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

OCT 8 1993

MEMORANDUM:

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Chlorpropham, Reregistration. Chlorpropham Task Force Submission of Supplemental Data: Nature of the Residue in Animals.
No MRID No. CBRS No. 12452. DP Barcode No. D194640.

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The Chlorpropham Task Force, on behalf of registrants Aceto Agricultural Chemicals Corporation and Elf Atochem North America, Inc., has submitted supplemental data on the nature of the residue in animals in response to a previous review (CBRS 8942ff, 3/10/93, J. Abbotts).

Tolerances are established for combined residues of the plant regulator and herbicide chlorpropham, isopropyl m-chlorocarbanilate (CIPC), and its metabolite 1-hydroxy-2-propyl 3'-chlorocarbanilate, calculated as CIPC, in or on potatoes (post-harvest) at 50 ppm, and soybeans at 0.2 ppm (40 CFR 180.181). Interim tolerances are established for residues of parent chlorpropham in animal commodities at 0.05 ppm, and in or on other plant commodities, pending establishment of permanent tolerances (40 CFR 180.319). Chlorpropham is a List A Chemical. A Registration Standard (Guidance Document) was issued 12/87; an Update to the Residue Chemistry Chapter was issued 10/16/91.

Conclusions

1. Conclusions below are numbered to be consistent with Conclusions requiring resolution in the previous review (CBRS 8942ff, 3/10/93, J. Abbotts).

2a. The data provided in the present submission are sufficient to assign 11% of the TRR in goat liver to 3-chloroaniline; because of the severity of the extraction procedure which released these residues, they may have been conjugated in tissue.

2b. Registrant should explain the utility of the "reconstructed" HPLC profiles and describe how these were generated. The relationship between the profiles in Attachments 23 and 24 of the present submission should be explained. If registrant can provide adequate explanations for these items, then no further work on the goat metabolism study is required.

3b. For the poultry metabolism study, for any extract containing 0.05 ppm or greater which was stored at reduced temperature (0 to 5°C) prior to analysis, registrant should provide available data on chromatographic profiles which give confidence that radioactive residues were stable over the duration of storage at reduced temperature.

Recommendations

Additional information should be provided to resolve Conclusions 2b and 3b above. Previous review (CBRS 8942ff, 3/10/93, J. Abbotts) concluded that data on magnitude of the residue in poultry and eggs will not be required if the only chlorpropham use allowed is post-harvest treatment of potatoes, because potato waste and cull potatoes are not a significant poultry feed item. If registration is subsequently requested on a significant poultry feed item, then a poultry feeding study would be required and additional poultry metabolism data may be required. These conclusions remain in effect regardless of the registrant's ability to resolve Conclusion 3b here.

Background

Data on the nature of the residue in ruminant and poultry have been reviewed (CBRS 8942, 9137, 9166, 9171, 3/10/93, J. Abbotts). This previous review identified deficiencies in the animal metabolism studies, which are reiterated here:

2b. In lactating goats, residues were greater than 0.06 ppm only in milk and liver (0.19-0.34 ppm), and residues in liver were not adequately characterized. In liver, only 9.43% of the radioactive residue was characterized. Aqueous and organic soluble residues identified in liver accounted for 4.68-9.41% of the TRR and included 3-chloroacetanilide (3.23% TRR), 4-hydroxychlorpropham (3.95% TRR), 1,4-dihydroxychlorpropham (1.11% TRR), 3-chloro-4-hydroxyacetanilide-O-sulfonic acid (0.5% TRR), and chlorpropham carboxylic acid (0.64% TRR). Collagenase/protease released residues accounted for 53.95%-63.69% of the total radioactive residue (TRR) in goat liver; these residues were not identified. Acid hydrolysis released

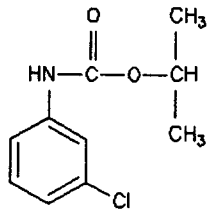
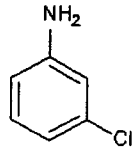
20.4-23.2% of TRR in liver; the major peak of radioactivity from this fraction co-chromatographed with 3-chloroaniline, but identity was not confirmed and %TRR was not reported.

