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

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

**MEMORANDUM**

SUBJECT: Chlorothalonil: PP#6F4611: Reregistration Case No. 0097: Chemical No. 08190: MRID Nos. 438324-01, -02, -03 and -04: CBRS No. 16482: DP Barcode D220825.

FROM: William O. Smith, Chemist
Chemistry Pilot Review Team
Chemistry Branch II-Reregistration Support (CBRS)
Health Effects Division (7509C)

THROUGH: Edward Zager, Chief
Chemistry Branch II-Reregistration Support
Health Effects Division

TO: Rose Kearns/Cynthia Giles-Parker (PM 22)
Registration Division

and

Andrew Ertman/Walter Waldrop
Reregistration Branch
Special Review & Reregistration Division (7508W)

In response to reregistration data requirements ISK Biosciences has submitted a petition to establish the following tolerances for 4-hydroxy-2,5,6-trichloroisophthalonitrile (SDS-3701), a metabolite of chlorothalonil.

| | |
|---|---|
| milk | 0.1 ppm |
| Fat | 0.1 ppm |
| kidney | 0.5 ppm |
| mbyp (except kidney) | 0.05 ppm |
| meat | 0.03 ppm |

The meat tolerances apply to cattle, goats, hogs, horses and sheep.

## BACKGROUND

CBRS has previously approved a protocol for a feeding study to establish appropriate meat and milk tolerances (D. McNeilly, 7/11/91, D162243). It was agreed that the registrant would dose the animals with both chlorothalonil and SDS-3701; the suitable dosing levels were also agreed upon. Subsequently the registrant proposed to modify the feeding study, analyzing only for SDS-3701 and excluding chlorothalonil from the tolerance expression. The registrant supported this request with information demonstrating that chlorothalonil is very rapidly metabolized once ingested by livestock while SDS-3701 is not. In our review of this request (W. Smith, 10/14/94, D199685) we concluded that detectable residues of chlorothalonil are not likely to transfer from the diet of livestock to meat and milk commodities and the feeding study could be modified to analyze only for SDS-3701; however, in conformance with Branch policy, chlorothalonil must remain in the tolerance expression and enforcement methodology for both chlorothalonil and SDS-3701 must be validated for animal commodities.

The present submission includes a ruminant feeding study, a description of the analytical method for SDS-3701, a storage stability study for SDS-3701 and two studies concerning the instability of chlorothalonil in bovine tissues.

## CONCLUSIONS

1. Residues of chlorothalonil, per se, are not expected to transfer from feed items to meat and milk but residues of the 4-hydroxy metabolite (SDS-3701) could occur in these commodities.

2. Due to the instability of chlorothalonil per se in meat and milk tissues, residues would not be expected to occur even from misuse of chlorothalonil.

3. The chlorothalonil residue of concern in meat and milk is SDS-3701. It is not practical to regulate based on residues of chlorothalonil in these commodities.

4. The analytical method submitted by the registrant is adequate for analysis of SDS-3701 in meat and milk; however, a complete description of a method, along with an independent laboratory validation, must be submitted for consideration by the Agency for inclusion in PAM Vol II.

5. The submitted feeding study is adequate for determination of appropriate tolerance levels in meat and milk. The analytical results are supported by adequate frozen storage stability data. No significant losses of SDS-3701 residues occurred during the frozen storage of analytical samples.

6. The proposed tolerances are adequate to cover residues of SDS-3701 that might occur in meat and milk as the result of chlorothalonil uses on animal feed items.

## RECOMMENDATIONS

The registrant should be informed that a complete description of a suitable enforcement method for SDS-3701 in meat and milk must be submitted. This method must be validated by an independent laboratory as specified in PR 88-5.

Contingent upon approval of a suitable enforcement analytical method for residues of SDS-3701 in meat and milk, CBRS recommends in favor of establishment of tolerances as petitioned by the registrant.

## DETAILED DISCUSSION

Nature of the Residue in Plants

The qualitative nature of the residues in plants is adequately understood based on metabolism studies with carrots, celery, lettuce, snap beans, and tomatoes. The residues of concern are chlorothalonil and its 4-hydroxy metabolite (W. Smith, 12/15/93, D185139).

