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

MAR 10 1993

MEMORANDUM:

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Chlorpropham. Chlorpropham Task Force Response to the Reregistration Standard: Nature of the Residue in Postharvest Potatoes (MRID 42085601); Nature of the Residue in Ruminants (MRID 42112201) and Poultry (MRID 42130401); Analytical Methods for Potatoes (MRID 42123101).
CBRS Nos. 8942, 9137, 9166, 9171.
DP Barcode Nos. D171613, D172569, D172742, D172739.

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Attached is a review of residue chemistry data for nature of the residue in potatoes treated post-harvest, nature of the residue in ruminants and poultry, and analytical method for potatoes, submitted by the Chlorpropham Task Force in response to the Guidance Document (12/87) and the Update to the Residue Chemistry Chapter (10/16/91). This information was reviewed by Acurex Corporation under supervision of CBRS, HED. The data assessment has undergone secondary review in the branch and has been revised to reflect branch policies.

The review reached the following conclusions:
--The nature of the residue in stored potatoes treated post-harvest is adequately understood.
--The ruminant metabolism data can be upgraded to an acceptable status with additional data on liver.
--The poultry metabolism study is acceptable, provided adequate data are submitted on storage conditions and storage stability of residues.
--The analytical method is acceptable for recovery of parent chlorpropham and two metabolites; further data will be required

if additional metabolites are designated residues to be regulated. Additional requirements for establishing an enforcement method remain outstanding.
--The requirement for feeding studies (Guideline 171-4(j)) is changed from reserved to required.

In addition, numerous tolerances for uses not being supported by registrants should be revoked; these tolerances can be revoked promptly, without waiting for additional reregistration data.

If you need additional input please advise.

Attachment: Review of Chlorpropham Residue Chemistry Data

cc (with Attachment):Circ, Abbotts, Reg. Std. File, SF, Acurex,
cc (without any Attachments):RF
RDI:FBSuhre:3/8/93:MSMetzger:3/9/93:EZager:3/10/93
H7509C:CBII-RS:JAbbotts:CM-2:Rm805A:305-6230:3/11/93

CHLORPROPHAM
(Chemical Code No. 018301)
(CBRS Nos. 8942, 9137, 9166, and 9171; DP Barcodes D171613, D172569, D172742, and D172739)

TASK 3

Registrant's Response to Residue Chemistry Data Requirements

April 16, 1992

Contract No. 68-DO-0142

Submitted to:

U.S. Environmental Protection Agency
Arlington, VA 22202

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CHLORPROPHAM

(Chemical Code 018301)

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REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

Task 3

BACKGROUND

The Chlorpropham Guidance Document dated 12/87 concluded, and the Chlorpropham Reregistration Standard Update dated 10/91 reiterated, that the nature of the residue in animals is not adequately understood. Data were required depicting the metabolism of chlorpropham in ruminants and poultry. Analyses of samples from the required metabolism studies using current enforcement methods were also required. The Agency also reevaluated the requirements, taking into account the voluntary cancellation of chlorpropham use of all commodities except potatoes following harvest, and concluded that metabolism data for animals are still required because culled potatoes are a livestock feed item (CBRS No. 6272, 4/4/90, H. Fonouni).

The Chlorpropham Residue Chemistry Chapter dated 8/87 concluded that the nature of the residue in field-treated, growing plants is adequately understood, and the major residues are chlorpropham (I), isopropyl 3-chloro-6-hydroxycarbanilate (II), isopropyl 3-chloro-4-hydroxycarbanilate (III), 1-hydroxy-2-propyl-3-chlorocarbanilate (IV), and isopropyl 3-chloro-2-hydroxycarbanilate (V). In addition, the Guidance Document required, and the Update reiterated, that data are required depicting the uptake, distribution, and metabolism of ring-labeled [^{14}C]chlorpropham in stored potatoes following postharvest application. Analyses of representative samples from the metabolism studies were required. In addition, the Update reiterated conclusions of the Guidance Document concerning residue analytical methods. The conclusions required data collection and enforcement methodology to include pre-hydrolysis extraction and hydrolysis steps to allow for the detection of free as well as conjugated metabolites, including validation with weathered ^{14}C-residues.

The Chlorpropham Task Force submitted data (1991; MRIDs 42112201 and 42130401) on the metabolism of [^{14}C]chlorpropham in goats and poultry, potato metabolism data (1991; MRID 42085601), and residue analytical methodology for potatoes (1991; MRID 42123101). These data are reviewed here for their adequacy in fulfilling outstanding data requirements.

The qualitative nature of the residue in plants is adequately understood, as of this review. As currently defined (40 CFR §180.181), the residues of concern in plant commodities are chlorpropham (CIPC) and its metabolite 1-hydroxy-2-propyl-3'-chlorocarbanilate. The

Agency will reevaluate the metabolites to be regulated, based on the metabolism study results now available. There is no adequate enforcement methodology for the currently regulated residues in or on plant commodities. The registrant has been required to select and designate specific method(s) for tolerance enforcement, and provide independent laboratory validation prior to submittance for Agency validation trials.

CONCLUSIONS

1a. The nature of residues of [^{14}C]chlorpropham applied as a postharvest treatment to stored potatoes is adequately understood. The major residue in potatoes stored for 52 weeks posttreatment was chlorpropham (95.91% of the total residues). Minor components of the residue identified in pulp and peel included 3-chloroaniline (VI), 3-chloroaniline-N-glucosylamine (XIX), p-methoxychlorpropham (X), oligosaccharide conjugates of 4-hydroxychlorpropham (XXI) and 1-hydroxychlorpropham (XX), and an amino acid conjugate of 4-hydroxychlorpropham (XXII), each accounting for 0.03-1.25% of the total residues (MRID 42085601). These data indicate that in plants, chlorpropham can undergo hydroxylation of the benzene ring or the isopropyl side chain and subsequently form conjugates with carbohydrates or amino acids. Chlorpropham can also undergo decarbanilation to form 3-chloroaniline (VI), which subsequently conjugates with natural plant products. Plant metabolites and their molecular structures are presented in Table 1.

1b. The present tolerance for chlorpropham (40 CFR 180.181) is expressed as residues of parent and 1-hydroxy-2-propyl-3'-chlorocarbanilate (IV). Metabolite IV was not identified as part of the TRR due to post-harvest treatment of potatoes, but an oligosaccharide conjugate (XX) was identified as a small portion of the TRR. The results of this metabolism study will be referred to the HED Metabolism Committee to determine the residues to be regulated due to post-harvest treatment of potatoes.

2a. In lactating goats, radioactive residues in milk (0.483 ppm), fat (0.061 ppm), and kidney (0.041 ppm) were adequately characterized; unknown metabolites represented less than 0.05 ppm in all these tissues, and in muscle. The principle metabolite isolated from milk was 4-hydroxychlorpropham-O-sulfonic acid (XIV), which accounted for 77.92% of the total radioactive residue (TRR). Minor amounts of 4-hydroxychlorpropham (III) (1.78% TRR), 1,4-dihydroxychlorpropham-4-O-sulfonic acid (XV) (5.72% TRR), 3-chloro-4-hydroxyacetanilide-O-sulfonic acid (XVI) (6.77% TRR), and 4-hydroxychlorpropham-O-glucuronic acid (XVIII) (3.02% TRR) were also detected. In fat, the principle radioactive residue was the parent compound, chlorpropham (I), which accounted for 83.85% of the TRR. In kidney tissue, the major metabolite was 4-hydroxychlorpropham-O-sulfonic acid (XIV) (31.91% TRR). In addition, trace amounts of chlorpropham (I) (2.11% TRR), p-methoxychlorpropham (X) (2.17% TRR), 3-chloroacetanilide (VIII) (2.64% TRR), 3-chloro-4-hydroxyacetanilide (VII) (1.46% TRR), 3-chloro-6-hydroxyaniline (XXIV) (1.3% TRR), 1,4-dihydroxychlorpropham (XII) (1.09% TRR),

1,4-dihydroxychlorpropham-4-O-sulfonic acid (XV) (0.51% TRR), 3-chloro-4-hydroxyacetanilide-O-sulfonic acid (XVI) (4.44% TRR), and 4-hydroxychlorpropham-O-glucuronic acid (XVIII) (0.61% TRR) were characterized. Metabolite B-6, an unknown in rat urine, represented 4.27% TRR in kidney. (MRID 42112201) Metabolites and their molecular structures are presented in Table 1.

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 (VIII) (3.23% TRR), 4-hydroxychlorpropham (III) (3.95% TRR), 1,4-dihydroxychlorpropham (XII) (1.11% TRR), 3-chloro-4-hydroxyacetanilide-O-sulfonic acid (XVI) (0.5% TRR), and chlorpropham carboxylic acid (XVII) (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). About 64% (0.301 ppm) of the TRR in liver, and 25% (0.144 ppm) of the TRR in kidney were described as 3-chloro-4-hydroxyaniline related compounds, but were not further identified. Additional identification of this portion of the TRR will not be required. The identified metabolites provide sufficient evidence of the metabolic path (Conclusion 4). The data show that the principle metabolite isolated from kidney was 4-hydroxychlorpropham-O-glucuronide (XVIII) representing 9.25% of the TRR. The major ^{14}C-residue isolated from skin and fat was chlorpropham (I), representing 68.08%, and 91.78% of the TRR, respectively. In addition, 4-hydroxychlorpropham-O-sulfonic acid (XIV) represented 18.95% of the TRR in skin tissues. The major metabolite in egg whites was 3-chloro-4-hydroxyaniline-O-sulfonic acid (XI), representing 22.24% of the TRR. Egg whites, liver, and kidney also contained chlorpropham at 3.05%, 0.51%, and 7.36% of the TRR, respectively. The major metabolites in egg yolks were chlorpropham (I) (19.91% of the TRR) and 4-hydroxychlorpropham-O-sulfonic acid (XIV) (31.65% of the TRR).

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.

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4. The registrant has proposed a metabolic pathway in which chlorpropham may be oxidized to 4-hydroxychlorpropham or degraded to 3-chloroaniline. The aniline is subsequently metabolized to 3-chloro-4-hydroxyaniline-O-sulfonic acid. The hydroxy chlorpropham is further metabolized to 4-hydroxychloropropham-O-sulfonic acid or 4-hydroxychlorpropham-O-glucuronide.

5. The method adequately recovers chlorpropham (I), *p*-hydroxychlorpropham (III), and *p*-methoxychlorpropham (X) from fortified potato samples. However, the method as described would not be suitable for data collection or enforcement of tolerances, if the residues to be regulated were to include 3-chloroaniline (VI) or additional metabolites. After the residues to be regulated in potatoes due to post-harvest treatment are determined, the registrant must submit an adequate enforcement method along with supporting data on all residues to be regulated, following an independent laboratory validation. The method must include pre-hydrolysis extraction and hydrolysis steps that allow for the detection of free as well as conjugated metabolites, if conjugated metabolites are included in the residues to be regulated. Limits of detection must be reported for each residue to be regulated. Validation data with weathered, radiolabeled samples from metabolism studies must be included.

6a. Magnitude of the residue in meat and milk studies (171-4(j)) will now be required. Results of the plant and animal nature of the residue studies show similar metabolic paths. Therefore, the test ruminant animals should be fed chlorpropham only. At least three feeding levels are required, 1X, 3X, and 10X the maximum anticipated dietary burden. When the nature of the residue in ruminants is adequately understood, and the residues to be regulated in animal commodities are determined, the registrant should conduct cattle feeding studies.

6b. Magnitude of the residue in poultry and eggs will not be required 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.

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.

Additional data as indicated are required to resolve Conclusions 3b, 5, and 6a.

The tolerance expressions (40 CFR 180.181; 40 CFR 180.319) require revision. The interim tolerances for alfalfa, clover, grass, alfalfa hay, clover hay, grass hay, beans, blackberries, blueberries, cranberries, peas, raspberries, sugar beet tops, garlic, onions, rice grain, safflower seed, sugar beet roots, and tomatoes must be revoked. The tolerance for soybeans must be revoked. The registrant is not supporting these uses. The tolerance for poultry and eggs should be revoked. The one remaining use of chlorpropham, post-harvest application to potatoes, does not present a significant poultry feed item. All these tolerances above can be revoked promptly, without waiting for additional reregistration data. Revised tolerance expressions may be required for potatoes (see Conclusion 1b) and for animal commodities once the nature of the residue in ruminants is adequately understood.

