C. Furlow
PIB/FOD

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

OCT 2 1990

MEMORANDUM

SUBJECT: Chevron Chemical Co.: Response to the Captan Reregistration Standard: Residue Chemistry Requirements (No MRID #'s 406580-02,-03,-04,-05, and -06, and 413930-01, 414069-01 and 413865-01, DEB # 's 6525 and 6526.)

FROM: R. B. Perfetti, Ph.D., Chemist
Reregistration Section
Dietary Exposure Branch
Health Effects Division (H7509C)

RBPerfetti

THRU: W. J. Boodee, Section Head
Reregistration Section
Dietary Exposure Branch
Health Effects Division (H7509C)

RBPgee

TO: Reto Engler, Ph.D., Chief
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

and

L. Rossi, Chief
Reregistration Branch
Special Review and Reregistration Division (H7508C)

Attached is a review of Chevron Chemical Co.'s response to the captan reregistration standard with respect to residue chemistry data requirements for plant and animal metabolism and analytical methodology.

This response was reviewed by Dynamac Corporation under supervision of Dietary Exposure Branch, HED.

This report has undergone secondary review in Dietary Exposure Branch and has been revised to reflect the Branch policies.

Please see our conclusions in the attachment regarding our response to the studies submitted by the Registrant.

If you need additional input please advise.

Attachment 1 : Review of Chevron Chemical Co.'s response to the Captan Reregistration Standard.

cc: With Attachment 1: R. B. Perfetti, E. Saito (TOX), J. Burrell (PIB/FOD), Captan Reregistration Standard File, Captan Subject File, C. Furlow (PIB/FOD).

cc: Without Attachment: P. Fenner-Crisp (HED), M. Hawkins (HED), Circulation (7), R. Schmitt and RF.

DYNAMAC
CORPORATION

Final Report

CAPTAN (DEB Nos. 6525/26) Task 4: Registrant's Response to Residue Chemistry Data Requirements

September 28, 1990

Contract No. 68-D8-0080

Submitted to:
Environmental Protection Agency
Arlington, VA 22202

Submitted by:
Dynamac Corporation
The Dynamac Building
11140 Rockville Pike
Rockville, MD 20852

CAPTAN

REGISTRANT'S RESPONSE TO RESIDUE CHEMISTRY DATA REQUIREMENTS

Task - 4

BACKGROUND

The Captan Guidance Document dated 3/86 concludes that the metabolism of captan in plants and animals is not adequately understood. Additional data are required depicting the nature of the residue in mature potatoes and lettuce following foliar and postharvest applications in separate tests with [trichloromethyl (TCM)-^{14}C]captan and [cyclohexene(CHR)-1,2-^{14}C]captan. Since the registrant elected to cancel the registered foliar and postharvest uses on potatoes, DEB agreed to consider metabolism studies using tomatoes rather than potatoes (DEB Memo dated 10/1/86 by L. Bradley). Additional data are also required depicting the nature of the residue in ruminants and poultry following administration of [trichloromethyl-^{14}C]captan and [cyclohexene-1,2-^{14}C]captan, in separate tests, in the diet for a minimum of 3 days. In response to data requirements for plant and animal metabolism studies, Chevron Chemical Co. has submitted five volumes of data (MRIDs 40658002, 40658003, 40658004, 40658005, and 40658006 under DEB No. 6525/26) which are reviewed here for their adequacy in fulfilling the data requirements.

An Addendum to the Captan Registration Standard (DEB No. 2317 dated 4/22/88 by N. Gray) concludes that, although the qualitative nature of the residue in plants and animals is not understood, significant residues in plants are known to be both captan and its metabolite THPI; significant residues in animals are known to be captan and its metabolites THPI, 3-OH-THPI, and 5-OH-THPI. The current tolerances for residues in food/feed commodities are expressed in terms of captan only (40 CFR 180.103[a] and [b], 185.500 and 186.500). Chevron Chemical Co. has submitted three volumes of validation data (MRIDs 41386501, 41393001, and 41406901) on analytical methods for determination of residues of THPI, 3-OH-THPI, and 5-OH-THPI in eggs and animal tissues which are also reviewed here.