3a. The qualitative nature of the residue in poultry is adequately understood for purposes of limited chlorpropham use (postharvest potatoes) and pending submission of storage stability data (Conclusion 3b)....

3b. The registrant did not report sample, sample extract or fraction storage intervals in the poultry report (MRID 42130401). Information provided indicates that the study began on 7/9/90 and was terminated on 10/29/91. Therefore, samples/extracts/fractions may have been stored for up to 16 months. The registrant must provide the dates of sample collection, extraction, and analysis. Storage stability data are required to support the storage conditions and intervals of this study.

Previous Recommendations: Further work is necessary before Conclusion 2b is resolved and the ruminant metabolism study can be considered acceptable. The residues released from liver by collagenase/protease treatment and acid hydrolysis should be further analyzed. Where feasible, fractions from goat No. 19 should be used since radioactive residues were higher in these samples. The identity of any putative metabolite whose combined level in the collagenase/protease and acid hydrolysis fractions represents 0.05 ppm or greater should be confirmed by a second method. Residues should be reported in ppm and %TRR; this applies to 3-chloroaniline, whether it is characterized by a single method or confirmed in identity by a second method.

Table 1. Chlorpropham and Metabolites.

Chemical Names (Common names)	Chemical Structure
isopropyl m-chlorocarbanilate isopropyl 3-chlorocarbanilate (chlorpropham; CIPC)	
3-chloroaniline (chloroaniline)	

Present Submission

By letter of 8/31/93 to Venus Eagle, SRRD, the Chlorpropham Task Force has submitted supplemental data in response to the previous review. The transmittal letter also refers to data on the analytical method which are to be submitted in the future, and a previous submission on independent laboratory validation of the method. We note that the independent laboratory validation was unsuccessful, and this item remains an outstanding data requirement (CBRS 11948, 7/8/93, J. Abbotts). The review below covers the supplemental data on poultry and ruminant metabolism, which were provided with the present submission, in a letter from the performing laboratory, Xenobiotics Laboratories, to the Chlorpropham Task Force, 8/27/93.

With regard to the goat metabolism study, the performing laboratory provided information on the analyses already performed and reported in MRID 42112201. The extraction protocol for liver and kidney is shown in Figure 1 for reference. Table 2 summarizes data from the previous review (CBRS 8942ff) on the distribution of radioactive residues from the extraction procedure. The recommendations in the previous review pertained to the radioactive residues remaining in the AQ-1 and AQ-2 fractions:

Table 2. Distribution of TRR in Goat Liver

Fraction	% TRR	ppm
Chloroform	6.7	0.023
Solids-1	(86.9)	(0.296)
AQ-1	63.7	0.217
Solids-2	(23.2)	(0.079)
AQ-2	20.4	0.070

Table notes: Data are shown for liver of Goat No. 19, which had the larger TRR; the distribution was similar for Goat No. 17. Values in parentheses indicate fractions that were further extracted. Refer to Figure 1 for fractions in the protocol.

In the present submission, the performing laboratory reported that efforts were made to characterize products in the AQ-1 and AQ-2 fractions from liver using reverse phase HPLC or normal phase TLC, followed by analytical TLC. This procedure was successfully used in analysis of organosoluble residues from tissues including liver. However, these techniques were unsuccessful in resolving metabolites from the aqueous fractions, a circumstance that the performing laboratory attributed to matrix effects. The AQ-1 and AQ-2 fractions were subjected to preparative reverse phase HPLC for cleanup before further analysis. With this procedure, the majority of radioactivity eluted in a broad band at retention times 30-50 min, with