DETAILED CONSIDERATIONS

Qualitative Nature of the Residue in Plants

The Chlorpropham Task Force submitted data (1991; MRID 42085601) pertaining to the metabolism of uniformly ring-labeled [^{14}C]chlorpropham (98.96% radiochemical purity) in stored potatoes. Potatoes were treated postharvest by rolling the tubers in a 1.5% solution of [^{14}C]chlorpropham with a specific activity of 1.584 mCi/g (3,516 dpm/μg). The application rate (40 ppm on a tuber-weight basis) was equivalent to 2.4x the maximum labeled rate. After treatment, excess solution was allowed to drain off and the tubers were air dried and stored in an incubator at 8° C until analysis. Potatoes were sampled for analysis at 1- to 4-week intervals from 0 to 52 weeks after treatment. Untreated potatoes were used as controls.

A sample of eight potatoes was taken at each sampling interval. Six potatoes were washed with methanol, and fractionated into peel (skin), the first layer of pulp beneath the peel, and the remaining pulp. Each fraction was diced, frozen in liquid nitrogen, and homogenized. The two remaining potatoes were also washed with methanol, and the whole tubers were diced, frozen, and homogenized.

Total Radioactive Residues (TRR)

Duplicate subsamples of the methanol washes and all liquid fractions were radioassayed by liquid scintillation spectrometry (LSS). Solid fractions were radioassayed in triplicate using ^{14}C-combustion/LSS analysis. The detection limit of the radioassays was 0.01 ppm. The TRR in or on unwashed whole potatoes, methanol washes, washed whole potatoes, peel, first layer of pulp, and pulp from 0 to 52 weeks posttreatment is presented in Table 2.

The TRR in or on unwashed tubers declined from 46.4 ppm at treatment to 29.0 ppm at 52 weeks after treatment. The registrant stated that the loss of radioactivity was due to volatilization of the parent compound during storage. This statement was supported by analysis of frost from the incubator, which detected [^{14}C]chlorpropham in the ice. For the entire storage period the majority of ^{14}C-residues (84.57-98.08% TRR) remained on the tuber

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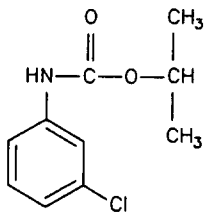
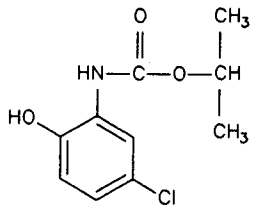
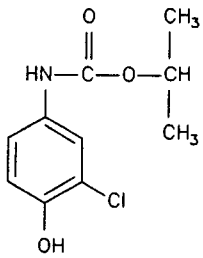
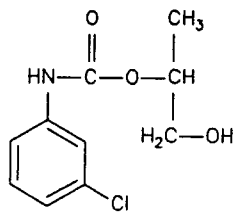
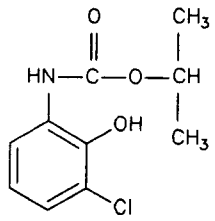
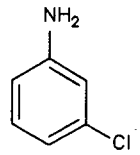
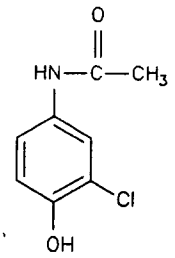
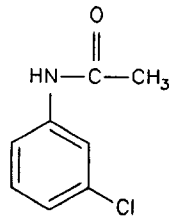
Table 1. Isolated metabolites of chlorpropham in plants and animals.

Code Number	Chemical Names (Common names)	Chemical Structure	Substrate; MRID	
I	isopropyl 3-chlorocarbanilate isopropyl m-chlorocarbanilate (chlorpropham; CIPC)		soybean potato egg white yolk hen liver kidney skin fat	00035485 00114794 00035480 42085601 42130401 42130401
II	isopropyl 3-chloro-6-hydroxycarbanilate (6-OH-CIPC)		soybean alfalfa orchard grass turnip 	00035485 00114794 00035480 00036628 00139680 00036395 00036640 00036638 00036639
III	isopropyl 3-chloro-4-hydroxycarbanilate 4-hydroxychlorpropham *p*-hydroxychlorpropham (4-OH-CIPC)		soybean alfalfa cucumber orchard grass turnip hen liver goat milk liver cows milk	00035485 00114794 00035480 00036628 00139680 00036629 00036395 00036640 00036638 00036639 42130401 42112201 00114739

(continued)

Table 1. (continued)

Code Number	Chemical Names (Common names)	Chemical Structure	Substrate; MRID
IV	1-hydroxy-2-propyl-3-chlorocarbanilate (40 CFR 180.181) (hydroxymethyl)ethyl-3-chlorocarbanilate 1-hydroxychlorpropham (isopropyl-OH-CIPC)		soybean 00035485 00114794 00035480 orchard grass 00036395 00036640 turnip 00036638 00036639 egg white 42130401 yolk
V	isopropyl 3-chloro-2-hydroxycarbanilate (2-OH-CIPC)		soybean 00035485 00035480 orchard grass 00036395 00036640 turnip 00036638 00036639
VI	3-chloroaniline (chloroaniline)		potato 42085601
VII	3-chloro-4-hydroxyacetanilide (4-OH-acetanilide)		Hen liver 42130401 kidney goat kidney 42112201 cows milk 00114739
VIII	3-chloroacetanilide		egg white 42130401 yolk goat liver 42112201 kidney

(continued)

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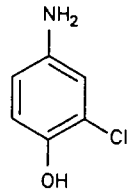
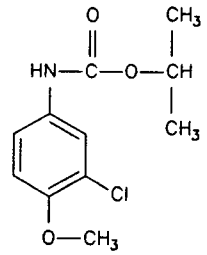
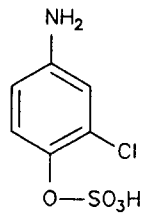
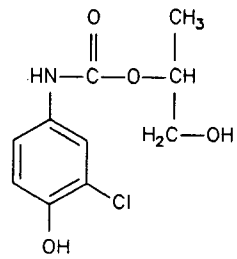
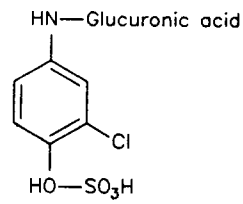
Table 1. (continued)

Code Number	Chemical Names (Common names)	Chemical Structure	Substrate; MRID
IX	3-chloro-4-hydroxyaniline		hen kidney 42130401
X	*p*-methoxychlorpropham		goat kidney 42112201 potato 42085601
XI	3-chloro-4-hydroxyaniline-O-sulfonic acid		egg white 42130401 hen kidney 42130401
XII	1,4-dihydroxychlorpropham		hen kidney 42130401 goat liver 42112201
XIII	3-chloro-4-hydroxyaniline-N-glucuronic acid-4-O-sulfonic acid		egg white 42130401

(continued)

Table 1. (continued)

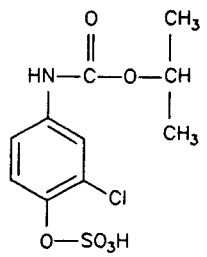
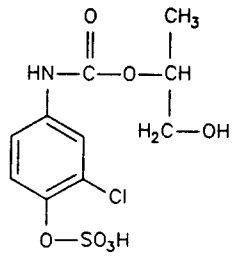
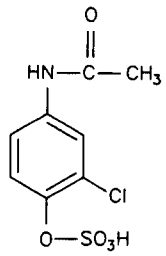
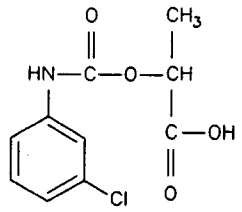
Code Number	Chemical Names (Common names)	Chemical Structure	Substrate; MRID
XIV	4-hydroxychlorpropham-O-sulfonic acid		hen liver 42130401 skin egg white 42130401 yolks goat milk 42112201 kidney
XV	1,4-dihydroxychlorpropham-4-O-sulfonic acid		egg white 42130401 goat milk 42112201 kidney
XVI	3-chloro-4-hydroxyacetanilide-O-sulfonic acid		hen kidney 42130401 goat milk 42112201 liver kidney
XVII	chlorpropham carboxylic acid		egg white 42130401 hen kidney 42130401 goat liver 42112201

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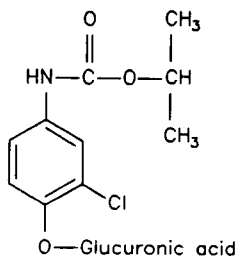
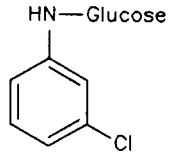
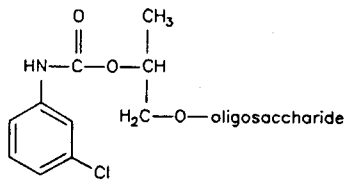
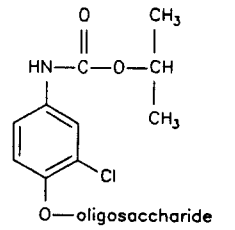
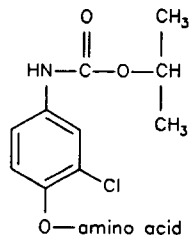
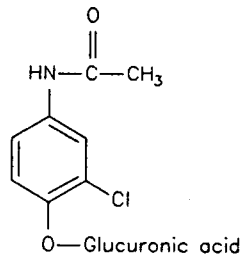
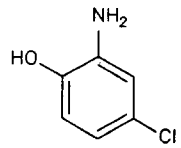
Table 1. (continued)

Code Number	Chemical Names (Common names)	Chemical Structure	Substrate; MRID
XVIII	4-hydroxychlorpropham-O-glucuronic acid		hen kidney 42130401 goat milk 42112201 kidney
XIX	3-chloroaniline-N-glucosylamine		potato 42085601
XX	oligosaccharide conjugate of 1-OH-CIPC		potato 42085601
XXI	oligosaccharide conjugate of 4-OH-CIPC		potato 42085601

(continued)

Table 1. (continued)

Code Number	Chemical Names (Common names)	Chemical Structure	Substrate; MRID
XXII	amino acid conjugate of 4-OH-CIPC		potato 42085601
XXIII	3-chloro-4-hydroxyacetanilide-O-glucuronic acid		hen kidney 42130401
XXIV	3-chloro-6-hydroxyaniline		goat kidney 42112201

In addition, the following analytical standards were used: 3-chloroaniline-N-sulfamic acid, 3-chloro-6-hydroxyaniline, and 3-chloro-6-hydroxyacetanilide.

surface and were extracted by the methanol wash. Of the ^{14}C-residues remaining in or on the washed tubers, the majority (1.86-9.77% TRR) was localized in the potato peel. In addition, the amount of ^{14}C-residues and the percentage of the TRR translocated into the peel, first pulp layer, and the remaining pulp increased as the storage interval increased.

Table 2. Total radioactive residues in or on whole potatoes and potato fractions at various intervals following a dip application of 1.5% [^{14}C]chlorpropham (2.4x rate).

		Fractions					
Week		Unwashed Tubers	Methanol Wash[a]	Washed Tuber	Peel[b]	First Layer	Pulp
0	%TRR	-	98.08	1.92	1.86	0.02	0.04
	PPM[c]	46.39	45.50	0.89	0.86	0.01	0.02
1	%TRR	-	97.31	2.70	2.62	0.03	0.05
	PPM	72.29	71.17	1.95	1.89	0.02	0.04
6	%TRR	-	96.21	3.79	3.41	0.11	0.27
	PPM	56.22	54.09	2.13	1.92	0.06	0.15
12	%TRR	-	93.72	6.28	5.56	0.16	0.56
	PPM	42.62	39.95	2.68	2.37	0.07	0.24
24	%TRR	-	91.53	8.48	6.94	0.42	1.12
	PPM	39.56	36.21	3.35	2.74	0.17	0.44
36	%TRR	-	86.79	13.21	9.24	0.87	3.10
	PPM	23.93	20.77	3.16	2.21	0.21	0.74
48	%TRR	-	84.97	15.03	11.44	0.75	2.84
	PPM	31.92	27.12	4.80	3.65	0.24	0.91
52	%TRR	-	86.44	13.56	9.77	0.90	2.89
	PPM	29.01	25.08	3.93	2.83	0.26	0.84

[a]PPM values for the MeOH wash are expressed on a whole tuber weight basis.