Nature of the Residue in Animals

The metabolism of chlorothalonil and 4-hydroxy chlorothalonil in ruminants is adequately understood based on goat metabolism studies. Little metabolism of the 4-hydroxy metabolite occurs in ruminants and the unchanged test substance accounted for 88-99% of the TRR in milk and edible tissues. The proposed pathway for chlorothalonil metabolism in ruminants involves substitution of one or more of the chlorine atoms with glutathione; these complexes may undergo further modification of the glutathione side chains to yield a variety of products (R. Perfetti, 1/30/91, CB No. 7112; P. Deschamp, 11/4/93, D174779). Although CBRS has concluded that the residue of concern in ruminants is chlorothalonil and 4-hydroxy chlorothalonil, we have also accepted the registrant's argument that there is not significant transfer of chlorothalonil to meat or milk from feed (W. Smith, 10/14/94, D199685).

Based on poultry metabolism studies using [$^{14}$C]chlorothalonil and 4-hydroxy-[$^{14}$C]chlorothalonil, CBRS concluded that there is no significant transfer of chlorothalonil to poultry tissues or eggs, and that the levels of transfer of the 4-hydroxy metabolite are too low to require feeding studies or tolerances for poultry commodities (W. Smith, 11/2/94, D196755).

Analytical Methods

The registrant submitted a description of an analytical method for determination of residues of SDS-3701 in meat and milk (MRID 43832403). Residues of SDS-3701 were extracted from milk and tissues with acid acetone and partitioned into diethyl ether. Residues of SDS-3701 were methylated and cleaned up with alumina column chromatography prior to

quantitation by GLC using an electron capture detector. The procedures are described in detail in MRID 43832403. The limit of quantitation, which was determined as the lowest concentration fortified sample analyzed, was 0.01 ppm. The validation data for this method are summarized in Table 1.

Table 1. Method Validation data for SDS-3701. Fortification levels for all matrices were 0.01 ppm to 1.00 ppm.

| Sample Matrix | Recovery (%) | | | |
|---|---|---|---|---|
| | Minimum | Maximum | Mean | Std Dev (n) |
| Milk | 80 | 100 | 92 | 6.5 (8) |
| Butterfat | 80 | 120 | 100 | 13.5 (6) |
| Skim Milk | 106 | 124 | 115 | 7.1 (6) |
| Loin Muscle | 91 | 110 | 99 | 8.4 (6) |
| Round Muscle | 95 | 110 | 104 | 5.7 (8) |
| Fat | 110 | 120 | 117 | 5.8 (3) |
| Kidney | 77 | 110 | 96 | 16.9 (4) |
| Liver | 80 | 96 | 87 | 6.4 (6) |

Recovery data were also obtained concurrently with analysis of the meat and milk samples and controls. A summary of these recoveries is included in Table 2.

Table 2. A summary of concurrent recoveries of SDS-3701 conducted in conjunction with analyses of meat and milk commodities.

| Matrix | Fortification Range (ppm) | Number Samples Analyzed | % Recovery | | | |
|---|---|---|---|---|---|---|
| | | | Min. | Max. | Mean | Std Dev (n) |
| Milk | 0.01-1.00 | 116 | 70 | 120 | 99 | 9.6 (116) |
| Skim Milk | 0.01-0.80 | 16 | 86 | 112 | 97 | 6.9 (16) |
| Butterfat | 0.01-0.50 | 16 | 84 | 110 | 97 | 6.9 (16) |
| Omental fat | 0.01-0.50 | 3 | 82 | 100 | 91 | 9.0 (3) |
| Perirenal fat | 0.01-0.50 | 4 | 71 | 96 | 83 | 10.4 (4) |
| Loin Muscle | 0.01-0.20 | 4 | 78 | 103 | 89 | 12.0 4() |
| Round Muscle | 0.01-0.50 | 4 | 90 | 110 | 102 | 8.7 (4) |
| Liver | 0.01-0.50 | 4 | 76 | 106 | 94 | 14.2 (4) |
| Kidney | 0.01-5.00 | 4 | 94 | 120 | 101 | 13.0 (4) |

The submitted method is adequate for residue collection; however, in order to determine its usefulness for enforcement purposes, the registrant must provide a satisfactory independent laboratory validation and a complete description of this method suitable for inclusion in PAM, Vol II.

