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

SEP 23 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: **Tetrachlorvinphos.** Addendum to RED. PTRL Response to EPA Review of Nature of Residue Study (MRID Nos. 428288-01, 428288-02, and 428288-03). Request Dated August 11, 1994
CBRS No.:14227; DP Barcode: D206721; No MRID No.; Case No. 0321.

FROM: David J. Miller, SA HSO, US Public Health Service DJM
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TO: Dennis McNeilly, PM Team 73
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In a letter dated August 11, 1994, PTRL East, Inc. (PTRL) is responding to an EPA review of the Nature of the Residue in ruminants (oral administration), Nature of the Residue in ruminants (dermal application) and Nature of the Residue in Poultry (dermal application) reviewed by J. Abbotts (10/5/93, CBRS No. 12240). CBRS had previously judged these studies to be upgradable, pending submission of additional information.

In the present submission, the registrant has provided responses to the following CBRS requests for additional information:

- an explanation of why metabolite profiles in liver and kidney were considered stable during frozen storage, since direct comparisons over the duration of storage could not be made.

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- thirty percent of the TRR was lost during pronase digestion of the Soxhlet pellet and portioning with ethyl acetate. The registrant should provide data to explain why it is justified in assuming that the chromatographic profile of the organic pronase fraction accurately represents the lost residues as well.

- identification and/or characterization of residues in eggs with the highest TRR (i.e., in the Day 6 post-treatment sample as opposed to the 24 hr.-Sacrifice sample).

- Metabolite M7 represented 0.087 ppm and 24.2% of TRR in egg and should be identified.

CONCLUSIONS:

The registrant has satisfactorily addressed the concerns identified in the J. Abbotts 10/5/93 review. CBRS now makes the following conclusions:

1. The registrant has shown that liver and kidney extracts were stable over the frozen storage interval. The requirement expressed in Conclusion 1f of the J. Abbotts review is thus satisfied, and the goat metabolism study (oral administration) is now considered acceptable.

2. The registrant extracted a new (Month 12) fat sample taken from frozen storage, which adequately addresses the mass-balance concerns raised by the J. Abbotts review: reanalysis of the fat sample after 12 months of frozen storage demonstrated a nearly identical metabolite profile, without any recovery losses seen during sample preparation. The registrant has adequately addressed the requirement raised in Conclusion 3d of the J. Abbotts review.

3. The registrant has shown that no *new* metabolites were identified in the Day 6 egg samples when compared to the 24 hr.-Sacrifice samples. CBRS thus concludes that the chromatographic profile from eggs collected at Day 6 are essentially the same as the profiles from eggs collected just prior to sacrifice, and that Conclusion 3e of the J. Abbotts review is satisfied.

4. The registrant has provided adequate evidence that Metabolite M7 was a contaminant in the original study, and thus further identification of this compound is not necessary. Conclusion 3f of the J. Abbotts review has thus been adequately addressed. In accordance with the decision reached by the HED Metabolism Committee, CBRS concludes that the residues to be regulated are tetrachlorvinphos; des-O-methyltetrachlorvinphos; 1-(2,4,5-trichlorophenyl)-ethanol in free and conjugated form; 2,4,5-

trichloroacetophenone; and 1-(2,4,5-trichlorophenyl)-ethanediol.

RECOMMENDATIONS:

The registrant should be informed that Conclusions 1f, 3d, 3e, and 3f of the J. Abbotts 10/5/93 review (CBRS No. 12240) have been adequately addressed. CBRS thus concludes that the requirements for goat metabolism (oral dosage) and poultry metabolism (dermal application) studies have been fulfilled.

The RED document should be updated to reflect this additional information. The necessary changes are indicated (via redlining and strikeouts) in the Attachment.

DETAILED ANALYSIS

PTRL had earlier submitted a series of nature of the residue studies in ruminants (oral and dermal exposure) and poultry (dermal exposure only) for tetrachlorvinphos (MRIDs 42828801, 42828802, and 42828803). Upon review of this earlier studies, CBRS concluded that further work would be necessary to upgrade certain aspects of these studies. The registrant was required to perform this additional work and submit this information to CBRS.

The following is a summary of the original J. Abbotts review statements, PTRL comments, and CBRS's response for each study.

Ruminant, Oral Administration (MRID No. 42828801)

Reviewer Statement:

> "The registrant should provide an explanation of why metabolite profiles in liver and kidney were considered stable during frozen storage, since direct comparisons over the duration of storage could not be made."