[b]For purposes of comparison, PPM values for the peel, first layer, and pulp fractions were recalculated by the reviewer and are expressed on a whole tuber weight basis.

[c][^{14}C]chlorpropham equivalents.

Extraction and hydrolysis of residues

Samples of whole potato were homogenized and extracted with methanol/water/chloroform (11:5:5, v/v/v). The homogenates were filtered and the solid residues were reextracted with chloroform and filtered. The filtrates were combined and the insoluble residues (fraction-1) were radioassayed. The filtrates were separated into chloroform and methanol/water fractions and radioassayed. The chloroform fraction was concentrated and partitioned with acetonitrile/hexane (1:1, v/v) to yield acetonitrile and hexane fractions. The distribution of ^{14}C-residues in the extract fractions of whole potatoes at various storage intervals is presented in Table 3.

Of the radioactivity remaining in the tubers after the methanol wash, the majority (0.98-17.19% of the TRR) partitioned into chlorform and the majority of this radioactivity subsequently partitioned into acetonitrile (0.91-15.17% of the TRR). Radioactive residues partitioning into the methanol/water fraction increased at longer storage intervals but never accounted for more than 2.6% of the TRR. Unextracted ^{14}C-residues accounted for <2% of the TRR.

Table 3. Distribution of ^{14}C-residues in methanol washes, chloroform, acetonitrile, hexane, methanol/water, and insoluble solid fractions from whole potatoes stored for 0 to 52 weeks after treatment.

	Percent Total Radioactive Residue at Designated Weeks after Treatment							
Fraction	0	1	6	12	24	36	48	52
Methanol Wash	98.88	96.76	96.14	93.80	91.45	88.22	78.22	85.43
Chloroform	0.98	3.05	3.52	5.78	7.49	8.84	17.19	10.42
Acetonitrile	0.91	2.91	3.35	5.46	7.00	8.27	15.17	9.74
Hexane	0.07	0.14	0.17	0.32	0.49	0.57	2.02	0.68
Methanol/water	0.06	0.08	0.10	0.20	0.52	1.73	2.59	2.31
Solids-1	0.07	0.09	0.23	0.21	0.53	1.20	1.99	1.83

For the analysis of ^{14}C-residues in methanol washed potatoes, potato fractions (peel, first layer, and pulp) were extracted as described above at each sampling interval. These data (not shown) displayed the same trends in the distribution of ^{14}C-residues among extract fractions as those shown in Table 3 for whole potatoes. As the first layer fraction accounted for <1% of the TRR at any storage interval, extract fractions from this layer were not further analyzed.

Hexane and acetonitrile extract fractions from peel and acetonitrile fractions from pulp were analyzed using 2-dimensional (2-D) thin layer chromatography (TLC) and/or high performance liquid chromatography (HPLC). The hexane fraction from pulp contained low

residues and was not further analyzed. For further analysis of the methanol/water fractions, peel and pulp samples from 40-, 44-, 48-, and 52-week sampling intervals were utilized. The methanol/water fractions were concentrated to yield aqueous fractions. Each aqueous fraction was loaded onto a set of cation exchange and anion exchange cartridges linked in tandem. The linked columns were eluted with methanol and distilled water to yield a neutral fraction. The cartridges were separated and the anion exchange cartridge was eluted with 1N hydrochloric acid to yield an acidic fraction. The cation exchange cartridge was eluted with a 20% sodium hydroxide solution to yield a basic fraction. The ^{14}C-residues in these fractions from the 52-week samples of peel and pulp are shown in Table 4. The registrant reported the %TRR and ppm values for peel and pulp based on the sample weight and radioactivity in the peel and pulp fractions, respectively. For purposes of comparison, for this review, the %TRR was recalculated based on radioactivity in the whole tuber. Values for ppm equivalents of [^{14}C]chlorpropham in peel and pulp were not recalculated and are based on peel and pulp sample weight, respectively.

Table 4. Distribution and fractionation of ^{14}C-residues in peel and pulp from potatoes sampled 52 weeks after treatment.

	Peel		Pulp	
Fraction	%TRR[a]	PPM[b]	%TRR	PPM
Chloroform	(7.60)	(15.573)	(1.48)	(0.637)
Acetonitrile	6.97	14.283	1.43	0.615
Hexane	0.63	1.290	0.05	0.022
Methanol/water	(0.92)	(1.887)	(1.14)	(0.488)
Acidic	0.04	0.084	0.03	0.014
Basic	0.06	0.124	0.03	0.011
Neutral	(0.82)	(1.679)	(1.08)	(0.463)
Ethyl acetate-1	0.58	1.182	0.64	0.274
AQ-1	(0.24)	(0.497)	(0.44)	(0.189)
Butanol-1	0.23	0.463	0.35	0.152
AQ-2	0.02	0.034	0.08	0.037
Solids-1	1.25	2.572	0.26	0.112
Total	9.78	20.032	2.89	1.237

[a]Peel and pulp values for %TRR were recalculated by the reviewer based on radioactivity in whole unwashed potatoes.

[b]PPM equivalents of [^{14}C]chlorpropham are based on peel and pulp sample weights, respectively.

The majority of radioactivity in both peel and pulp methanol/water fractions eluted in the neutral fraction. The acidic and basic fractions were not analyzed further. The neutral fraction from each matrix was partitioned with ethyl acetate to yield an ethyl acetate-1 fraction and an aqueous (AQ-1) fraction. The AQ-1 fraction was further partitioned with n-butanol to yield butanol-1 and AQ-2 fractions. The majority of radioactivity partitioned into the ethyl acetate-1 and butanol-1 fractions. The ethyl acetate-1 fraction from peel was analyzed by 1-D and 2-D TLC and HPLC. The butanol-1 fraction from peel was analyzed by 1-D TLC and HPLC. The ethyl acetate-1 and butanol-1 fractions from pulp were analyzed by 2-D and 1-D TLC, respectively.

Insoluble ^{14}C-residues were analyzed using the Solids-1 fractions from peel and pulp fractions sampled at 52-weeks posttreatment. The Solids-1 fraction from the peel was hydrolyzed with cellulase in a acetate buffer (pH 5.1) at 37°C for 24 hours. The hydrolysate was filtered to yield aqueous (AQ-3) and insoluble (solids-2) fractions. The AQ-3 fraction was partitioned with ethyl acetate and yielded an ethyl acetate-2 soluble fraction and an aqueous fraction (AQ-4). The AQ-4 fraction was further partitioned with butanol to yield a butanol-2 fraction and an aqueous fraction (AQ-5). The distribution of ^{14}C-residues in the above fractions is presented in Table 5. After hydrolysis, the majority of radioactivity remained insoluble. Of the solubilized radioactivity, the majority partitioned into the ethyl acetate-2 and butanol-2 fractions, which were further characterized by 2-D TLC.

The Solids-2 fraction from the peel and the Solids-1 fraction from pulp were heated in an acetate buffer (pH 5.5) and hydrolyzed with α-amyloglucosidase at 55°C for 24 hours. The samples were centrifuged to yield aqueous (AQ-6) and insoluble (Solids-3) fractions. For both peel and pulp samples, the majority of the radioactivity remained in the insoluble residues. The AQ-6 fractions were partitioned with ethyl acetate to yield an ethyl acetate-3 and aqueous (AQ-7) fractions. The ethyl acetate-3 fractions of peel and pulp were analyzed by 2-D TLC. The AQ-7 fractions and the insoluble residue from the pulp sample were not further analyzed.

The Solids-3 fraction from peel was hydrolyzed by boiling in a 20% sodium hydroxide solution for 12 hours, and extracted with hexane in a distillation-extraction tube. The hexane fraction (hexane-2) and the aqueous hydrolysate fraction were separated. The hexane-2 fraction was analyzed by 2-D TLC. The aqueous fraction (AQ-8) was neutralized with hydrochloric acid, filtered, and partitioned with ethyl acetate. The resulting ethyl acetate-4 fraction was analyzed by 2-D TLC. The remaining aqueous fraction (AQ-9) was loaded onto a XAD-2 column and eluted with methanol. The resulting methanol fraction (0.15% TRR) was analyzed by 2-D TLC.

Table 5. Hydrolysis and fractionation of insoluble ^{14}C-residues in peel and pulp fractions from potatoes sampled 52 weeks after treatment.

Fraction	Peel		Pulp[a]	
	%TRR[b]	PPM[c]	%TRR	PPM
Cellulase Hydrolysis				
AQ-3	(0.27)	(0.551)	-	-
Ethyl acetate-2	0.21	0.425	-	-
AQ-4	(0.06)	(0.126)	-	-
Butanol-2	0.03	0.054	-	-
AQ-5	0.04	0.072	-	-
Solids-2	(0.99)	(2.021)	-	-
α-Amyloglucosidase Hydrolysis				
AQ-6	(0.12)	(0.250)	(0.07)	(0.028)
Ethyl acetate-3	0.08	0.166	0.03	0.013
AQ-7	0.04	0.084	0.04	0.016
Solids-3	(0.86)	(1.771)	0.19	0.083
NaOH Hydrolysis				
Hexane-2	0.35	0.719	-	-
AQ-8	(0.51)	(1.052)	-	-
Ethyl acetate-4	0.09	0.184	-	-
AQ-9	0.42	0.867	-	-
Total	1.26	2.571	0.26	0.112

[a]The Solids-1 fraction (0.26% TRR) from the pulp sample was only hydrolyzed with α-amyloglucosidase.

[b]Peel and pulp values for %TRR were recalculated by the reviewer based on radioactivity in whole unwashed potatoes.

[c]PPM equivalents of [^{14}C]chlorpropham are based on peel and pulp sample weights, respectively.

Characterization of residues

Radioactive residues were characterized using 1-D and 2-D TLC, HPLC, and GC/MS. TLC analyses were performed on silica gel plates using six different solvent systems.

Reference standards were spotted beside sample fractions and visualized using a UV lamp. Radioactive residues were detected using a TLC plate scanner and by scraping and counting

radioactive zones by LSS. HPLC analyses used a non-linear gradient of phosphoric acid and acetonitrile and detected reference standards and radioactive residues with a UV (254 nm) detector and an in-line radioactivity monitor. Radioactive fractions were also counted by LSS. Radioactive residues were identified by co-chromatography with reference standards.

Methanol washes from each sampling interval were analyzed by 2-D TLC and/or HPLC. The registrant indicated that chlorpropham was the only compound detected in the methanol washes; however, no representative TLC or HPLC radiochromatograms were presented. At 52-weeks posttreatment, chlorpropham in the methanol wash accounted for 86.44% of the TRR in or on whole unwashed potatoes (Table 6).

The acetonitrile and hexane extract fractions from the 52-week peel sample were analyzed by 2-D TLC. The only compound detected in these fractions was chlorpropham (6.97% TRR in acetonitrile, and 0.63% TRR in hexane). The acetonitrile fraction from pulp was also analyzed by 2-D TLC. Chlorpropham (I) (1.19% TRR), p-methoxychlorpropham (X) (0.05% TRR), and 3-chloroaniline-N-glucosylamine (XIX) (0.03% TRR) were detected by comparison to reference standards and their identities were confirmed by subsequent 2-D TLC and by HPLC. In addition, two unknown metabolites together accounting for not >0.15% of the TRR, were isolated from the pulp acetonitrile fraction.