Storage Stability of SDS-3701

A study was submitted concerning the stability of SDS-3701 in the presence of whole milk, muscle, liver and fat stored under frozen conditions (MRID No 43832402, Ricerca, Inc. study No. 5927-93-0329). Samples were fortified with SDS-3701, sealed in jars with teflon lined caps and placed in frozen storage. The fortification levels were 0.20 ppm for muscle and fat and 1.00 ppm for milk and liver. At sampling intervals of 1 day, 7 days, 14 days, 1 month, 2 months, 3 months, 6 months, 9 months and one year, stored samples, along with controls fortified on the day of analysis, were analyzed. Results demonstrated that residues of SDS-3701 were stable in milk under frozen conditions for at least one year. During one year of frozen storage, residues of SDS-3701 declined in muscle by 8 %, fat by 9 % and liver by 17 %. The maximum storage intervals for each of the tissues were 219 days for

milk, 62 days for muscle, 20 days for fat, 61 days for liver and 27 days for fat. We conclude that this study adequately supports the analytical results in the accompanying meat and milk study and that no significant losses of SDS-3701 residues occurred during the frozen storage of these samples.

Stability of Chlorothalonil in Meat and Milk.

A study was submitted concerning the freezer storage stability of chlorothalonil in milk and cow tissues (MRID 43832401). Samples of whole milk, muscle, liver, kidney and body fat were fortified with chlorothalonil at 0.20 ppm and placed in a freezer. At sampling intervals up to 29 days samples were removed from frozen storage and analyzed along with the appropriate controls and concurrent recovery samples. A summary of the results, taken from the registrant's study, is shown in Table 3.

Table 3. Decline of Chlorothalonil Residues During Frozen Storage in Meat and Milk.

| Sample Matrix | Mean Percent Decline of Chlorothalonil Uncorrected Values | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 hr | 8 hr | 16 hr | 24 hr | 48 hr | 4 day | 7 day | 14 day | 29 day |
| Liver | 27 | 91 | 94 | 100 | - | - | - | - | - |
| Muscle | 19 | 75 | 90 | 90 | 95 | - | - | - | - |
| Kidney | 21 | 74 | 87 | 87 | 94 | - | - | - | - |
| Milk | 0 | 13 | 51 | 59 | 82 | 91 | - | - | - |
| Fat | 23 | 22 | 27 | 26 | 28 | 33 | 29 | 32 | 41 |

The loss of chlorothalonil was very rapid in all tissues except fat.

A study was submitted (MRID 43832404) entitled "Reaction Kinetics of Chlorothalonil with Ruminant Tissues". A preliminary submission of these data was reviewed previously (W. Smith, 10/14/94, D199685). Chlorothalonil was incubated with bovine tissue homogenates, blood and model compounds at physiological temperatures. The half-life of chlorothalonil was one minute or less in the tissues studied. The products from incubations consisted primarily of water soluble metabolites, which were examined by reverse phase HPLC. The major product eluted identically to the diglutathione conjugate of chlorothalonil. Lesser amounts of metabolites matched the monoglutathione and triglutathione conjugates in their elution patterns.

The model compounds included reduced glutathione and the N-acetyl derivatives of histidine, lysine, arginine, tryptophan, tyrosine, and cysteine. Only glutathione and N-acetyl cysteine reacted at a measurable rate with half-lives for chlorothalonil of ca. 1 min. and 3-5 min.

The registrant concludes that chlorothalonil reacts very rapidly with thiol groups of cellular components under physiological conditions of temperature and pH. Chlorothalonil will be metabolized to other products within seconds or minutes after absorption from the gut; therefore, chlorothalonil will not be transmitted as an intact residue in meat or milk.

CBRS has previously accepted the registrant's argument, based on preliminary reports of the above two studies, that detectable residues of chlorothalonil are not likely to transfer from the diet of livestock to meat or milk commodities (W. Smith, 10/14/94, D199685) and we continue to agree with this conclusion.

In our earlier memorandum CBRS also concluded that for enforcement purposes it is generally Agency policy that the parent compound is included in tolerance expressions, even in those cases where it is not expected to occur under normal use practices. Therefore, we stated that "in conformance with Branch policy, chlorothalonil must remain in the tolerance expression and enforcement methodology for both chlorothalonil and SDS-3701 must be validated for animal commodities." On review of the complete data packages it is clear that it would not be practical to regulate chlorothalonil residues in meat and milk commodities because the likelihood of ever detecting chlorothalonil residues in these commodities from misuse would be exceedingly small. Therefore, we agree with the registrant that meat and milk tolerances should be expressed in terms of SDS-3701 only.