Deficiencies Remaining to be Resolved

The remaining residue chemistry data gaps on storage stability, residue analytical methods, and crop residue data that were identified in the Guidance Document have not been addressed in these submissions and are still unresolved.

The Conclusions and Recommendations stated below apply only to the plant and animal metabolism and residue analytical methods data contained in these submissions. The other data gaps indicated above are not stated in this document again, but still remain outstanding.

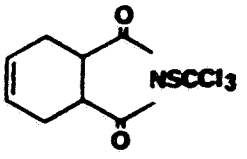
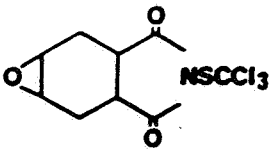
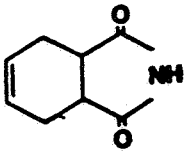
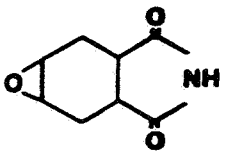
CONCLUSIONS

Plants: Plant metabolism data submitted in response to the Guidance Document do not adequately explain the qualitative nature of the residue in plants because characterized/identified residue components were not quantified on a ppm basis and as a percentage of the total recovered radioactivity (combustion analysis) in the plant sample at the time of harvest. Although insufficient, data in the current submission indicate that in plants, captan is cleaved at the N-S bond to form THPI which is further metabolized. The metabolite of the cleaved side chain is CO_2 which may be reincorporated into plant constituents. The major residue in tomato fruit and lettuce is captan comprising 55-81% of the TRR; minor residues are THPI (4-9%), captan epoxide (0.4%), and THPI epoxide (0.9%). Disulfide, 3-OH-THPI, and 5-OH-THPI may be present at very low levels. Chemical structures of metabolites can be found in Table 1.

Animals (ruminants): The qualitative nature of the residue in ruminants is not adequately understood for the following reasons: (i) a significant portion (ca. 70%) of the administered radioactivity was not recovered in excreta or tissues and the explanation that the balance of ^{14}C-activity is excreted as carbon dioxide or organic volatiles must be further substantiated; (ii) the ^{14}C-residues in milk, liver, and kidney which were separated into hexane-soluble, methanol-soluble, and insoluble fractions must be further characterized to identify the terminal residues; (iii) total ^{14}C-residues in samples of fat and muscle were not characterized or identified; and (iv) the storage interval from collection/sacrifice to analysis of samples of milk and tissues from goats was not reported.

Animals (poultry): The qualitative nature of the residue in poultry is not adequately understood for the following reasons: (i) a significant portion (54% of [TCM-^{14}C]captan and 28% of [CHR-^{14}C]captan) of the administered radioactivity was not recovered in excreta or tissues and the explanation that the balance of ^{14}C-activity is excreted as carbon dioxide or organic volatiles must be further substantiated; (ii) virtually none of the total ^{14}C-residues in eggs and kidney from hens dosed with [TCM-^{14}C]captan were identified, and only 10.6% of the TRR present in liver was tentatively identified as thiazolidine-2-thione-4-carboxylic (TTC); (iii) total ^{14}C-residues in samples of fat and muscle were not characterized or identified; and (iv) the storage interval from collection/sacrifice to analysis of samples of eggs and tissues from poultry was not reported.

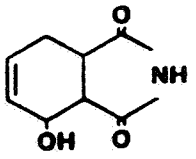
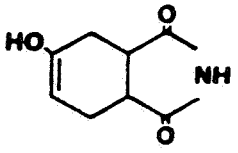
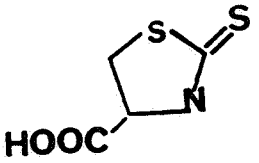
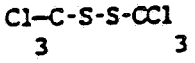
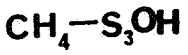
Table 1. Captan and its metabolites in plants and animals.