The methanol/water fraction from peel and the ethyl acetate-1 fraction were analyzed by 1-D TLC and HPLC. The metabolites identified included: chlorpropham (I) (0.37% TRR), 3-chloroaniline-N-glucosylamine (XIX) (0.05% TRR), metabolite #1 (0.06% TRR), metabolite #2 (0.03% TRR), and metabolite #3 (0.05% TRR). The butanol-1 fraction from peel was analyzed by 1-D TLC and HPLC and the major metabolite isolated was metabolite #1 (0.23% TRR).

The ethyl acetate-1 fraction from the methanol/water fraction of pulp was analyzed by 2-D TLC. The predominant metabolites identified were 3-chloroaniline-N-glucosylamine (XIX) (0.13% TRR), metabolite #3 (0.37% TRR), and metabolite #1 (0.14% TRR). The major metabolite in the butanol-1 fraction was identified as metabolite #1 (0.35% TRR) by 1-D TLC using solvent system C.

The ethyl acetate-2 fraction from the cellulase hydrolysis of the 52-week peel sample was analyzed by 2-D TLC. Chlorpropham (0.20% TRR) was the only ^{14}C-residue detected in the fraction. The butanol-2 fraction from this hydrolysis contained metabolite #1 (0.03% TRR), which was detected by 2-D TLC. Following α-amyloglucosidase hydrolysis of the peel solids-2 fraction, the ethyl acetate-3 fraction was analyzed by 2-D TLC. The fraction contained chlorpropham (0.08%). Further NaOH hydrolysis of the peel Solids-3 fraction released 3-chloroaniline (VI) (0.35%) into the hexane-2 fraction, which was detected by 2-D TLC. Analysis of the ethyl acetate-4 fraction (0.09% TRR) from this hydrolysis was unsuccessful due to matrix interference. However, an analysis of the purified AQ-9 fraction using the same TLC system detected metabolite #1 (0.42% TRR).

The ethyl acetate-3 fraction from α-amyloglucosidase hydrolysis of the pulp Solids-1 fraction was analyzed by 2-D TLC. The analysis identified chlorpropham (I) (0.01% TRR), metabolite #3 (0.01% TRR), and 3-chloroaniline-N-glucosylamine (XIX) (0.006%).

Identification of metabolites #1, #2, and #3

For identification of metabolites #1 and #2, ethyl acetate-1 fractions from peel and pulp 40-, 44-, 48-, and 52-week samples were pooled and separated into 5 discrete bands of radioactivity by 1-D TLC. The band at the origin (metabolite #1) was scraped, eluted, and pooled with butanol-1 fractions from the same samples. The pooled fraction was concentrated and hydrolyzed with cellulase as described above. The hydrolysate was partitioned with ethyl acetate and the ethyl acetate fraction was analyzed by 2-D TLC. The major metabolite detected was 4-hydroxy-chlorpropham (III), which accounted for 82% of radioactivity in the ethyl acetate fraction. Other metabolites detected included 1-hydroxychlorpropham (IV) and metabolites #1 and #2. The partitioned aqueous fraction was analyzed by 2-D TLC. The analyses detected metabolite #1. Based on these results the registrant concluded that metabolite #1 is an oligosaccharide conjugate of 4-hydroxychlorpropham (XXI) and metabolite #2 is an oligosaccharide conjugate of 1-hydroxychlorpropham (XX).

For identification, metabolite #3 was isolated from acetonitrile and ethyl acetate-1 fractions of pulp by 1-D TLC. Bands of radioactivity from ethyl acetate-1 fractions corresponding to metabolite #3 were pooled and a portion of the radioactivity was transesterified using hydrochloric acid/methanol. Transesterified metabolite #3 was analyzed by EI/GC/MS. In addition, metabolite #3 isolated from the acetonitrile fractions was purified by HPLC and analyzed by Fast Atom Bombardment/MS, Direct Insertion Probe/MS, and Desorption Chemical Ionization/MS. These analyses identified 4-hydroxychlorpropham (XXI) as a constituent of metabolite #3, indicating that metabolite #3 is an amino acid conjugate of 4-hydroxychlorpropham (XXII).

In summary, the majority of ^{14}C-residues (84.57-98.08% TRR) remained on the tuber surface at all storage intervals and were extractable in the methanol wash. Of the ^{14}C-residues remaining in or on the washed tubers, the majority (1.86-9.77% TRR) was localized in the potato peel. The amount of ^{14}C-residues and the percentage of the TRR that translocated into the peel, first pulp layer, and the remaining pulp increased as the storage interval increased. Following analysis, >95% of the TRR in or on potatoes treated postharvest with [^{14}C]chlorpropham was identified. The major ^{14}C-residue isolated from potatoes stored for up to 52 weeks after treatment was the parent compound. Chlorpropham (I) accounted for all the radioactivity in the methanol wash (86.44% TRR) and the majority of radioactivity in both the pulp (1.21% TRR) and peel (8.26% TRR). Other minor components of the ^{14}C-residue identified in pulp and peel included 3-chloroaniline (VI), 3-chloroaniline-N-glucosylamine (XIX), p-methoxychlorpropham (X), oligosaccharide conjugates of 4-hydroxychlorpropham (XXI) and 1-hydroxychlorpropham (XX), and an amino acid conjugate of 4-hydroxychlorpropham (XXII).

Table 6. Identification and characterization of radioactive residues in or on potatoes treated postharvest with [^{14}C]chlorpropham and stored at 8°C for 52 weeks.

Metabolite	Pulp		Peel		Methanol Wash		Total	
	%TRR	PPM[a]	%TRR	PPM	%TRR	PPM	%TRR	PPM
chlorpropham	1.21	0.351	8.26	2.396	86.44	25.075	95.91	27.822
p-methoxychlorpropham (X)	0.05	0.015	-	-	-	-	0.05	0.015
3-chloroaniline (VI)	-	-	0.35	0.102	-	-	0.35	0.102
3-chloroaniline-N-glucosylamine (XIX)	0.18	0.052	0.05	0.015	-	-	0.23	0.067
4-hydroxychlorpropham oligosaccharide conjugate (XXI)	0.51	0.148	0.74	0.215	-	-	1.25	0.363
1-hydroxychlorpropham oligosaccharide conjugate (XX)	-	-	0.03	0.009	-	-	0.03	0.009
4-hydroxychlorpropham amino acid conjugate (XXII)	0.51	0.148	0.06	0.017	-	-	0.57	0.165
Unknowns								
Polar unidentified	0.15	0.044	0.12	0.035	-	-	0.27	0.079
Hexane soluble	0.05	0.015	-	-	-	-	0.05	0.015
Enzyme-hydrolyzed aqueous	0.04	0.012	0.17	0.049	-	-	0.21	0.061
Unextracted residues	0.19	0.055	-	-	-	-	0.19	0.055
Total	2.89	0.838	9.78	2.838	86.44	25.075	99.11	28.751

[a] The ppm is expressed on the basis of weight of pulp or peel.

CBRS Conclusions, Potatoes

The Chlorpropham Guidance Document (12/87) required specific data on residues of 3-chloroaniline, a probable mutagen which the Chlorpropham Residue Chemistry Chapter (8/14/87) identified in several plant species as a chlorpropham metabolite due to field treatment of growing plants. The current submission identified the presence of 3-chloroaniline and a glucose conjugate of 3-chloroaniline, although both were present as a small portion of the TRR. The present tolerance expression (40 CFR 180.181) includes parent and its metabolite 1-hydroxy-2-propyl 3'-chlorocarbanilate (IV). The current submission did not identify metabolite IV as part of the TRR due to post-harvest treatment of potatoes, but it did identify its oligosaccharide conjugate (XX) as a small portion of the TRR.

Conclusion 1a: The nature of residues of [^{14}C]chlorpropham applied as a postharvest treatment to stored potatoes is adequately understood. The major residue in potatoes stored for 52 weeks posttreatment was chlorpropham (95.91% of the total residues). Minor components of the residue identified in pulp and peel included 3-chloroaniline (VI), 3-chloroaniline-N-glucosylamine (XIX), p-methoxychlorpropham (X), oligosaccharide conjugates of 4-hydroxychlorpropham (XXI) and 1-hydroxychlorpropham (XX), and an amino acid conjugate of 4-hydroxychlorpropham (XXII), each accounting for 0.03-1.25% of the total residues (MRID 42085601). These data indicate that in plants, chlorpropham can undergo hydroxylation of the benzene ring or the isopropyl side chain and subsequently form conjugates with carbohydrates or amino acids. Chlorpropham can also undergo decarbanilation to form 3-chloroaniline (VI), which subsequently conjugates with natural plant products. Plant metabolites and their molecular structures are presented in Table 1.

Conclusion 1b: The present tolerance expression for chlorpropham (40 CFR 180.181) includes parent and 1-hydroxy-2-propyl-3'-chlorocarbanilate (IV). Metabolite IV was not identified as part of the TRR due to post-harvest treatment of potatoes, but an oligosaccharide conjugate (XX) was identified as a small portion of the TRR. The results of this metabolism study will be referred to the HED Metabolism Committee to determine the residues to be regulated.

Qualitative Nature of the Residue in Animals

Goats:

The Chlorpropham Task Force submitted data (1991; MRID 42112201) pertaining to the metabolism of uniformly ring-labeled [^{14}C]chlorpropham (98.13% radiochemical purity) in lactating goats. Two goats (designated Nos. 17 and 19) were each dosed orally once a day with a capsule containing 74.95 mg of [^{14}C]chlorpropham (specific activity of 19.8 μCi/mg; radiochemical purity 99.3%) for 7 consecutive days. A third goat was used as a control. The daily dose of [^{14}C]chlorpropham was equivalent to 31.5 and 35.7 ppm in the feed or 1.6 and 1.9 mg/kg body weight for goats #17 and #19, respectively. This dose is equivalent to 1.1-1.3x the maximum theoretical exposure possible from the consumption of tolerance level residues. During the treatment period, goats were milked twice daily and the two samples combined into a single daily sample for each goat. Milk samples were stored at -15°C. The goats were sacrificed 24 hours after the final dose and liver, kidney, muscle (leg and loin), and fat samples were collected. The tissue samples were diced, frozen and stored at -15°C until analysis 2-3 months after sacrifice.

Total Radioactive Residues (TRR)

Duplicate subsamples of liquid fractions were radioassayed by liquid scintillation spectrometry (LSS). Triplicate subsamples of tissues were homogenized in liquid nitrogen, combusted, and analyzed by LSS. Radioactive residues in milk and tissue samples were determined 2 days after sacrifice and again 2-3 months after sacrifice when the samples were

extracted for analysis. Detection limits were 0.003 ppm in milk, 0.03 ppm in liver, kidney, and muscle, and 0.06 ppm in fat. Results from both analyses were similar. The TRR in the sampled tissues and milk are reported in Table 7. Radioactive residues in the milk reached a plateau within 1-2 days of the initial dose.

Table 7. Total radioactive residues in milk and tissues from two lactating goats that received ca. 33 ppm of [^{14}C]chlorpropham for 7 days.

Sample	Goat #17 TRR (ppm)[a]	Goat #19 TRR (ppm)
Milk[b]	0.483	0.325
Liver	0.188	0.341
Kidney	0.061	0.060
Muscle		
leg	<0.03	<0.03
loin	<0.03	<0.03
Fat	0.041	0.031

[a][^{14}C]chlorpropham equivalents (specific activity 43,910 dpm/μg).

[b]The TRR in milk reported from each goat represents the maximum level of ^{14}C-residues detected, day 6 for goat #17 and day 1 for goat #19.

Extraction and Hydrolysis of Residues

Milk

Subsamples of day-6 milk from goats #17 (0.483 ppm) and #19 (0.272 ppm) were extracted sequentially with acetonitrile, acetonitrile/water (5:1 v/v), and hexane. The acetonitrile containing fractions were combined and concentrated. Radioactive residues in the combined acetonitrile/water fractions accounted for 99-99.5% of the TRR (0.481 and 0.269 ppm, respectively); no radioactive residues were detected in the hexane extracts. The hexane extracts and the remaining solids, which contained $\leq$1% of the TRR, were not further analyzed. The concentrated acetonitrile/water fractions were partitioned with hexane to yield aqueous (AQ-2) and hexane fractions. As <1% of the TRR partitioned into the hexane fractions, these fractions were not further analyzed. The AQ-2 fractions were evaporated to dryness and extracted with methanol. The resulting methanol fractions accounted for 98.4-99.2% of the TRR (0.479 and 0.268 ppm, respectively) and were analyzed by 1- and 2-D TLC and HPLC.

