Instructions. This latter copy of the Registrant's submission had the supplement identified with MRID # 41715501. TB II suggests that the Submission be identified by only one MRID #.