Code	Chemical name Structure	Substrate	MRID Common name
I.	N-[Trichloromethylthio]cyclohex-4-ene-1,2-dicarboximide 	tomato fruit lettuce	40658005 40658006 captan
II.	N-[Trichloromethylthio]-7-oxabicyclo-[2.2.1]heptane-2,3-dicarboximide 	tomato fruit lettuce	40658005 40658006 captan epoxide
III.	1,2,3,6-Tetrahydrophthalimide 	tomato fruit lettuce poultry liver, kidney, muscle, fat, and eggs	40658005 40658006 40658004 THPI
IV.	7-Oxabicyclo[2.2.1]heptane-2,3-dicarboximide 	lettuce	40658006 THPI epoxide

(Continued).

6

Table 1. (Continued).

Code	Chemical name Structure	Substrate Common name	MRID
V.	3-Hydroxy-1,2,6-trihydrophthalimide 	tomato fruit lettuce poultry liver, kidney, muscle, fat and eggs 3-OH-THPI	40658005 40658006 40658004
VI.	5-Hydroxy-1,2,6-trihydrophthalimide 	tomato fruit lettuce poultry liver, kidney, muscle, fat and eggs 5-OH-THPI	40658005 40658006 40658004
VII.	Thiazolidine-2-thione-4-carboxylic 	goat liver goat urine TTC	40658002 40658002
VIII.	Bis-(trichloromethyl) disulfide 	tomato fruit lettuce Disulfide	40658005 40658006
IX.	Dithio-bis-methanesulfonic acid 	goat urine DMS	40658002

7

Residue Analytical Methods: Independent validation of ICI's Method No. 152 by Battelle Labs was unsuccessful. The method tended to recover excessively high residue levels at the 0.03 ppm fortification level and recoveries were extremely variable regardless of the metabolite used or the fortification level. The validating laboratory concluded that accuracy "may be close to acceptable" but that "precision may be a problem." Thus, we conclude that ICI Agrochemicals Residue Analytical Methods 152 and 166 are adequate for data collection but not for enforcement of anticipated tolerances for residues of THPI, 3-OH-THPI, and 5-OH-THPI, in eggs, milk, and animal tissues.

RECOMMENDATIONS

Plants: The registrant should be informed that the submitted data do not fulfill the requirements regarding metabolism in plants. The registrant must recalculate and resubmit the extraction and metabolite data, clearly presenting each characterized/identified component both on a ppm basis (combustion analysis) and as a percentage of the total radioactivity in the tissue or sample fraction analyzed.

Animals (ruminants and poultry): The registrant should be informed that the submitted data do not fulfill the requirements regarding metabolism in ruminants and poultry. The 70% loss of radioactivity administered to goats must be satisfactorily explained. The 28-54% loss of ^{14}C-material administered to hens must also be satisfactorily explained. Organosoluble and aqueous-soluble residues in eggs, milk, liver, and kidney tissues should be thoroughly partitioned and characterized. Data demonstrating the quantitative incorporation of ^{14}C-activity into natural constituents of milk (lipids, lactose, and casein) is required. Both polar and non-polar metabolites will be solubilized in the polar solvents (methanol and acetonitrile) used for residue partitioning (with hexane) in the submitted studies. Addition of chloroform (or a similar non-polar solvent) to acetonitrile and methanol extracts, followed by partitioning with water, should adequately separate organosoluble and aqueous-soluble metabolites. Unextractable and aqueous-soluble residues should be hydrolyzed to release bound and/or conjugated residues. Metabolite identification using HPLC must be confirmed by a second method (TLC or MS) and supported by representative HPLC and UV chromatograms.

Residue Analytical Methods: We recommend that the registrant revise Method No. 152 for determination of THPI, 3-OH-THPI, and 5-OH-THPI in milk and animal tissues and run a second confirmatory trial using a different laboratory. Recommended revisions, including those made by Battelle Labs in the first confirmatory trial are as follows: (i) increase the amount of derivatizing agent (BSTFA) used, and increase the time of the

derivatizing step from 10 minutes to 20-30 minutes; (ii) inject an acetonitrile blank between each sample injection to improve column performance during GC/MSD analysis; (iii) provide additional details concerning GC/MSD operating conditions; (iv) raise the column temperature to 110 C and change the temperature increase rate to 15 degrees per minute to eliminate peak splitting and improve analyte resolution; and (v) reduce the level of the calibration standard to the equivalent of the expected residue levels in the samples.