Fat

Fat samples from goats #17 and #19 were extracted sequentially with hexane and methanol. The hexane fractions accounted for 88.8-97.1% of the TRR (0.028 and 0.04 ppm, respectively) and the methanol fractions accounted for 0-4.56% of the TRR. Radioactive residues in the solid fractions accounted for 2.9-6.6% of the TRR. The hexane extracts were partitioned with acetonitrile. The majority of ^{14}C-residues partitioned into the acetonitrile fractions (83.9-93.1% TRR). The ^{14}C-residues remaining in the hexane fractions accounted for 4-5% of the TRR. The hexane and solid fraction were not further analyzed and the acetonitrile fraction was analyzed by 2-D TLC and HPLC.

Kidney

Kidney samples were extracted with methanol/water/chloroform (11:5:5, v/v/v). The extracts were centrifuged and filtered, and the solid residues reextracted with chloroform, centrifuged and filtered. The resulting solid fractions were radioassayed. The filtrates from each sample were combined and then separated into methanol/water and chloroform fractions and radioassayed. The methanol/water and the chloroform fractions were each concentrated and the chloroform fraction was partitioned with acetonitrile/hexane (1:1 v/v). The resulting distribution of ^{14}C-residues in the fractions are presented in Table 8. The acetonitrile and methanol/water fractions, which together accounted for 38.8-64% of the TRR, were analyzed by TLC and HPLC. The hexane fractions (<2% TRR) were not further analyzed.

The insoluble residues (Solids-1) were dissolved in Tris buffer (pH 7.4) and hydrolyzed at 37°C with collagenase for 1-2 hours and protease for 24 hours. The hydrolysates were centrifuged and the resulting supernatants (AQ-1) were concentrated and desalted with ethanol to yield ethanol/(AQ-1) fractions and precipitates. The precipitates were not further analyzed and the ethanol/(AQ-1) fractions were analyzed by HPLC and TLC.

The insoluble residues (Solids-2) remaining after enzymatic hydrolysis were further hydrolyzed using 6N hydrochloric acid. The resulting insoluble (Solids-3) fractions were not further analyzed. The solubilized radioactivity (AQ-2 fractions) were analyzed by HPLC and TLC.

A subsample of the Solids-1 fraction from kidney tissue of goat #17 was steam distilled/extracted by refluxing in 6N sodium hydroxide and extracted into isooctane. The hydrolysate was filtered and neutralized. The remaining insoluble ^{14}C-residues accounted for 17.79% of the TRR (0.011 ppm) and the aqueous fraction accounted for 7.78% of the TRR (0.005 ppm). The radioactivity extracted in isooctane accounted for 9.4% of the TRR (0.006 ppm). None of these fractions was further analyzed.

Table 8. Distribution of radioactive residues in kidney tissue from goats that received ca. 33 ppm of [^{14}C]chlorpropham for 7 days.

	Goat #17		Goat #19	
Fraction	%TRR	PPM	%TRR	PPM
Chloroform	15.92	0.010	10.64	0.006
Hexane	1.86	0.001	1.59	0.001
Acetonitrile	14.06	0.009	9.05	0.005
Methanol/water	49.90	0.030	29.70	0.018
Solids-1	34.18	0.021	59.66	0.036
Collagenase/Protease digestion				
AQ-1	16.26	0.010	28.93	0.017
Ethanol/(AQ-1) precipitate	11.56	0.007	23.52	0.014
Solids-2	4.70	0.003	5.41	0.003
6N hydrochloric acid hydrolysis	17.92	0.011	30.73	0.018
AQ-2	14.25	0.009	16.89	0.010
Solids-3	3.67	0.002	13.84	0.009

Liver

Liver samples were extracted with methanol/water/chloroform (11:5:5, v/v/v) as described previously for kidney. Of the TRR in liver, <20% was organic or aqueous soluble. The distribution of ^{14}C-residues in the liver fractions is presented in Table 9. The acetonitrile fractions were analyzed by HPLC and TLC and the hexane fractions were not further analyzed. Prior to analysis, the methanol/water fractions were hydrolyzed in acetate buffer (pH 5.0) with glucuronidase at 37°C for 2 hours. The hydrolysates were partitioned with ethyl acetate. Approximately half of the radioactivity (goat #17, 4.05% TRR; goat #19, 2.96% TRR) in the methanol/water fractions was extracted by ethyl acetate after hydrolysis. The aqueous fractions were not further analyzed; the ethyl acetate fractions were analyzed by TLC.

The insoluble residues (Solids-1) from the liver samples were hydrolyzed with collagenase and protease using the conditions described for kidney tissue. The hydrolysates were centrifuged and the resulting supernatants (AQ-1) concentrated. The majority of ^{14}C-residues were solubilized by the enzyme hydrolysis procedure (Table 9). The resulting aqueous fraction (AQ-1) from goat #19 was partitioned with ethyl acetate; however, as only 2.35% of the TRR was extracted by this step, the ethyl acetate fraction was not analyzed and the partitioning step was not used for the goat #17 liver sample.

The partitioned aqueous fraction (AQ-1a) from goat #19 and the AQ-1 fraction from goat #17 were desalted with ethanol to yield ethanol/(AQ-1) fractions and precipitate fractions. The precipitate fractions were not further analyzed and the ethanol/(AQ-1) fractions were analyzed by HPLC.

The insoluble residues (Solids-2) remaining after enzymatic hydrolysis were further hydrolyzed using 6N hydrochloric acid. The resulting insoluble (Solids-3) fractions were not further analyzed. The solubilized radioactivity (AQ-2 fractions) were analyzed by HPLC.

To further characterize insoluble ^{14}C-residues, subsamples of Solids-1, ethanol/(AQ-1), and AQ-2 fractions from liver of goat #17 were steam distilled/extracted by refluxing in 6N sodium hydroxide and collected in isooctane. The hydrolysate was filtered and neutralized. The distribution of radioactive residues between the insoluble residue, the aqueous hydrolysate, and the isooctane extract for each sample is presented in Table 10. The isooctane extract from the AQ-2 fraction was concentrated and analyzed by HPLC.

Table 9. Distribution of radioactive residues in liver tissue from goats that received ca. 33 ppm of [^{14}C]chlorpropham for 7 days.

	Goat #17		Goat #19	
Fraction	%TRR	PPM	%TRR	PPM
Chloroform	7.85	0.015	6.69	0.023
Acetonitrile	1.90	0.004	2.51	0.009
Hexane	5.95	0.011	4.18	0.014
Methanol/water	8.99	0.017	6.45	0.022
Solids-1	83.16	0.156	86.86	0.296
Collagenase/Protease digestion				
AQ-1	53.95	0.101	63.69	0.217
Ethyl acetate	NA	NA	2.35	0.008
AQ-1a	NA	NA	61.34	0.209
Ethanol/(AQ-1)	37.33	0.070	54.19	0.185
precipitate	16.62	0.031	7.15	0.024
Solids-2	29.21	0.055	23.17	0.079
6N hydrochloric acid hydrolysis				
AQ-2	23.20	0.044	20.40	0.070
Solids-3	6.01	0.011	2.77	0.009

Table 10. Distribution of radioactive residues from sodium hydroxide hydrolysis and distillation/extraction of liver fractions from goat #17.

	Isooctane Extract		Aqueous Hydrolysate		Insoluble Residue	
Fraction	%TRR	PPM	%TRR	PPM	%TRR	PPM
Solids-1	19.09	0.036	37.96	0.071	27.97	0.053
Ethanol/ (AQ-1)	16.25	0.031	12.46	0.023	8.60	0.016
AQ-2	12.12	0.022	7.63	0.014	3.44	0.006

Characterization of Residues

Radioactive residues were characterized using 1-D and 2-D TLC and HPLC. TLC analyses used silica gel plates and four different solvent systems.

Sample fractions were cochromatographed with reference standards. Radioactivity on TLC plates was detected and quantified using a TLC plate scanner or by scraping radioactive zones and counting by LSS. Reference standards were visualized using short wavelength UV. HPLC analyses used a non-linear gradient of phosphoric acid to acetonitrile and detected reference standards and radioactive residues with a UV (254 nm) detector and an in-line radioactivity monitor. Radioactive fractions were also assayed by LSS.

Milk

HPLC analysis of the methanol fraction detected 4-hydroxychlorpropham (III), 4-hydroxychlorpropham-O-sulfonic acid (XIV), 1,4-dihydroxychlorpropham-4-O-sulfonic acid (XV), 3-chloro-4-hydroxyacetanilide-O-sulfonic acid (XVI), and 4-hydroxychlorpropham-O-glucuronic acid (XVIII) in milk (Table 11).

The identity of the major milk metabolite, 4-hydroxychlorpropham-O-sulfonic acid (XIV) (77.9% TRR), was verified by analysis of the principle band of radioactivity resulting from a TLC analysis of the methanol fraction. The band of radioactivity was scraped, eluted in methanol, and concentrated. The fraction was dissolved in acetate buffer (pH 5) and hydrolyzed at 37°C for 1 hour using sulfatase enzyme. The hydrolysate was partitioned with ethyl acetate (69.6% TRR). Analysis of the ethyl acetate fraction by HPLC and 1-D TLC identified 4-hydroxychlorpropham (III) as the product of the sulfatase hydrolysis.

The identities of the other metabolites isolated from milk were confirmed by collecting and pooling their respective HPLC peaks and analyzing the pooled peaks by 1-D TLC.

Fat

The acetonitrile fractions from fat were analyzed by 2-D TLC. Chlorpropham was the only ^{14}C-residue detected (83.9 and 93.1% TRR) and its identity was confirmed by HPLC and TLC analysis.

Kidney

The acetonitrile fraction from the chloroform fraction of kidney tissue (goat #17) was purified by preparative HPLC. The major area of radioactivity was collected and analyzed, along with the acetonitrile fraction from goat #19, by TLC analysis. Kidney metabolites (goat #17) identified were chlorpropham (I) (2.11% TRR), p-methoxychlorpropham (X) (2.17% TRR), 3-chloroacetanilide (VIII) (2.64% TRR), and at least three unknown metabolites (each accounting for <5% TRR). In the acetonitrile fraction from goat #19 kidney tissue, 3-chloro-6-hydroxychlorpropham (II) (1.30% TRR) and 1,4-dihydroxychlorpropham (XII) (1.09% TRR) were identified along with four unknowns, each of which accounted for <3% of the TRR.

The methanol/water fraction from kidney was concentrated and desalted with ethanol and the resulting ethanol/water fraction was purified by 1-D TLC. Four bands of radioactivity were detected in the fraction. Bands 1 and 2 were analyzed by HPLC and resulted in two peaks of radioactivity. Subsequent analysis of these two peaks by 1-D TLC identified 3-chloro-4-hydroxyacetanilide (VII) (1.46% TRR), 1,4-dihydroxychlorpropham-4-O-sulfonic acid (XV) (0.51% TRR), 3-chloro-4-hydroxyacetanilide-O-sulfonic acid (XVI) (4.44% TRR), and 4-hydroxychlorpropham-O-sulfonic acid (XIV) (31.91% TRR). Band 3 from the preparative TLC analysis was further analyzed by 1-D TLC. The analysis identified 4-hydroxychlorpropham-O-glucuronic acid (XVIII) (0.61% TRR) and an unknown metabolite (B-6; 4.27% TRR) previously isolated from rat urine. Other unknowns isolated from the methanol/water fraction accounted for 1.65% of the TRR. The data cited are from the analysis of the fraction from goat #17, but are also representative of the data from goat #19. A summary of aqueous and organic soluble metabolites identified in kidney tissue is presented in Table 11.

