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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Chlorothalonil; ruminant feeding study protocol.
[DEB No.: 7760; MRID: n/a; DP Barcode: D162243]

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SRRD forwarded to CBRS a chlorothalonil Livestock Feeding Study protocol for review. The study protocol was submitted by Fermenta ASC Corporation in response to the Chlorothalonil Reregistration Standard. The protocol was prepared by Ricerca Inc, Painesville, OH.

Chlorothalonil ((2,4,5,6-tetrachloro-isophthalonitrile) is a fungicide marketed by Fermenta ASC Corporation. A Reg. Std. and FRSTR have been issued for chlorothalonil. The Residue Chemistry chapter is dated 3/30/84, the FRSTR is dated 3/11/88, and the Guidance Document is dated 9/88. Hexachlorobenzene (HCB) is an impurity in technical chlorothalonil at an upper certified limit of 0.05%.

Tolerances have been established (40 CFR 180.275) for the combined residue of the parent compound, chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile) and its metabolite (4-hydroxy-2,5,6-trichloro-isophthalonitrile) in or on numerous raw agricultural commodities (RACs) ranging from 0.05 ppm in or on

cocoa beans to 15 ppm in or on celery and papayas. No tolerances have been established for meat, milk, poultry, or eggs.

The chlorothalonil Guidance Document (9/88) concluded that the qualitative nature of the residue in animals was not adequately understood, and that data defining the nature of the terminal residue in poultry and ruminants were required. The Guidance Document specified dosing animals with [$^{14}$C]chlorothalonil, **only**. The previously issued Guidance Document dated 9/84 included a requirement for dosing with the 4-hydroxy metabolite as well as the parent.

**Summary of the Proposed Protocol:**

In response to deficiencies cited in the Reg. Std. the petitioner, Fermenta ASC has agreed to repeat the ruminant feeding study. A protocol entitled "Livestock Feeding Study, Lactating Cow Feeding Study with Chlorothalonil and SDS-3710" was submitted. The objective of the proposed feeding study is to determine the magnitude of the residue in meat, milk, poultry, and eggs resulting from chlorothalonil residues in livestock feed items.

**Test Substances**

Fermenta ASC is proposing dosing the animals with technical chlorothalonil (97.5% pure) and SDS-3701 (unspecified analytical purity). They state that technical grade chlorothalonil will contain no more than 0.04 ppm of hexachlorobenzene (HCB), the present analytical limit of detection for that compound. They state that the technical chlorothanonil used in the study will be characterized and the actual amount of HCB determined within the limits of the analytical method.

The test materials will be dispersed in a water-based suspension in which the test material is stable. The concentration of this suspension will be confirmed by separate analysis. Dosing will be accomplished by the use of gelatin capsules. The capsule will be administered as a bolus approximately at the AM and PM feedings. Gelatin capsules will be analyzed to confirm the identity and concentration of the dose.

**Summary of the Protocol Experimental Design**

Dairy cows producing near the average amount of milk per day for that particular dairy species (breed not specified) will be selected and put through an appropriate acclimation period prior to study initiation. Animals will be divided into five groups (four cows in each group), i.e.: control, 0.5X, 1X, 3X, and 10X as defined by the feeding level.

The registrant is proposing 3 ppm of chlorothalonil and 0.2 ppm of the 4-OH metabolite as the 1X feeding level. The following table explains how the registrant estimated the 1X feeding level.

| Feed Item | % of Diet | Max. Residue of Chlorotha-lonil in ppm | Max. Residue of SDS-3701 in ppm |
|---|---|---|---|
| Peanut, hay | 60 | 5.0 | 0.3 |
| Soybean, meal | 25 | 0.02 | 0.03 |
| Corn, field (untreated) | 15 | 0.0 | 0.0 |
| Total | 100 | | |
| Weighted Avg. | | 3.0 | 0.19 |

Table 1. Estimation of the 1X feeding level.

CBRS has previously estimated the 1X dietary burden to be 2.1 (see R. Perfetti Memorandum, 3/30/91). This estimation was based on established tolerances and feeding rates for a combination of the following feed items: dried tomato pomace (5 ppm, 25%), spent mint hay (2 ppm, 40%), and soybean seed (0.2 ppm, 35%). CBRS therefore accepts 3 ppm feeding level as the 1X dietary burden for chlorothalonil.

The registrant has proposed dosing both the parent compound chlorothalonil and its metabolite, SDS-3701 (4-hydroxy-chlorothalonil). Recently submitted metabolism studies suggest that SDS-3701 may constitute the majority of the total residue in tissue and milk (see R. Perfetti Memorandum, 1/30/91). In contrast the chlorothalonil FRSTR indicates that the parent compound constitutes 90% of the TRR in plants. The FRSTR also indicates that SDS-3701 is 4.2% of the residue in celery, 2% of the total residue in the Brassica leafy vegetables group, and 2.5% of the total residue in beans. Therefore, CBRS believes that a 0.2 ppm feeding level for SDS-3701 is adequate.

In fact, according to current CBRS/CBTS policy if the plant and animal metabolites of a pesticide are the same, animal feeding studies are required for the parent compound only. However, since the results of goat metabolism studies for chlorothalonil and its 4-OH metabolite (see R. Perfetti Memorandum, 1/30/91) suggest SDS-3701 residues in/on plants may constitute the major source of SDS-3701 residues in tissue and milk, CBRS supports dosing livestock with both the parent and its 4-OH metabolite.