DETAILED CONSIDERATIONS

Qualitative Nature of the Residue in Plants - DEB No. 6526

Chevron Chemical Co. submitted two studies (1988; MRIDs 40658005 and 40658006) concerning the metabolism of captan in tomato and lettuce. [Trichloromethyl-^{14}C]captan (specific activity 38.0 mCi/mMole; radiochemical purity 99.6%) and [cyclohexene-1,2-^{14}C]captan (specific activity 16.6 mCi/mMole; radiochemical purity 98.7%) were each mixed with non-radioactive technical captan. These two radiolabeled mixtures, each with a final specific activity of 2.29 mCi/mMole, were foliarly applied in separate experiments to greenhouse-grown plants contained in 1-gallon pots. Treatments were made using a hand sprayer at a field-equivalent rate of 4 lb ai/A. Tomato and lettuce plants received four applications at 7-day intervals; tomato plants bore 1-inch diameter fruit and 12-16 leaves when the first treatment was made. Mature plants were harvested 3 hours after the final treatment and separated into leaves, stems, roots, and tomato fractions. All samples were stored at -20 C, combusted for total ^{14}C-determination, and extracted within two weeks postharvest.

Total Radioactive Residue

The total radioactive residue (TRR) in samples of tomato and lettuce was determined by liquid scintillation spectrometry (LSS) following combustion. The limit of detection was reported as 30-40 dpm (0.02 ppm, calculated by the study reviewer). Total ^{14}C-residues, determined by LSS and expressed as ppm captan equivalents, were given only for selected samples of tomato and lettuce (Table 2).

Table 2. Radioactive residues in selected samples of tomato and lettuce following foliar applications of [TCM-^{14}C]captan and [CHR-^{14}C]captan.

Component	^{14}C-residues (ppm captan equivalents)			
	Tomato		Lettuce	
	[TCM-^{14}C]	[CHR-^{14}C]	[TCM-^{14}C]	[CHR-^{14}C]
Leaves	125.9	201.6	70.59	56.49
Stems	21.3	24.87	NA[a]	NA

[a] NA = Not applicable.

The registrant presented additional data (see Table 3) on the distribution of ^{14}C-activity in samples of tomato and lettuce, but it is unclear how the values for total ^{14}C-residue concentration (ppm) and percentage of the total recovered ^{14}C-activity were determined. The registrant states that the TRR values reported for whole fruit were calculated by addition of the TRR present in the acetone wash, juice, and pulp. Tomatoes were washed with acetone to remove surface residues, homogenized, and centrifuged to separate the juice and pulp; aliquots of all fractions were combusted to determine total radioactivity. However, the reported percentages given parenthetically in Table 3 are not based on the sum (e.g., leaves + roots) of reported total ^{14}C-activity for each sample, nor on the representative combustion data. The registrant must base the distribution (percentage) of the total plant radioactivity on the total recovered (combusted) radioactivity remaining in the plant at time of sampling.

Table 3. Distribution of ^{14}C-activity in tomato and lettuce plants following foliar applications of [TCM-^{14}C]captan and [CHR-^{14}C]captan.

Component	^{14}C-residues, ppm captan equivalents (% TRR)			
	Tomato		Lettuce	
	[TCM-^{14}C]	[CHR-^{14}C]	[TCM-^{14}C]	[CHR-^{14}C]
Leaves	128.5(62.1)	202.1(70.4)	68.5(98.7)	64.4(99.7)
Stems	21.8(9.2)	30.1(9.7)	NA	NA
Roots	0.2(0.2)	0.2(0.2)	1.3(1.3)	0.3(0.3)
Fruit[a]	6.9(28.5)	6.7(19.7)	NA	NA
Total	157.4	239.1	69.8	64.7
Tomato Fruit				
Surface	2.74(79.6)	4.83(88.9)	NA	NA
Juice	1.22(14.7)	0.71(8.9)	NA	NA
Pulp	2.94(5.7)	1.18(2.2)	NA	NA

[a] The TRR values reported for whole tomato fruit were calculated by addition of the TRR present in acetone wash, juice, and pulp.