The ethanol/(AQ-1) fraction (0.007-0.014 ppm) from the enzymatic hydrolysis and the AQ-2 fraction (0.002-0.009 ppm) from the acid hydrolysis were analyzed with HPLC. However, radioactive peaks were inadequately resolved and no ^{14}C-residues were identified.

Liver

The acetonitrile fractions from the liver chloroform fraction (goat #17 and #19) were analyzed by HPLC and TLC. Liver metabolites from goats #17 and #19 identified by cochromatography included 3-chloroacetanilide (VIII) (3.23% and 0.67% TRR) and 4-hydroxychlorpropham (III) (2.12% and 1.33% TRR). In addition, at least three unknowns, each accounting for ≤1.1% of the TRR, were isolated from the acetonitrile fractions.

Table 11. Identification and characterization of [^{14}C]chlorpropham residues in tissues and milk from a lactating goat dosed with 31.5 ppm of [^{14}C]chlorpropham for 7 days.[a]

Metabolite	Milk		Fat		Kidney[b]		Liver	
	%TRR	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM
chlorpropham	-	-	83.85	0.026	2.11	0.001	-	-
p-methoxychlorpropham (X)	-	-	-	-	2.17	0.001	-	-
3-chloroacetanilide (VIII)	-	-	-	-	2.64	0.002	3.23	0.006
4-hydroxychlorpropham (III)	1.78	0.009	-	-	-	-	3.95	0.007
3-chloro-4-hydroxyacetanilide (VII)	-	-	-	-	1.46	0.001	-	-
1,4-dihydroxychlorpropham (XII)	-	-	-	-	-	-	1.11	0.002
4-hydroxychlorpropham-O-sulfonic acid (XIV)	77.92	0.376	-	-	31.91	0.019	-	-
1,4-dihydroxychlorpropham-4-O-sulfonic acid (XV)	5.72	0.028	-	-	0.51	<0.001	-	-
3-chloro-4-hydroxyacetanilide-O-sulfonic acid (XVI)	6.77	0.033	-	-	4.44	0.003	0.50	0.001
chlorpropham carboxylic acid (XVII)	-	-	-	-	-	-	0.64	0.001
4-hydroxychlorpropham-O-glucuronic acid (XVIII)	3.02	0.015	-	-	0.61	<0.001	-	-
Total Identified	95.21	0.461	83.85	0.026	45.85	0.028	9.43	0.017
Unknowns								
B-6[c]	-	-	-	-	4.27	0.003	-	-
Isolated unknowns	4.03	0.019	-	-	13.78	0.008	0.60	0.001
Hexane soluble residues	0.27	0.001	4.99	0.002	1.86	0.001	1.90	0.004
Methanol soluble residues	-	-	4.56	0.001	-	-	-	-
Aqueous soluble residues	-	-	-	-	-	-	4.94	0.009
Protease hydrolyzable residues	-	-	-	-	16.26	0.010	53.95	0.101
Acid hydrolyzable residues	-	-	-	-	14.25	0.009	23.20[b]	0.044
Unextracted Residues	0.49	0.002	6.60	0.002	3.67	0.002	6.01	0.011
Total	100.00	0.483	100.00	0.031	99.94	0.061	100.03	0.187

[a]Data are from goat #17 and are representative of the data from goat #19.

[b]In kidney tissue from goat #19, trace (0.001 ppm) amounts of 3-chloro-6-hydroxyaniline (XXIV) and 1,4'-dihydroxychlorpropham (XII) were also identified. The major constituent, level unreported, of acid hydrolyzable residues in liver cochromatographed with 3-chloroaniline (VI).

[c]Metabolite of an unknown structure, found in rat urine.

The ethyl acetate fractions from the glucuronidase hydrolysis of the methanol/water fraction were analyzed by 1-D TLC. Metabolites identified from goats #17 and #19 included 4-hydroxychlorpropham (III) (1.81% and 1.10% TRR, respectively), 1,4-dihydroxychlorpropham (XII) (1.11% and 0.93% TRR), 3-chloro-4-hydroxyacetanilide-O-sulfonic acid (XVI) (0.50% and 0.30% TRR), and chlorpropham carboxylic acid (XVII) (0.64% and 0.35% TRR). A summary of aqueous and organic soluble metabolites identified in liver tissue is presented in Table 11.

The ethanol/(AQ-1) fraction from the enzymatic hydrolysis and the AQ-2 fraction from the acid hydrolysis were analyzed by HPLC; however, radioactive peaks were inadequately resolved and no ^{14}C-residues were identified.

The isooctane extract from distillation/extraction of the AQ-2 fraction from goat #17 liver was analyzed by HPLC. The major peak of radioactivity cochromatographed with 3-chloroaniline (VI). Registrant did not report levels of this residue in ppm or its TRR.

CBRS Comments, Goat Metabolism:

Conclusion 2a: In lactating goats, radioactive residues in milk (0.483 ppm), fat (0.061 ppm), and kidney (0.041 ppm) were adequately characterized; unknown metabolites represented less than 0.05 ppm in all these tissues, and in muscle. The principle metabolite isolated from milk was 4-hydroxychlorpropham-O-sulfonic acid (XIV), which accounted for 77.92% of the total radioactive residue (TRR). Minor amounts of 4-hydroxychlorpropham (III) (1.78% TRR), 1,4-dihydroxychlorpropham-4-O-sulfonic acid (XV) (5.72% TRR), 3-chloro-4-hydroxyacetanilide-O-sulfonic acid (XVI) (6.77% TRR), and 4-hydroxychlorpropham-O-glucuronic acid (XVIII) (3.02% TRR) were also detected. In fat, the principle radioactive residue was the parent compound, chlorpropham (I), which accounted for 83.85% of the TRR. In kidney tissue, the major metabolite was 4-hydroxychlorpropham-O-sulfonic acid (XIV) (31.91% TRR). In addition, trace amounts of chlorpropham (I) (2.11% TRR), p-methoxychlorpropham (X) (2.17% TRR), 3-chloroacetanilide (VIII) (2.64% TRR), 3-chloro-4-hydroxyacetanilide (VII) (1.46% TRR), 3-chloro-6-hydroxyaniline (XXIV) (1.3% TRR), 1,4-dihydroxychlorpropham (XII) (1.09% TRR), 1,4-dihydroxychlorpropham-4-O-sulfonic acid (XV) (0.51% TRR), 3-chloro-4-hydroxyacetanilide-O-sulfonic acid (XVI) (4.44% TRR), and 4-hydroxychlorpropham-O-glucuronic acid (XVIII) (0.61% TRR) were identified. Metabolite B-6, an unknown in rat urine, represented 4.27% TRR in kidney. (MRID 42112201) Metabolites and their molecular structures are presented in Table 1.

Conclusion 2b: In lactating goats, residues were greater than 0.06 ppm only in milk and liver, 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 (VIII) (3.23% TRR), 4-hydroxychlorpropham (III) (3.95% TRR), 1,4-dihydroxychlorpropham (XII) (1.11% TRR), 3-chloro-4-hydroxyacetanilide-O-sulfonic acid (XVI) (0.5% TRR), and chlorpropham

carboxylic acid (XVII) (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.

Recommendation: Further work is necessary before 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.

Poultry:

The Chlorpropham Task Force submitted data (1991; MRID 42130401) on the metabolism of [^{14}C]chlorpropham in poultry. Ten white leghorn laying hens were each dosed once daily for 7 days with a capsule containing 6 mg of [^{14}C]chlorpropham (equivalent to 50 ppm in the feed). The daily chlorpropham dose represented approximately 5x the maximum theoretical dietary exposure. [^{14}C]Chlorpropham was uniformly ring-labeled and had a specific activity of 21.2 μCi/mg with a radiochemical purity of 98%. Eggs were collected twice daily, and egg whites and yolks were separated, homogenized, and stored frozen. The animals were sacrificed 8 hours after the last dose; liver, kidney, breast muscle, thigh muscle, fat, and skin were collected. Tissues were diced and stored frozen. Samples were shipped frozen to the analysis laboratory where they were stored frozen (ca. -15 °C) for an unspecified period of time (not more than 16 months).

Total Radioactive Residues (TRR)

Subsamples of eggs, tissues, and solids were homogenized in liquid nitrogen, combusted and radioassayed by liquid scintillation spectrometry (LSS). Total radioactive residues were 0.468 ppm in liver, 0.455 ppm in kidney, <0.028 ppm in both breast and thigh muscle, 0.186 ppm in fat, and 0.153 ppm in skin. The Day 6 egg yolk and white samples contained the highest combined residues (0.074, 0.199 respectively) and were chosen for residue isolation and analyses. The radioassay (LSS) limit of detection was reportedly 0.028 ppm. Residues were reported as low as 0.001 ppm for chromatographic analyses, but no detection limit was reported. Sample calculations were provided.

Extraction and Hydrolysis of Residues

Residues were extracted from thawed egg whites and yolks with acetonitrile and hexane (1:1, v/v). The solids were separated, combusted and radioassayed. The liquid phases were

separated, using water, and the hexane layer was cleaned up using Florisil and analyzed by TLC. The acetonitrile was evaporated and the residues contained in the remaining water layer were partitioned into ethyl acetate. In addition, the yolk solids were hydrolyzed with protease Type I (pH 7.5, 37 °C, 4 hours) and partitioned into ethyl acetate. The resulting water and ethyl acetate layers were analyzed by TLC and radiochromatography. The aqueous layer was further partitioned into ethanol, and resulted in an ethanol/aqueous fraction and a precipitate. The precipitate was combusted and radioassayed. The ethanol fraction was refluxed with hydrochloric acid, and partitioned into ethyl acetate at basic, acidic, and neutral pHs.

Residues were extracted from thawed liver, kidney, and muscle with methanol:water:chloroform (11:5:5, v/v). The solids were separated, combusted and radioassayed. The liquid phases were separated into methanol/water and chloroform fractions. The chloroform layer was concentrated, and residues were partitioned into hexane and acetonitrile. The hexane fraction was cleaned up using Florisil prior to TLC analysis. The liver and kidney solids were hydrolyzed with collagenase Type IV (pH 7.4, 37 °C, 1-2 hours) followed by protease Type I (24 hours). The solids were separated and hydrolyzed with hydrochloric acid, resulting in an aqueous solution that was analyzed by HPLC. The aqueous phase (liver only) was partitioned with ethyl acetate. The aqueous layer was concentrated and redissolved in ethanol, resulting in an ethanol/aqueous layer and a precipitate. The precipitate was combusted and radioassayed. The aqueous fraction was hydrolyzed with hydrochloric acid and partitioned into ethyl acetate at basic, acidic and neutral pHs.

Residues were extracted from thawed skin and fat with hexane. The solids were separated, combusted and radioassayed. The hexane layer was partitioned with acetonitrile. The solids were extracted with methanol, and the methanol fraction was analyzed using TLC, HPLC, and radiochromatography. The distribution of ^{14}C-activity in extracts of tissues and eggs is summarized in Table 12.

Table 12. Distribution of total radioactive residues (TRR) in egg whites and yolks, liver, kidney, thigh muscle, skin and fat extracts from poultry dosed with [^{14}C]chlorpropham.