Magnitude of SDS-3701 Residues in Meat and Milk

The in-life portion of this study was conducted by Bio-Life Associates, Ltd, Neillsville, WI. All analyses were conducted by Ricerca, Inc., Department of Residue Analysis, Painesville, OH.

Twenty Holstein dairy cows were randomly divided into groups of 4 and dosed with 0.5X, 1X, 3X, or 10X levels. The 1X dose was 3 ppm chlorothalonil and 0.2 ppm SDS-3701. Animals were dosed once a day for 28 days. Milk samples were collected twice daily from each cow and pooled in proportion to production. Animals were sacrificed at 28 days and frozen samples of milk, muscle, liver, fat and kidneys were shipped to Ricerca for analysis.

The SDS-3701 level in milk reached a plateau in about 10 days (Table 4). Processing of milk into butterfat and skim milk did not result in concentration of residues in either fraction.

Table 4. Average Daily Residues of SDS-3701 in Milk

| Day | Residues of SDS-3701 in Milk (ppm) | | | |
|---|---|---|---|---|
| | 0.5X Dose | 1X Dose | 3X Dose | 10X Dose |
| 1 | ND | ND | 0.01 | 0.02 |
| 2 | ND | 0.01 | 0.04 | 0.10 |
| 3 | 0.02 | 0.02 | 0.08 | 0.16 |
| 4 | 0.01 | 0.03 | 0.11 | 0.23 |
| 5 | 0.02 | 0.03 | 0.11 | 0.24 |
| 6 | 0.02 | 0.02 | 0.14 | 0.32 |
| 7 | 0.02 | 0.04 | 0.17 | 0.33 |
| 8 | 0.03 | 0.05 | 0.16 | 0.40 |
| 9 | 0.03 | 0.04 | 0.16 | 0.39 |
| 10 | 0.03 | 0.05 | 0.16 | 0.44 |
| 11 | 0.03 | 0.05 | 0.16 | 0.42 |
| 12 | 0.02 | 0.04 | 0.19 | 0.43 |
| 13 | 0.03 | 0.05 | 0.20 | 0.47 |
| 14 | 0.03 | 0.06 | 0.20 | 0.50 |
| 15 | 0.02 | 0.06 | 0.22 | 0.43 |
| 16 | 0.03 | 0.05 | 0.19 | 0.51 |
| 17 | 0.03 | 0.05 | 0.22 | 0.46 |
| 18 | 0.03 | 0.06 | 0.23 | 0.44 |
| 19 | 0.03 | 0.06 | 0.22 | 0.49 |
| 20 | 0.03 | 0.06 | 0.22 | 0.47 |
| 21 | 0.03 | 0.05 | 0.16 | 0.46 |
| 22 | 0.03 | 0.05 | 0.17 | 0.53 |
| 23 | 0.03 | 0.06 | 0.20 | 0.47 |
| 24 | 0.04 | 0.07 | 0.18 | 0.52 |
| 25 | 0.03 | 0.06 | 0.20 | 0.55 |
| 26 | 0.04 | 0.07 | 0.20 | 0.51 |
| 27 | 0.04 | 0.07 | 0.22 | 0.48 |
| 28 | 0.03 | 0.07 | 0.21 | 0.49 |

Maximum residues in the milk and tissues at all four dosing levels are summarized below in Table 5.

Table 5. Maximum residues of SDS-3701 in meat and milk as a function of feeding level.

| Tissue | Maximum SDS-3701 Residues (ppm) | | | |
|---|---|---|---|---|
| | 0.5X Dose | 1X Dose | 3X Dose | 10X Dose |
| Milk | 0.04 | 0.10 | 0.31 | 0.65 |
| Muscle | ND | 0.02 | 0.09 | 0.24 |
| Fat | 0.03 | 0.07 | 0.08 | 0.85 |
| Liver | 0.03 | 0.04 | 0.18 | 0.55 |
| Kidney | 0.14 | 0.28 | 0.55 | 1.19 |

Based on these results the registrant is proposing tolerances of 0.10 ppm for milk, 0.03 ppm for meat, 0.05 ppm for liver and meat by-products, 0.10 ppm for fat and 0.5 ppm for kidney. These proposals are appropriate.

cc: W. Smith (CBRS), M. Clock (HED/RCAB,) Reg Std File, SF, RF, Circ.

7509C:CB-II:WOS:wos:Rm805A:CM2:305-5353:02/29/96
RDI: Team(02/29/96) EZager(03/01/96).