10

Extraction procedures

Tomato juice was extracted with ethyl acetate. Tomato pulp and all other plant fractions (except roots, which were not further analyzed) were homogenized with dry ice in a blender and extracted with acetone, methanol, methanol:water (1:1, v/v), and acidic methanol (0.2 N HCl); the last extraction solvent step was omitted for samples treated with [CHR-^{14}C]-captan. Solvent extracts were separated from the residue by centrifugation and concentrated under reduced pressure; nonextractable residues were combusted for ^{14}C-determination. Total ^{14}C-activity expressed as radioactive counts or ppm was not provided. Table 4 details the radiolabel distribution obtained by solvent extraction.

Table 4. Distribution of total radioactivity in extracts of tomato fruit, tomato foliage, and lettuce leaves following foliar treatments with [TCM-^{14}C]captan and [CHR-^{14}C]captan.

	% of ^{14}C-activity in solvent fractions				
Component	Acetone	MeOH[a]	MeOH/H_2O	Acidic MeOH	Insoluble
		[TCM-^{14}C]captan			
Tomato leaves	85.5	2.3	3.2	2.2	6.8
Tomato stems	81.8	3.7	3.6	1.4	9.5
Tomato juice	12.8[b]	NA[c]	NA	NA	87.2[d]
Tomato pulp	22.0	4.1	9.1	6.5	58.3
Lettuce leaves	81.7	0.1	2.8	1.7	13.7
		[CHR-^{14}C]captan			
Tomato leaves	81.8	3.5	5.9	NA	8.8
Tomato stems	83.5	3.4	4.2	NA	8.9
Tomato juice	42.2[b]	NA	NA	NA	57.8[d]
Tomato pulp	46.7	4.1	7.0	NA	42.2
Lettuce leaves	94.0	1.6	1.4	NA	3.0

[a] MeOH = methanol.
[b] Tomato juice was extracted with ethyl acetate.
[c] NA = not applicable.
[d] Remaining juice after ethyl acetate extraction.

11

Hydrolysis

Polar and/or conjugated metabolites present in selected soluble fractions were subjected to acid (1 N HCl) or base (1 N NaOH) hydrolysis for 1-2 hours at 100 C in sealed ampules. Base hydrolysates were acidified with concentrated HCl to pH 1. Hydrolysates were extracted with diethyl ether, saturated with ammonium sulfate, and extracted with diethyl ether:ethanol (3:1, v/v). Insoluble residues of lettuce leaves and tomato pulp were hydrolyzed with 1 N HCl (at 100 C for 2 hours) followed by 20% NaOH (at 100 C for 24 hours). The base hydrolysate was acidified with concentrated HCl to pH 1 and the precipitate collected by centrifugation. The acid hydrolysate contained carbohydrates, the precipitate after base hydrolysis and centrifugation contained lignin, and the aqueous fraction contained proteins (amino acids). Total ^{14}C-activity expressed as radioactive counts or ppm was not provided; thus, it is not possible to determine what portion of the TRR present in these tissues may be attributable to natural plant constituents. Table 5 details the reported radiolabel distribution.

Table 5. Fractionation of insoluble residues from tomato pulp and lettuce leaves into natural plant constituents.

Component	% of ^{14}C-activity in insoluble residues		
	Tomato pulp		Lettuce leaves
	[TCM-^{14}C]	[CHR-^{14}C]	[TCM-^{14}C]
Carbohydrates	15.3	71.2	48.0
Proteins (amino acids)	14.6	17.6	10.2
Lignin	3.7	3.3	1.7
Remaining residue	17.8	7.9	5.1
Loss during hydrolysis	48.6	--[a]	35.0
Total	100.0	100.0	100.0

[a] None reported.

A 31.9% loss of ^{14}C-activity from tomato pulp occurred during the acid treatment, and an additional 13.7% was lost during acidification of the 20% NaOH hydroyslate. A 35% loss of ^{14}C-activity from lettuce was also reported during acidification of the base hydroyslate.