Fraction	Egg white[a]		Egg yolk[a]		Liver[b]		Kidney[b]		Thigh muscle[c]		Skin		Fat	
	%TRR[f]	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM
Acetonitrile	(83.60)	(0.062)	(66.42)	(0.132)	-	-	-	-	-	-	-	-	-	-
Ethyl acetate	12.97	0.010	36.81	0.073	-	-	-	-	-	-	-	-	-	-
Water	70.63	0.052	29.61	0.059	-	-	-	-	-	-	-	-	-	-
Hexane	-	-	(12.73)	(0.025)	-	-	-	-	-	-	(73.28)	(0.112)	(98.05)	(0.182)
Hexane	-	-	-	-	-	-	-	-	-	-	5.20	0.008	6.27	0.012
Acetonitrile	-	-	-	-	-	-	-	-	-	-	68.08	0.104	91.78	0.171
Methanol	-	-	-	-	(17.31)	(0.081)	(38.61)	(0.176)	(28.06)	(0.004)	(18.95)	(0.029)	(1.06)	(0.002)
Chloroform	-	-	-	-	(7.38)	(0.035)	(19.78)	(0.090)	(51.39)	(0.008)	-	-	-	-
Hexane	-	-	-	-	2.63	0.012	11.94	0.054	4.08	0.001	-	-	-	-
Acetonitrile	-	-	-	-	4.75	0.022	7.84	0.036	47.31	0.007	-	-	-	-
Solids	16.40	0.012	20.85	0.041	75.31	0.352	41.61	0.189	20.55	0.003	7.7	0.012	0.89	0.002
Protease supernatant	-	-	19.15	0.038	-	-	-	-	-	-	-	-	-	-
Ethanol[d]	-	-	(16.69)	(0.033)	-	-	-	-	-	-	-	-	-	-
Precipitate	-	-	2.46	0.005	-	-	-	-	-	-	-	-	-	-
Ethyl acetate	-	-	(1.70)	(0.003)	-	-	-	-	-	-	-	-	-	-
Collagenase/Protease supernatant	-	-	-	-	44.03	0.206	21.42	0.097	-	-	-	-	-	-
Ethyl acetate	-	-	-	-	(3.77)	(0.018)	-	-	-	-	-	-	-	-
Aqueous	-	-	-	-	40.26	0.188	-	-	-	-	-	-	-	-
Ethanol	-	-	-	-	(35.32)	(0.165)	(19.67)	0.089	-	-	-	-	-	-
Precipitate	-	-	-	-	4.94	0.023	1.75	0.008	-	-	-	-	-	-
Solids[e]	-	-	-	-	(31.28)	(0.146)	(20.19)	(0.092)	-	-	-	-	-	-
Total Extractable	83.60	0.062	97.54	0.194	95.06	0.445	98.25	0.447	79.45	0.012	92.23	0.144	99.11	0.184
Total Unextractable	16.40	0.012	2.46	0.005	4.94	0.023	1.75	0.008	20.55	0.003	7.7	0.012	0.89	0.002

[a]Data from day-6 egg samples are presented. [b]A second sample was extracted, as the first, and the results (not reported here) were similar . [c]Detection limit for LSS reportedly 0.028 ppm; total radioactive residues in thigh muscle were below the detection limit, and were not characterized further. [d]Hydrolyzed with hydrochloric acid, partitioned with ethyl acetate, and analyzed. [e]Solids hydrolyzed with hydrochloric acid, releasing all residues, and analyzed by HPLC. [f]Sum of values presented in parentheses above.

Characterization of residues

Ethyl acetate and water fractions from egg yolk extractions were analyzed by both TLC and HPLC. Egg white ethyl acetate and water fractions were analyzed by TLC and HPLC, respectively. Egg yolk hexane soluble residues were cleaned up using Florisil and analyzed by TLC. Methanol fractions from liver and kidney extracts were cleaned up using C_{18} cartridges and analyzed by HPLC. Liver and kidney acetonitrile soluble residues were analyzed by TLC, and hexane soluble residues were cleaned up with Florisil and analyzed by TLC. Liver residues contained in ethyl acetate following enzyme hydrolysis were analyzed by TLC. Liver and kidney solids remaining after enzyme hydrolysis were acid hydrolyzed and then analyzed by HPLC. Acetonitrile soluble skin and fat residues and methanol soluble skin residues were analyzed by TLC.

A summary of the characterization of radioactive residues in egg whites and yolks, liver, kidney, skin and fat samples from hens fed [^{14}C]chlorpropham is presented in Table 13.

The principle metabolite isolated from kidney was 4-hydroxychlorpropham-O-glucuronic acid (XVIII) representing 9.25% of the TRR. Kidney and liver residues, representing 25.08% (0.144 ppm) and 64.36% (0.301 ppm) of the TRR respectively, were described by the registrant as 3-chloro-4-hydroxyaniline (IX) related compounds. The major ^{14}C-residue isolated from skin and fat was chlorpropham (I), representing 68.08%, and 91.78% of the TRR, respectively. In addition, 4-hydroxy-chlorpropham-O-sulfonic acid (XIV) represented 18.95% of the TRR in skin tissues. The major metabolite in egg whites was 3-chloro-4-hydroxyaniline-O-sulfonic acid (XI), representing 22.24% of the TRR. Egg whites, liver, and kidney also contained chlorpropham (I) at 3.05%, 0.51%, and 7.36% of the TRR, respectively. The major metabolites in egg yolks were chlorpropham (I) (19.91% of the TRR) and 4-hydroxychlorpropham-O-sulfonic acid (XIV) (31.65% of the TRR).

Table 13. Characterization of radioactive residues in egg whites and yolks, liver, kidney, skin and fat samples from hens fed [^{14}C]chlorpropham.

Component	Egg White[a]		Egg Yolk[a]		Liver		Kidney		Skin		Fat	
	%TRR	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM	%TRR	PPM
Chlorpropham (I)	3.05	0.002	19.91	0.040	0.51	0.002	7.36	0.033	68.08	0.104	91.78	0.171
1-hydroxychlorpropham (IV)	2.25	0.002	3.38	0.007	-	-	-	-	-	-	-	-
3-chloroacetanilide (VIII)	1.39	0.001	1.51	0.003	-	-	-	-	-	-	-	-
3-chloro-4-hydroxyacetanilide (VII)	-	-	-	-	0.35	0.002	0.40	0.002	-	-	-	-
3-chloro-4-hydroxyaniline (IX)	-	-	-	-	-	-	3.37	0.015	-	-	-	-
3-chloro-4-hydroxyaniline-O-sulfonic acid (XI)	22.24	0.016	-	-	-	-	3.37	0.015	-	-	-	-
4-hydroxychlorpropham (III)	-	-	-	-	3.66	0.017	-	-	-	-	-	-
1,4-dihydroxychlorpropham (XII)	-	-	-	-	-	-	5.03	0.023	-	-	-	-
3-chloro-4-hydroxyaniline-N-glucuronic acid-4-O-sulfonic acid (XIII)	3.88	0.003	-	-	-	-	-	-	-	-	-	-
4-hydroxychlorpropham-O-sulfonic acid (XIV)	7.72	0.006	31.65	0.063	4.30	0.020	-	-	18.95	0.029	-	-
1,4-dihydroxychlorpropham-4-O-sulfonic acid (XV)	1.10	0.001	-	-	-	-	-	-	-	-	-	-
3-chloro-4-hydroxyacetanilide-O-sulfonic acid (XVI)	-	-	-	-	-	-	3.66	0.017	-	-	-	-
chlorpropham carboxylic acid (XVII)	3.26	0.002	-	-	-	-	3.04	0.014	-	-	-	-
4-hydroxychlorpropham-O-glucuronic acid (XVIII)	-	-	-	-	-	-	9.25	0.042	-	-	-	-
3-chloro-4-hydroxyacetanilide-O-glucuronic acid (XIII)	-	-	-	-	-	-	8.07	0.037	-	-	-	-
Unknowns (found in rat urine)	-	-	-	-	-	-	5.54	0.025	-	-	-	-
Unknowns (organo or aqueous soluble)	38.78	0.029	9.97	0.020	11.82	0.055	5.96	0.029	-	-	1.06	0.002
Unknowns (lipophilic)	-	-	12.73	0.025	2.48	0.012	-	-	5.20	0.008	6.27	0.012
Unknowns (Protease released)	-	-	20.85	0.041	-	-	-	-	-	-	-	-
Unknowns (3-chloro-4-hydroxy-aniline related)[b]	-	-	-	-	64.36	0.301	25.08	0.144	-	-	-	-
Unknowns	-	-	-	-	12.52	0.059	19.86	0.090	-	-	-	-
Total Identified	44.89	0.033	56.45	0.113	8.82	0.041	43.55	0.198	87.03	0.133	91.78	0.171

[a]Day-6 data presented. [b]Residues released following enzyme or acid hydrolysis.

CBRS Comments, Poultry Metabolism

Conclusion 3a: The qualitative nature of the residue in poultry is adequately understood for purposes of supporting the limited chlorpropham use (postharvest potatoes) and pending submission of storage stability data (Conclusion 3b). About 64% (0.301 ppm) of the TRR in liver, and 25% (0.144 ppm) of the TRR in kidney were described as 3-chloro-4-hydroxyaniline related compounds, but were not further identified. Additional identification of this portion of the TRR will not be required. The identified metabolites provide sufficient evidence of the metabolic path (Conclusion 4). The data show that the principle metabolite isolated from kidney was 4-hydroxychlorpropham-O-glucuronide (XVIII) representing 9.25% of the TRR. The major ^{14}C-residue isolated from skin and fat was chlorpropham (I), representing 68.08%, and 91.78% of the TRR, respectively. In addition, 4-hydroxychlorpropham-O-sulfonic acid (XIV) represented 18.95% of the TRR in skin tissues. The major metabolite in egg whites was 3-chloro-4-hydroxyaniline-O-sulfonic acid (XI), representing 22.24% of the TRR. Egg whites, liver, and kidney also contained chlorpropham at 3.05%, 0.51%, and 7.36% of the TRR, respectively. The major metabolites in egg yolks were chlorpropham (I) (19.91% of the TRR) and 4-hydroxychlorpropham-O-sulfonic acid (XIV) (31.65% of the TRR).

Conclusion 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.

Conclusion 4: The registrant has proposed a metabolic pathway in which chlorpropham may be oxidized to 4-hydroxychlorpropham or degraded to 3-chloroaniline. The aniline is subsequently metabolized to 3-chloro-4-hydroxyaniline-O-sulfonic acid. The hydroxy chlorpropham is further metabolized to 4-hydroxychloropropham-O-sulfonic acid or 4-hydroxychlorpropham-O-glucuronide.

Residue Analytical Methods

The Chlorpropham Task Force submitted a GLC/NPD analytical method and supporting data (1991; MRID 42123101) for the determination of residues in potatoes. The method determines 3-chloroaniline (VI), chlorpropham (I), *p*-hydroxychlorpropham (III), and *p*-methoxychlorpropham (X). The method involves first washing the potato samples in tap water to remove soil. The samples are then mixed and finely chopped while extracting the residues into methanol:0.5N hydrochloric acid (1:1, v/v), microwave heated (reportedly to release conjugated residues), and partitioned with hexane:ethyl acetate (1:1, v/v) including adding potassium phosphate buffer and sodium hydroxide to neutralize the solution. Residues are collected in the organic fraction, and analyzed by GLC/NPD. Apparent residues in controls were not reported; however, a control chromatogram did not contain

peaks at the calibration standard retention times. The method detection limit was not reported. However, the representative chromatograms, including the low calibration standard and control, indicate that the detection limit would at least approach 0.2 ppm. The method's limits of detection must be established. Recovery data are summarized in Table 14.

Table 14. Recovery of 3-chloroaniline, chlorpropham, *p*-hydroxychlorpropham, and *p*-methoxychlorpropham from potato samples.

Fortification level (sample number)	Percent Recovery			
	3-chloroaniline (VI)	chlorpropham (I)	*p*-hydroxychlorpropham (III)	*p*-methoxychlorpropham (X)
0.4 ppm (7)	21.8-47.8	53.5-83.0	71.0-105	62.8-93.2
average	38.0	68.3	86.9	77.7
1.2 ppm (6)	33.3-53.1	59.7-80.4	73.2-93.4	69.9-90.1
average	41.0	68.7	82.8	78.2

CBRS Comments, Method

The Update to the Residue Chemistry Chapter (10/16/91) noted that data collection and enforcement methodology should include pre-hydrolysis extraction and hydrolysis steps in order to detect free and conjugated metabolites of concern. In addition, methods must be tested with regard to their efficiency in extracting bound residues, including residues of 3-chloroaniline, if appropriate. To this end, it was recommended that methods be validated with weathered radioactive residues in conjunction with required metabolism studies.