PTRL Comments:

> PTRL does admit that since extraction and chromatographic methods were modified during this time period to improve extractability and chromatographic resolution, direct comparisons between the Month 1 and Month 8 samples were not possible due to minor changes in extraction technique and chromatographic conditions. PTRL does, however, emphasize that the two sets of analyses were more similar than they were different: both procedures used methanol as the primary extraction solvent, and chromatographic analyses were performed with the same HPLC C18 reverse phase column with modified water:organic solvent gradients as the mobile phase. HPLC System I (used on the one month liver extract) showed a single major radioactive peak accounting for 85.9% of the sample radioactivity (which was subsequently identified as 2,4,5-trichloroacetophenone) with a minor component eluting near the void volume accounting for ca. 15% of sample radioactivity. The finding, the registrant suggests, is comparable to the HPLC System 2 analysis of the month 8 liver extract which showed that the only metabolite present in the primary extract was 2,4,5-trichloroacetophenone. The registrant attributes the presence of the minor component in the Month 1 extract and its absence in the Month 8 extract to the use of a higher polarity solvent with the Month 1 sample: the higher polarity solvent used to extract the Month 1 sample resulted in the partial extraction of the more polar 1-(2,4,5-trichlorophenyl) ethanol glucuronide present in the sample. Based on similar findings between the Month 1 and Month 8 samples, the registrant concludes that the residues in goat liver were stable over the duration of the 8 month frozen storage period.

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The registrant also states that the stability finding for kidney were similar to those for liver. HPLC System 1 analysis of the Month 1 kidney extract showed a single major radioactive peak (91.1% of sample radioactivity) with a minor component (8.9%) eluting near the void volume. The major residue of this Month 1 extract did not co-elute with any of the standards: this finding was nearly identical to the HPLC analysis of the Month 8 kidney extract which showed the presence of only one unknown metabolite which was subsequently identified as 1-(2,4,5-trichlorophenyl)-ethanol glucuronide during the definitive analysis.

Based on the chromatographic findings and simplicity in the metabolic profiles in kidney and liver (i.e., only one major metabolite), the registrant concludes that the incurred residues in goat liver and kidney were stable over the duration of the 8-month storage period.

CBRS Response:

CBRS is in agreement with the registrant's contentions and thus concludes that Conclusion 1f of the J. Abbotts 10/5/93 review is resolved. Therefore, the goat oral metabolism study is judged acceptable.

Poultry Dermal Application (MRID No. 42828802)

Reviewer Statement:

"The highest TRR was found in fat at 13.5 ppm. However, 30% of the TRR was lost during pronase digestion of the Soxhlet pellet and portioning with ethyl acetate. Registrant should provide data to explain why it is justified in assuming that the chromatographic profile of the organic pronase fraction accurately represents the lost residues as well."

PTRL Comments:

The performing laboratory extracted a new (Month 12) fat sample taken from frozen storage, and extracted and analyzed this sample in the same manner as the original fat sample.

The laboratory reported that extraction results from the Month 12 sample were significantly different from the original extraction results reviewed by J. Abbotts: in the Month 12 sample, 97.8% of the TRR was accounted for in the primary organic extract, whereas only 40.8% was accounted for in the original (Month 0-2) analysis. While this finding would normally suggest that the nature of the fat matrix had changed during frozen storage in such a way that incurred residues were more easily

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extracted, chromatographic analysis of the Month 12 sample showed a similar metabolic profile to the original profile generated from the Month 0-2 sample. This comparison is shown below in Table 1.

Table 1. Comparison of Residue Profile found in Original (Month 0-2) Analysis and New (Month 12) Analysis.

Residue/Metabolite	Residue Level, % extract radioactivity	
	Month 0-2 Sample	Month 12 Sample
Tetrachlorvinphos	82.9%	75.7%
2,4,5-trichloroacetophenone	3.5%	3.5%
1-(2,4,5-trichlorophenyl)-ethanol	6.1%	7.7%
1-(2,4,5-trichlorophenyl)-ethanediol	1.8%	2.0%

The performing laboratory concludes that the reported loss of 30% TRR seen during the original (Month 0-2) pronase digestion of the fat Soxhlet pellet was an experimental artifact. Reanalysis of the fat sample after 12 months of frozen storage demonstrated a nearly identical metabolite profile, without any recovery losses seen during sample preparation.

CBRS Response:

The registrant extracted a new (Month 12) fat sample taken from frozen storage. In this extraction mass balance was achieved, and CBRS concludes that the requirement that the registrant is justified in its assumption that the chromatographic profile of its organic pronase fraction accurately represents the lost residues as well is fulfilled.

Reviewer Statement:

"In accordance with the approved protocol, the registrant should identify and/or characterize residues in eggs with the highest TRR. If data indicate that chromatographic profiles of fractions from eggs collected at day 6 post-treatment or later are essentially the same as eggs collected just prior to sacrifice, then this Conclusion would be resolved."

PTRL Comments:

The performing laboratory removed a Day 6 egg sample from frozen storage and

extracted and analyzed this in the same manner as the original (24 hr.-Sacrifice) egg sample. A new (24 hour-Sacrifice) egg sample removed from frozen storage was also analyzed at this time for comparative purposes.

The laboratory found that extraction from the Day 6 egg sample were different from the original 24 hr-Sacrifice sample in two ways. Firstly, extraction efficiency was higher in the Day 6 sample (83.8% of TRR accounted for in primary extracts) than in the original sample (55.4% of TRR accounted for in primary extracts). Secondly, the nature of the extracted residues in the Day 6 sample was different since a large proportion (20.9%) of the residues remained in the aqueous fraction.