Based on results from previous studies with this chemical and with similar exogenous compounds, the registrant states that most of the nonextractable ^{14}C-residues resulted from the captan side chain releasing $^{14}CO_2$ (from the disulfide), followed by reincorporation of $^{14}CO_2$ into plant constituents. Data from this

study indicates that most tissues from plants treated with [TCM-^{14}C]captan did container higher levels of nonextractable ^{14}C-material; however the increase was too small to account for a majority of the radiolabel in the insoluble residues.

Residue characterization

Captan metabolites were separated by thin-layer chromatography (TLC) utilizing silica gel (normal or silanized) and developed with three solvent systems: chloroform:acetic acid (4:1, or 5:1, or 40:1 v/v), methylene chloride:acetone (5:1, v/v), or butan-1-ol:acetic acid:water (4:1:1, v/v/v). Radioactivity was located on the thin-layer plates with autoradiography by exposing the developed TLC plates to x-ray film. For quantitation, the radioactive spots were scraped off the plate for liquid scintillation counting. Metabolite structures were characterized by cochromatography with the following standards:

a. Captan per se
b. 1,2,3,6-tetrahydropthalimide (THPI)
c. 6-carbamoyl-3-cyclohexene-1-carboxylic acid (THPAM)
d. N-[trichloromethylthio]-7-oxabicyclo[2.2.1]heptane-2,3-dicarboximide (captan epoxide)
e. 7-oxabicyclo[2.2.1]heptane-2,3-dicarboximide (THPI epoxide)
f. 3-hydroxy-1,2,6-trihydrophthalimide (3-OH-THPI)
g. 5-hydroxy-1,2,6-trihydrophthalimide (5-OH-THPI)
h. 3-cyclohexene-1,3-dicarboxylic acid (THPAL)
i. thiazolidine-2-thione-4-carboxylic acid (thiazolidine)
j. bis-(trichoromethyl) disulfide (disulfide)

The presence of captan per se and THPI in acetone extracts from tomato leaves, stems, surface extractions, and from lettuce leaves was confirmed by HPLC equipped with a radioactive flow detector and a reversed phase column. The binary solvent system was 0.5% (v/v) 70% perchloric acid in HPLC water and acetonitrile. Elution was by a linear solvent gradient at a flow rate of 1.0 mL/minute. Direct probe MS in the CI and EI modes was also utilized for structural confirmation of captan and THPI isolated from unspecified plant tissues. HPLC retention times and TLC R_f values were given for selected standards but representative chromatograms were not included in this submission. The results of residue analyses in tomato plants, tomato fruit, and lettuce leaves are detailed by label position in Tables 6 and 7 and summarized in Table 8.

Table 6. Characterization of ^{14}C-residues in tomato plants, tomato fruits, and lettuce leaves following foliar application with [TCM-^{14}C].

Component	% of total ^{14}C-residues (ppm captan equivalents) Tomato Plant[a]	Tomato Fruit	Lettuce Leaves
Captan	61.7 (92.7)	76.6 (5.29)	76.2 (52.2)
Captan epoxide	0.2 (0.34)	0.2 (0.014)	0.3 (0.21)
Other free metabolites[b]	5.3 (7.91)	9.5 (0.66)	5.2 (3.56)
Polar and conjugates[b]	3.6 (5.50)	10.4 (0.72)	4.6 (3.15)
Nonextractable residue	5.5 (8.26)	3.3 (0.23)	13.7 (9.39)
Unaccounted for	23.7 (35.6)	--[c]	--
Total	100.0(150.3)	100.0 (6.91)	100.0 (68.51)

[a] Percent values were calculated by study reviewer based on the total ^{14}C-residues of 150.3 ppm present in tomato leaves and stems.
[b] Contains 4-9 unidentified metabolites.
[c] None reported.

Table 7. Characterization of ^{14}C-residues in tomato plants, tomato fruits, and lettuce leaves following foliar application with [CHR-^{14}C]captan.