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The feeding levels proposed by the registrant are listed in Table 2.

| Feeding Level | Chlorothalonil | 4-OH Metabolite (SDS-3701) |
|---|---|---|
| Control | 0 | 0 |
| 0.5 X | 1.5 | 0.1 |
| 1 X | 3.0 | 0.2 |
| 3 X | 9.0 | 0.6 |
| 10 X | 30.0 | 2.0 |

Table 2. Proposed feeding levels of chlorothalonil and SDS-3701.

Complete records will be kept on each individual animal during both acclimation and study periods including: age, breed, weight, milk production, feed consumption, health, and behavioral changes.

The animal diet will consist of a concentrate grain ration (circa 40% of total diet) fed twice a day plus roughage (alfalfa hay, circa 60% of total diet). Water will be fed ad libitum. The animals will be dosed by application of appropriate amounts of technical chlorothalonil and SDS-3701 at both the AM and PM feedings. The amount of food consumed per animal per day will be recorded.

**Sample Collection**

Milk will be collected from both the AM and PM milkings. The total amount produce will be measured and recorded for each individual cow. Aliquots of the AM and PM sample will be pooled per cow on a daily basis in the same ratio produced. Samples of milk fat will be collected from the pooled AM and PM samples and analyzed to determine the ratio of any detected residue between the whole milk and milk fat.

Animals will be sacrificed within 24 hours of the administration of the final dose. Muscle (from 2 or 3 separate locations), subcutaneous fat, perirenal fat, liver, and kidney will be collected. All samples will be frozen as soon as possible after collection.

Additionally, some samples of feed items, milk, milk fat, and or animal body fat from individual cows during acclimation will be analyzed for chlorothanonil, SDS-3701, and HCB if a background level of HCB is observed in milk fat.

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**Sample Analysis**

The registrant did not provided detailed information concerning the analytical method. The protocol does state that chlorothalonil, SDS-3701, and HCB will be extracted and partitioned into an organic solvent. Residues of chlorothalonil and HCB will be "cleaned-up" using column chromatography (unspecified type) prior to analysis using a GC equipped with an electron capture detector (GC/ECD). The protocol states that detailed information will appear in the protocol and that the analytical method will be validated prior to the assaying of study samples.

The intended analytical detection limits for chlorothalonil residues in meat, milk, and milk fat are provided in Table 3.

| Residue | Detection limits for meat | Detection limit for milk | Detection limit for milk fat |
|---|---|---|---|
| Chlorothalonil | 0.001 ppm | 0.001 ppm | 0.001 ppm |
| SDS-3701 | 0.01 ppm | 0.01 ppm | 0.01 ppm |
| HCB | 0.001 ppm | 0.001 ppm | 0.001 ppm |

Table 3. Intended analytical detecton limits for chlorothalonil, SDS-3701, and HCB residues in meat, milk, and milk fat.

The registrant states that complete details of the analytical method will be provided in the final study report. This report will include QC data such as fortification data, and control samples.

**CBRS Comments**

1. The submitted protocol is very general, therefore CBRS cannot provide comprehensive or specific detailed comments concerning the adequacy of the protocol.

2. Recently submitted metabolism studies for chlorothalonil in ruminants (goats, MRID #'s 41576001 and 41576002) do not entirely correct the deficiencies in the ruminant metabolism studies (see R. Perfetti Memorandum, 1/30/91). Only 45.4% of the residue in milk, 6% of the residue in liver, and 3% of the residue in kidney were conclusively identified. The 4-OH metabolite was the only compound identified. Nonextractable residues accounting for up to 33% of the residue in liver and 43% in the kidney, these residues were not characterized.

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3. Hexachlorobenzene (HCB) is a manufacturing impurity present in technical grade chlorothalonil at an upper certified limit of 0.05%. HCB is a $B_2$ oncogen, with a $Q^*$ of 1.7 $(mg/kg/day)^{-1}$, which is 150X that of chlorothalonil itself ($Q^*$ of 0.011 $(mg/kg/day)^{-1}$) (See D. Edwards, 4/11/89). Published studies in the open literature and data submitted by registrants for other pesticides show that HCB will transfer from livestock feed items to meat and will bioaccumulate in fat tissue.

   The registrant states that the HCB contamination used for these studies will contain no more than 40 ppb (0.04 ppm) HCB (limit of detection for HCB in technical grade chlorothalonil). CBRS recommends that the technical grade chlorothalonil used in the feeding study contain the maximum amount of HCB contamination [redacted] in the Statement of Formulation.

   Due to the high $Q^*$ of HCB, CBRS also recommends that all tissue samples, especially fat samples, be analyzed for HCB at a 0.001 ppm limit of detection if possible. CBRS also suggests that tissues from the control animals also be analyzed for HCB.

4. The registrant did not define how they would determine if a residue plateau level is reached in milk. CBRS suggests three consecutive days with the same magnitude of the residue.

**Recommendations**

CBRS recommends that the registrant be provided with a copy of this review and a copy of the Standard Evaluation Procedure for Residues in Meat, Milk, Poultry and Eggs: Feeding Studies/Feed-through be provided to the petitioner (attached).

Attachment: Standard Evaluation Procedure for Residues in Meat, Milk, Poultry and Eggs: Feeding Studies/Feed-through by R. A. Loranger, EPA 540/09/90-087, NTIS: PB 90-208943 (15 pages).

CC: Reviewer;SF;PMSD/PIB(C.Furlow);~~DRES~~;Circulation.
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Manufacturing Process Information is not Included