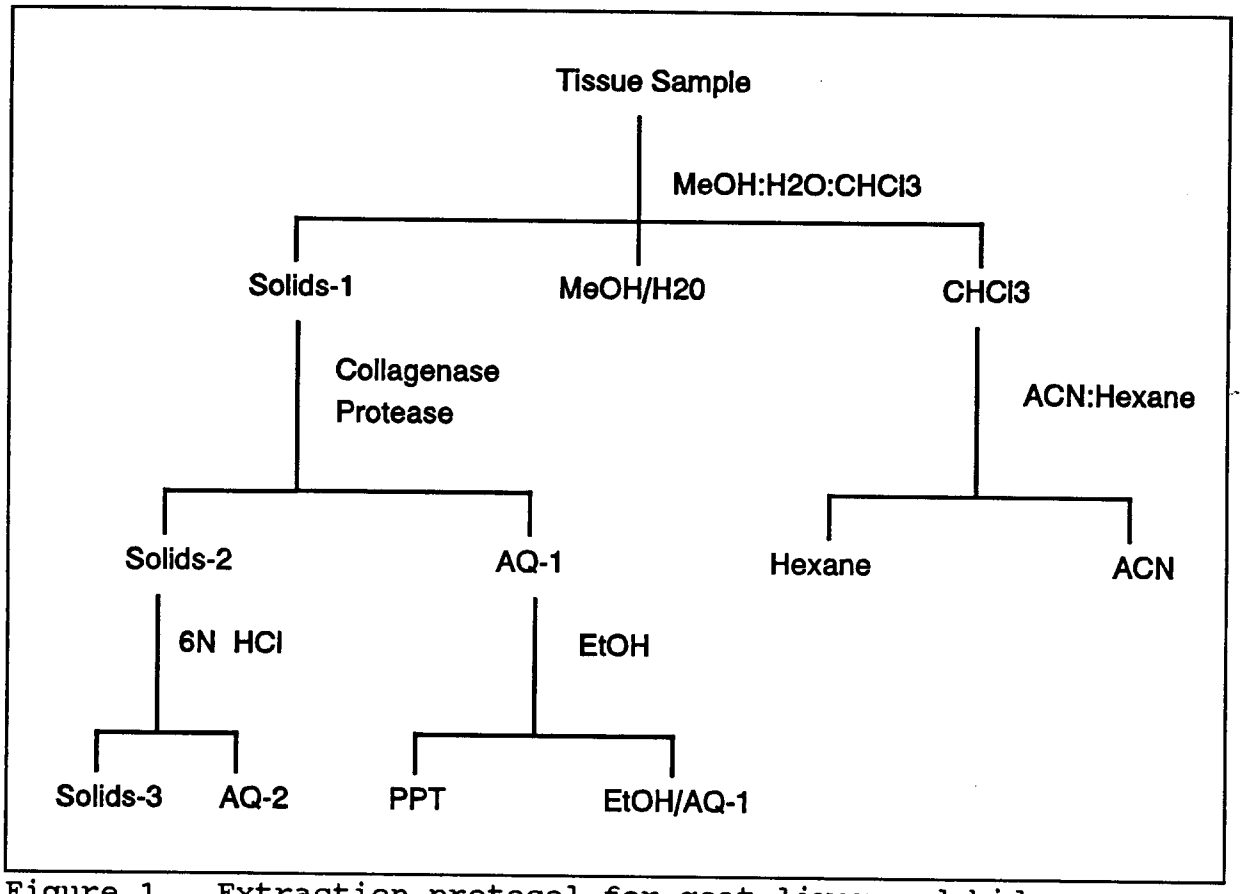


Figure 1. Extraction protocol for goat liver and kidney

secondary peaks at approximately 12 and 65 min. Similar results were seen with either AQ-1 or AQ-2 samples, and with samples from either goat.

In a further effort to gain information, the main peaks collected from preparative HPLC on the AQ-1 and AQ-2 fractions were analyzed by reverse phase HPLC. Eight sharp peaks were seen, along with minor peaks in addition. The performing laboratory provided copies of TLC analyses conducted on AQ-2 samples. Analysis with a relatively polar solvent indicated at least two major components of approximately equal intensity, and minor components as well.

The performing laboratory noted that a modified Bleidner procedure was used to release residues from the major preparative HPLC peak of AQ-1, or from the Solids-1 fraction. Under this procedure, previously described in MRID 42112201, sample was refluxed in 25% NaOH in a reaction flask connected to a continuous extractor with isooctane. As hydrolysis proceeded, the released organic compounds were steam distilled into the isooctane layer. As the previous review reported (CBRS 8942ff, 3/10/93, J. Abbotts, see Table 10), about 19% of TRR was

extracted into isooctane from the Solids-1 fraction. HPLC analysis indicated that the majority of the extracted residue showed mobility similar to a 3-chloroaniline standard. Attempts to confirm this assignment with a second method were inconclusive, which the performing laboratory also attributed to matrix effects. The performing laboratory assigned 58% of the recovered residue, or aproximately 11% TRR (0.02 ppm) to 3-chloroaniline; because of the severity of the extraction procedure, these residues may have been conjugated in tissue.