Component	% of total ^{14}C-residues (ppm captan equivalents) Tomato Plant[a]	Tomato Fruit	Lettuce Leaves
Captan	55.0 (127.6)	81.5 (5.48)	77.2 (49.7)
Captan epoxide	0.3 (0.73)	0.5 (0.03)	0.6 (0.39)
THPI	3.6 (8.34)	4.5 (0.30)	9.5 (6.12)
THPI epoxide	NA	NA	0.9 (0.58)
Other free metabolites[b]	5.4 (12.5)	5.2 (0.35)	4.3 (2.77)
Polar and/or conjugates[b]	6.9 (16.1)	7.4 (0.50)	4.5 (2.90)
Nonextractable residue	6.9 (16.0)	0.9 (0.06)	3.0 (1.93)
Unaccounted for	21.9 (50.9)	--[c]	--
Total	100.0 (232.2)	100.0 (6.72)	100.0 (64.39)

[a] Combination of tomato leaves and stems.
[b] Contains 3-9 unidentified metabolites.
[c] None reported.

Table 8. Summary of distribution and characterization of ^{14}C-residues in tomato and lettuce plants following foliar applications with [^{14}C]captan.

Component	Percent of total ^{14}C-residues					
	TCM-[^{14}C]			CHR-[^{14}C]		
	Tomato		Lettuce	Tomato		Lettuce
	Plant	Fruit	Leaf	Plant	Fruit	Leaf
Captan	61.7	76.6	76.2	55.0	81.5	77.2
Captan epoxide	0.2	0.2	0.3	0.3	0.4	0.6
THPI	NA	NA	NA	3.6	4.5	9.5
THPI epoxide	NA	NA	NA	ND[a]	ND	0.9
Total Identified	61.9	76.8	76.5	58.9	86.4	88.2
Other free metabolites	5.3	9.5	5.2	5.4	5.2	4.3
Polar and/or conjugates	3.6	10.4	4.6	6.9	7.5	4.5
Nonextractable residue	5.5	3.3	13.7	6.9	0.9	3.0
Total Unidentified	14.5	23.2	23.5	19.2	13.6	11.8
Unaccounted for	23.7	--[b]	--	21.9	--	--
Total	100.0	100.0	100.0	100.0	100.0	100.0

[a] None detected.
[b] None reported.

In tomato plants, 58.9-61.9% of the total radioactivity was characterized as captan (55.0-61.7%), captan epoxide (0.2-0.3%), and THPI (3.6%). The nonextractable fraction contained 5.5-6.9% of the total radioactivity. The registrant provided some data to support the contention that these residues are probably due to side chain release of $^{14}CO_2$ and reincorporation into carbohydrates, proteins, and lignin. The remaining 32.6-34.2% of the total radioactivity was relatively uncharacterized: 21.9-23.7% was unaccounted for, while 5.4% was in "other free metabolites", and 3.6-6.9% represented polar and/or conjugate residues with a combined total of about 15 additional metabolites.

In tomato fruit, 76.8-86.4% of the total radioactivity was characterized as captan (76.6-81.5%), captan epoxide (0.2-0.4%), and THPI (4.5%). The nonextractable fraction contained 0.9-3.3% in insoluble residues. The remaining 12.6-19.9% of the total radioactivity was relatively uncharacterized: 5.2-9.5% was in "other free metabolites" and 7.5-10.4% represented polar and/or conjugate residues with a combined total of about 13 additional metabolites.

In lettuce leaves, 76.5-88.2% of the total radioactivity was characterized as captan (76.2-77.2%), captan epoxide (0.3-0.6%), THPI (9.5%), and THPI epoxide (0.9%). The nonextractable

fraction contained 3.0-13.7% in insoluble residues. The remaining 8.8-9.8% of the total radioactivity was relatively uncharacterized: 4.3-5.2% was "other free metabolites" and 4.5% represented polar and/or conjugate residues with a combined total of about 10 additional metabolites.

Captan is cleaved at the N-S bond to form THPI which is further metabolized. The metabolite of the cleaved side chain is CO_2 which may be reincorporated into plant constituents. The major residue in tomato fruit and lettuce is captan, comprising 55-81% of the TRR; minor residues are THPI (4-9%), captan epoxide (0.4%), and THPI epoxide (0.9%). Disulfide, 3-OH-THPI, and 5-OH-THPI may be present at very low levels; these moieties were difficult to confirm due to the low levels of ^{14}C-activity and interference from plant contaminants.