Conclusion 5: The method adequately recovers chlorpropham (I), *p*-hydroxychlorpropham (III), and *p*-methoxychlorpropham (X) from fortified potato samples. However, the method as described would not be suitable for data collection or enforcement of tolerances, if the residues to be regulated were to include 3-chloroaniline (VI) or other metabolites. After the residues to be regulated in potatoes are determined, the registrant must submit an adequate enforcement method along with supporting data on all residues to be regulated, following an independent laboratory validation. The method must include pre-hydrolysis extraction and hydrolysis steps that allow for the detection of free as well as conjugated metabolites, if conjugated metabolites are included in the residues to be regulated. Limits of detection must be reported for each residue to be regulated. Validation data on weathered residues from metabolism studies must be included.

References

Citations for the MRID documents and Agency correspondence referred to in this review are presented below. Submissions reviewed in this document are indicated in shaded type.

00035480 Ecke, G.G.: Ferguson, C.E.; Mitten, M.E.; et al. (1973) Agriculutural Chemicals: CCLXXVII: Qualitative Investigation of CIPC Metabolites in Soybean Shoots: Research Report No. BR-18605. (Unpublished study received on unknown date under 4F1429; submitted by PPG Industries, Inc., Barberton, Ohio; CDL: 093808-K)

00035485 Gard, L.N.; Dahmer, L.; Wiedmann, J.L.; et al. (1973) Analysis of 14C-CIPC Treated Soybean plants for 2- and 4- Hydroxy CIPC: First and second scheduled samples grown at Fargo, North Dakota (1972): Research Report No. BTS-18580. Methods dated May 18, 1973. (Unpublished study received on unknown date under 4F1429; submitted by PPG Industries, Inc., Barberton, Ohio; CDL: 093808-P)

00036395 Hardies, D.E. (1973) Agricultural Chemicals, CCXXIV. Procedure for Growing, Sampling, and Shipping of Orchardgrass Grown in the Presence of 14C CIPC. A protocol submitted to Boyce Thompson for CIPC Metabolite Study: Research Report No. BAR-18317. (Unpublished study received on unknown date under 2F1276: submitted by PPG Industries, Inc., Barberton, Ohio; CDL: 092174-V)

00036628 Still, G.G.; Mansager, E.R. (1973) Soybean Shoot Metabolism of Isopropyl-3-Chlorocarbanilate: Ortho and Para Aryl Hydroxylation. Pesticide Biochemistry and Physiology 3(1):87-95. (Unpublished submission received Jan. 4, 1974 under 2F1276: submitted by PPG Industries, Inc., Barberton, Ohio; CDL:092175-Y)

00036629 Still, G.G.; Mansager, E.R. (1973) Metabolism of Isopropyl-3-Chlorocarbanilate by Cucumber Plants. Journal of Agricultural and Food Chemistry 21(5):787-791. (Unpublished submission received Jan. 4, 1974 under 2F1276; submitted by PPG Industries, Inc., Barberton, Ohio; CDL:092175-Z)

00036638 Wiedmann, J.L.; Mitten, M.E.; Pensyl, J.W. (1973) Preliminary Determination of CIPC and Metabolites I, II, III, and IV in Turnip Roots grown in the presence of 14C-CIPC at Boyance-Thompson in 1973: Research Report No. BR-18942. (Unpublished study received Jan. 4, 1974 under 2F1276; submitted by PPG Industries, Inc., Barberton, Ohio; CDL: 092175-AJ)

00036639 Wiedmann, J.L.; Mitten, M.E.; Pensyl, J.W. (1973) Preliminary Determination of CIPC and Metabolites I, II, III, and IV in Turnip Tops grown in 14C-CIPC treated soil at Boyce-Thompson during 1973: Research Report No. BR-18943.

(Unpublished study received Jan. 4, 1974 under 2F1276; submitted by PPG Industries, Inc., Barberton, Ohio; CDL:092175-AK)

00036640 Wiedmann, J.L.; Mitten, M.E.; Pensyl, J.W. (1973) Preliminary Determination of CIPC and Metabolites I, II, III, and IV in Orchard grass grown in 14C-CIPC treated soil at Boyce Thompson during 1973: Research Report No. BR-18957. (Unpublished study received Jan. 4,1974 under 2F1276; submitted by PPG Industries, Inc., Barberton, Ohio; CDL:092175-AL)

00114739 PPG Industries, Inc. (1974) Analyses for Residues of CIPC Chemicals in Various Products. (Compilation; unpublished study received on unknown date under 4F1429; CDL:098173-A)

00114794 PPG Industries, Inc. (1979) Analyses for Residues of Furloe 124 and Other Herbicides in Various Products. (Compilation; unpublished study received Jun. 13, 1979 under 748-220; CDL:238627-A)

00139680 Still, G.G. (1973) Letter sent to Warren H. Zick dated Dec. 21, 1973 Studies on the Metabolism of Chloropropham. (Unpublished study received Jan. 4, 1974 under 2F1276; submitted by PPG Industries, Inc., Barberton, Ohio; DCL:092175-X)

42085601 Kim-Kang, H. (1991) Metabolism of ^{14}C-Chlorpropham in Stored Potatoes - Nature of the Residue in Potatoes: Study No. XBL 89070. Unpublished study prepared by Xenobiotic Laboratories, Inc. 167p.

42112201 Wu, D. (1991) Metabolism of ^{14}C-Chlorpropham in Lactating Goats - Metabolite Analysis and Quanitation in Milk and Edible Tissues: Study No. XBL 90055. Unpublished study prepared by Xenobiotic Laboratories, Inc. 231p.

42123101 Moller, G. (1991) Analytical Method for Magnitude of Residue in Stored Potatoes from Postharvest Treatments of Chlorpropham: Final Report: Lab Project No. 91CIPC01. Unpublished study prepared by Univ. of Idaho Analytical Lab, Holm Research Ctr. 87 p.

42130401 Wu, D. (1991) Metabolism of Carbon 14-Chlorpropham in Laying Hens: Metabolite Analysis and Quantitation In Eggs and Tissues: Lab Project No. XBL 90053: RPT0073. Unpublished study prepared Xeno Biotic Labs, Inc. 228p.

Agency Memoranda:

CB No.: 6272
Subject: Chlorpropham Registration Standard Response to the Product and Residue Chemistry Data Requirements.
To: J. Coombs
From: H. Fonouni
Dated: 4/04/90
MRID(s): N/A

CHLORPROPHAM (CASE 0271/CODE 108301)
UNOFFICIAL RESIDUE CHEMISTRY DATA SUMMARY THROUGH 3/11/93[1]

REASSESSMENT OF U.S. TOLERANCES AND POTENTIAL FOR HARMONIZATION WITH CODEX[2]

Guideline Number and Topic[3]	Phase 5 data requirements satisfied?	MRID(s)[4]
171-3 Directions for use	No	
171-4(a) Plant Metabolism	Yes[5]	42085601
171-4(b) Animal Metabolism	No[6]	42112201,42130401
171-4(c) Residue Analytical Methods - Plants	No[7]	42123101
171-4(d) Residue Analytical Methods - Animals	Reserved	
171-4(e) Storage Stability	No	
171-4(k) Crop Field Trials		
171-4(k) Root and Tuber Vegetables Group		
Carrots	No[8]	
Potatoes	No[8]	
(Processed food/feed)	No	
Sugar beets [see 171-4(l)]	No	
171-4(k) Leaves of Root and Tuber Vegetables		
Sugar beet tops	No	
171-4(k) Bulb Vegetables Group		
Garlic	No	
Onions (green and dry bulb)	No	
171-4(k) Leafy Vegetables (except Brassica)		
Spinach	No[8]	
171-4(k) Legume Vegetables (succulent/dried)		
Beans (succulent and dried)	No	
Peas (succulent and dried)	No	
Soybeans [see 171-4(l)]	No	
171-4(k) Foliage of Legume Vegetables		
Bean vines and hay	No	
Pea vines and straw	No	
Soybean forage and hay	No	
171-4(k) Fruiting Vegetables Group		
Tomatoes [see 171-4(l)]	No	
171-4(k) Small Fruits and Berries Group		
Blackberries	No	
Blueberries	No	
Cranberries	No	
Raspberries	No	
171-4(k) Cereal Grains Group		
Rice [see 171-4(l)]	No	
171-4(k) Forage, Fodder, and Straw of Cereal Grains		
Rice straw	No	

CHLORPROPHAM (CASE 0271/CODE 108301) UNOFFICIAL RESIDUE CHEMISTRY DATA SUMMARY THROUGH 3/11/93[1] REASSESSMENT OF U.S. TOLERANCES AND POTENTIAL FOR HARMONIZATION WITH CODEX[2]		
Guideline Number and Topic[3]	**Phase 5 data requirements satisfied?**	**MRID(s)[4]**
171-4(k) Grass Forage, Fodder, and Hay Group		
Grass forage and hay	No	
171-4(k) Non-grass Animal Feeds		
Alfalfa [see 171-4(l)]	No	
Clover	No	
Trefoil	No	
171-4(k) Miscellaneous Commodities		
Safflower [see 171-4(l)]	No	
Tobacco	No	
171-4(j) Meat/Milk/Poultry/Eggs	No[10]	
171-4(f) Potable Water	Yes	
171-4(g) Fish	Yes	
171-4(h) Irrigated Crops	N/A	
171-4(i) Food Handling Establishments	N/A	
171-5 Reduction of Residues	N/A	
171-6 Tolerances	No[11]	

[1]Registration Standard issued 12/87. Reregistration Standard Update to the Residue Chemistry Chapter issued 10/16/91. This summary is unofficial and subject to correction.

[2]No Codex MRLs are established or proposed for chlorpropham.

[3]N/A = Guideline requirement not applicable.

[4]MRIDs that were reviewed in the current submission are designated in shaded type.

[5]CBRS 8942, 9137, 9166, 9171, 3/11/93, J. Abbotts: The nature of the residue in stored potatoes treated post-harvest is adequately understood. Residues to be regulated should be determined by the HED Metabolism Committee.

[6]CBRS 8942ff, 3/11/93, J. Abbotts: Additional work is necessary to upgrade the ruminant metabolism study; 80% of the extracted residue in liver was not identified. Considering that potato commodities are not significant feed items, the poultry metabolism study is adequate, provided adequate storage stability data are submitted.

[7]CBRS 8942ff, 3/11/93, J. Abbotts: The submitted method adequately recovers parent and other metabolites from fortified potato samples. The method is not adequate for 3-chloroaniline, and an improved method will be necessary if this or additional metabolites are designated residues to be regulated. Validation of the method for recovery of free and conjugated residues of concern remains an outstanding requirement. Enforcement methods must be validated by an independent laboratory.

[8]Update: In view of existing use on spinach permitted under SLN VA910004 and USDA's wish to support use on carrots and spinach, interim tolerances for carrots and spinach should remain in effect until appropriate permanent tolerances are established. A full complement of residue data is necessary to establish tolerances.

[9]CBRS 8580, 9/18/91, R. Perfetti: A protocol for the "4 lb" formulation was accepted.
CBRS 9013, 12/26/91, P. Deschamp: CBRS advised SRRD that data from residue tests in which warehouse-stored potatoes were treated with a "4 lb" formulation as a fog would support registration of a "7 lb" formulation, provided that the "4 lb" and "7 lb" formulations are identical types (e.g., both are RTU formulations), have the same application rate and timing, and that the prescribed methods of application are essentially identical. At the present time, products registered for postharvest use on potatoes include the 49.65% and 78.5% ready-to-use (RTU); 25, 36, and 46.5% emulsifiable concentrate (EC), and 46% soluble concentrate/liquid (SC/L) formulations.
CBRS 9278, 4/17/92, S. Funk: A protocol for the "7 lb" formulation was acceptable with revisions.

[10]CBRS 8942ff, 3/11/93, J. Abbotts: A ruminant feeding study is required, to be conducted after the nature of the residue in ruminants is adequately understood and residues to be regulated in animal commodities have been determined.

[11]Update: Registrant voluntarily canceled all uses except post-harvest treatment of potatoes. The permanent tolerance on soybeans and all interim tolerances on commodities not supported for reregistration should be revoked.

cc: Abbotts; Chlorpropham Reregistration Standard File; Lois Rossi, SRRD