Chromatographic analysis of the Day 6 *organic* extract showed two major residues which co-eluted with 1-(2,4,5-trichlorophenyl)-ethanediol and 1-(2,4,5-trichlorophenyl)ethanol. These residues accounted for 44.9% and 18.2% of the TRR, respectively. A minor residue was also tentatively identified as 2,4,5-trichloroacetophenone and accounted for 1.3% of the TRR. *[CBRS notes that these results contrast slightly with the results of the original extraction in which the ethanediol, ethanol, and acetophenone compounds comprised 36.3%, 8.6%, and 1.3%, respectively.]*

Chromatographic analysis of the Day 6 *aqueous* extract showed a single major radioactive peak comprising 15.8% of the TRR which was identified as comprised mainly of a conjugate of 1-(2,4,5-trichlorophenyl)ethanol, a minor residue identified as des-O-methyl-tetrachlorvinphos, a minor residue (4.0% of TRR) identified as 1-(2,4,5-trichlorophenyl)ethanediol, and two more unknown components.

The laboratory concluded that the only difference in incurred residues present in the Day 6 egg sample compared to the 24 hrs-Sacrifice egg sample was that the Day 6 sample contained a higher proportion of conjugated metabolites: the analytical results of the two samples were comparable and the residues were stable during frozen storage. Thus the primary metabolic processes and metabolic pathway were determined to be the same for both egg samples.

CBRS Response:

The HED Metabolism Committee has tentatively determined that the residues of concern in animal commodities are parent tetrachlorvinphos, des-O-methyltetrachlorvinphos, 1-(2,4,5-trichlorophenyl ethanol) (free and conjugated forms), 2,4,5-trichloroacetophenone, and 1-(2,4,5-trichlorophenyl)ethanediol. Since no *new* metabolites were identified in the Day 6 extracts (and only minor changes in the relative residue proportions were noted), CBRS concludes that the chromatographic profiles of fractions from eggs collected at Day 6--posttreatment are essentially the same as from eggs collected just prior to sacrifice, with differences due

chiefly to the increased presence of conjugated metabolites. CBRS finds that the deficiencies connected with the poultry dermal metabolism study are thus satisfied and the nature of the residue in poultry following dermal application of tetrachlorvinphos is understood. CBRS does note, however, that any radiovalidation of an enforcement analytical method should use the Day 6 sample since this contains both higher residue levels and more conjugated metabolites.

Reviewer Statement:

"Metabolite M7 represented 0.087 ppm and 24.2% of TRR in egg; this metabolite should be identified. M7 may be identified from eggs with higher TRRs collected from the withdrawal group. Identification of M7 in the organic acid fraction from fat may be translated to eggs, provided that identity of M7 in the fat fraction with M7 in eggs is demonstrated, for example by similar mobilities in two different systems."

PTRL Comments:

PTRL first notes that while a logical source of M7 would have been from the Day 6 egg sample analyzed as part of the requirement that eggs with the highest TRR be analyzed, no M7 was present in the primary organic extract of the Day 6 egg sample. Given this finding, the laboratory performed a new analysis on a (Month 12) 24-hr-Sacrifice egg sample from frozen storage. The distributions of residues into the various fractions from the original extraction and the new extraction were comparable, suggesting that the incurred residues were stable during the frozen storage period.

Comparing the HPLC metabolite profile of the original 24 hr.-Sacrifice egg sample with that of the newly prepared sample revealed that the two profiles were similar, <u>with the exception that there was no M7 in the newly prepared sample</u>. The laboratory confirmed that M7 was actually present in the original sample by reanalyzing the original extract after being stored frozen for 12 months. Results from this reanalysis demonstrated that M7 <u>was</u> present in the original sample and <u>was</u> stable during the 12 month frozen storage period.

The laboratory concluded that since M7 has been shown to be stable under frozen storage conditions and is not present in any of the newly prepared egg and fat samples recently analyzed, the presence of M7 in the original extracts can be attributed to contamination.

CBRS Response:

The registrant has submitted the following evidence in support of its contention that Metabolite M7 was a contaminant in the original tissue:

- no M7 was present in the Day 6 sample taken from frozen storage
- a new extraction of the 24 hr.-Sacrifice egg sample taken from frozen storage did not reveal the presence of Metabolite M7.
- reanalysis of the original (24 hr.-Sacrifice) sample taken from frozen storage did reveal the presence of Metabolite M7 (thus confirming the integrity of the original analysis and confirming its stability of the frozen storage period)

CBRS believes that the registrant has submitted sufficient evidence that the Metabolite M7 is indeed a contaminant.

cc: w/Attach.: SF, List A Rereg. File, J. Smith (CCB).
w/o Attach.: RF, Circ., DJM.
RDI: FSuhre: 9/16/94;MMetzger:9/20/94;EZager:9/20/94.

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