With regard to the poultry metabolism study, the performing laboratory reported that poultry tissue samples were stored at approximately -10°C prior to shipment, were shipped in dry ice by overnight carrier to the performing laboratory, and then stored frozen at the performing laboratory. Extracts and bound residues (post-extraction solids) were stored at reduced temperature (0-5°C) prior to and following analysis. Dates of pertinent actions were provided. TRRs in tissue samples were determined within 2 weeks of receipt (within two months of sample collection), and all samples were extracted within 3 months of receipt (within 5 months of sample collection). The duration from time of collection to time of chromatography ranged from 91 to 169 days for most samples. Tissue hexane fractions were stored at reduced temperature for 330-347 days prior to assay. Post extraction solids were stored at reduced temperature for 267 days prior to assay.

CBRS Comments

With regard to the goat metabolism study, treatments with strong acid, strong base, and enzymes have been conducted to release bound residues. Data provided by the performing laboratory are sufficient to suggest that no single component of the AQ-1 or AQ-2 fractions represents greater than 0.05 ppm; further analysis is not required. Data provided by the performing laboratory are sufficient to assign 11% of the TRR in liver to 3-chloroaniline; because of the severity of the extraction procedure which released these residues, they may have been conjugated in tissue.

Present Conclusion 1: Conclusions below are numbered to be consistent with Conclusions requiring resolution in the previous review (CBRS 8942ff, 3/10/93, J. Abbotts).

Present Conclusion 2a: The data provided in the present submission are sufficient to assign 11% of the TRR in goat liver to 3-chloroaniline; because of the severity of the extraction procedure which released these residues, they may have been conjugated in tissue.

In the present submission, the performing laboratory has also provided some information which is confusing. Data provided

include both raw HPLC traces and "reconstructed" HPLC chromatograms. In one case, the raw and reconstructed profiles do not seem to be consistent. Attachment 23 of the present submission is an HPLC profile of the EtOH/AQ-1 fraction (see Figure 1) from liver of Goat No. 19; Attachment 24 is the "reconstructed" profile. The raw data in Attachment 23 indicate minimal radioactivity counts at a retention time of about 25 minutes, and the highest radioactivity at retention times of 50-60 min. Attachment 24 is labeled to indicate that it is a reconstructed HPLC profile of the same data in Attachment 23, yet it shows a peak of radioactivity at about 25 min, and minimal radioactivity at retention times of 50-60 min. Registrant should explain this apparent inconsistency.

Present Conclusion 2b: Registrant should explain the utility of the "reconstructed" HPLC profiles and describe how these were generated. The relationship between the profiles in Attachments 23 and 24 of the present submission should be explained. If registrant can provide adequate explanations for these items, then no further work on the goat metabolism study is required.

With regard to storage conditions of the fractions from the poultry metabolism study, it is a matter of concern that extracts were stored at 0 to 5°C for times up to nearly a year before the chromatographic analysis was performed. TRRs, which were determined within two months of sample collection, were <0.03 ppm for breast and thigh muscle, and greater than 0.05 ppm in other tissues, up to 0.468 ppm in liver. Any extract containing 0.05 ppm or greater would therefore also contain greater than 10% TRR. For any extract containing 0.05 ppm or greater which was stored at reduced temperature (0 to 5°C) prior to analysis, registrant should provide available data on chromatographic profiles which give confidence that radioactive residues were stable over the duration of storage at reduced temperature.

Present Conclusion 3b: For the poultry metabolism study, for any extract containing 0.05 ppm or greater which was stored at reduced temperature (0 to 5°C) prior to analysis, registrant should provide available data on chromatographic profiles which give confidence that radioactive residues were stable over the duration of storage at reduced temperature.

cc:Circ, Abbotts, RF, List A File, SF
RDI:FBSuhre:10/7/93:MSMetzger:10/7/93:EZager:10/7/93
H7509C:CBII-RS:JAbbotts:CM-2:Rm805A:305-6230:10/8/93
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