Recovery of residues using an enforcement method

Samples of [CHR-^{14}C]captan-treated lettuce leaves were also analyzed using GLC method RM-1K-3 dated 3/27/86 for determination of captan *per se* and THPI in plants. Samples had been stored for about 1 year since the original LSS determinations were made; thus, total ^{14}C-activity by LSS was performed again. The results given in Table 9 indicate that this method is capable of detecting residues of captan and THPI in samples of lettuce; however, determination of THPI may not be sufficiently quantitative for enforcement purposes.

Table 9. Analysis of [CHR-^{14}C]- and [TCM-^{14}C]captan-treated lettuce leaves using GC Method RM-1K-3.

Residue	Label	Residues (ppm, captan equivalents) 1st LSS	2nd LSS	GC Method RM-1K-3
Captan	CHR	56.49	38.5	42.2
Captan	TCM	70.59	37.5	37.3
THPI	CHR	--[a]	4.2	1.49
THPI	TCM	--	--	1.71

[a] Not reported.

In summary, the qualitative nature of the residue in plants is not adequately understood. It is not clear how residues in identified components were quantified. It appears that the percentages of total ^{14}C-activity for identified and characterized components listed in Tables 6, 7, and 8 are based on the sum of dpm found in each fraction rather than on the amount of total radioactivity in that tissue. The registrant must provide data on amount of total radioactivity *in that tissue*, expressed as a percentage and in ppm.

Qualitative Nature of the Residue in Animals - DEB No. 6526

Lactating Goats: Chevron Chemical Co. (1988; MRID 40658002) submitted data pertaining to the metabolism of captan in lactating goats. A single lactating goat was dosed for three consecutive days with [TCM-^{14}C]captan (specific activity 2.59 mCi/mmol, radiochemical purity, 95%) via oral capsule; three equal doses of 0.47 mg/kg/capsule were administered for a total of 1.4 mg/kg/day. This dose is equivalent to 108 ppm in the diet based on the goat's weight (54 kg) and total daily feed consumption (0.7 kg). Water and alfalfa-grass hay were available ad libitum and 1 kg of a mixed grain ration was limit fed. Another goat served as an untreated control. Milk and excreta were collected twice daily, and animals were sacrificed for tissue analysis 3 hours after the last dose was administered. Tissue samples were rinsed in tap water prior to storage, and excreta, milk, and tissue samples were stored at an unspecified temperature below 0 C before and after analysis. The rinse water was not analyzed for radioactivity, and the interval between sampling and storage was not reported.

Total Radioactive Residue (TRR)

Samples were analyzed for total radioactivity by combustion/liquid scintillation spectrometry (LSS). Milk samples were lyophilized, and tissue samples were homogenized and lyophilized to a dry powder, prior to total radioactivity determinations. The registrant listed the method limit of detection as 0.009 ppm. Approximately 29% of the administered total dose was recovered in the excreta (26.5%), milk (1.5%), and all tested tissues (liver, kidney, muscle, fat, heart, gall bladder, bladder, and mammary gland, ca. 1%). The remaining 71% of the administered radioactivity was unaccounted for. Based on studies with rats, the authors believe that the balance of ^{14}C-activity is excreted as carbon dioxide or organic volatiles. The TRR in sampled tissues and milk is presented in Table 9.

Table 9. Total radioactive residue in milk and tissues from a lactating goat administered [TCM-^{14}C]captan.

Sample	TRR (ppm captan equivalents)
Milk[a]	0.30-1.70
Blood	0.25
Kidneys	1.57
Liver	2.01
Muscle	0.16
Fat (omental & renal)	0.02-0.03
Heart	0.27
Gall bladder	0.35
Bile	0.64
Mammary gland	0.92

[a] Residues increased from 0.30 ppm after the first dose to 1.70 ppm on the day of sacrifice.

Extraction and Characterization

Milk: Samples of milk containing total ^{14}C-residues of 1.7 ppm were lyophilized and sequentially extracted with hexane and methanol. The hexane extracts were partitioned into acetonitrile. Solids from the initial hexane and methanol extractions were solubilized in 0.2 N sodium hydroxide and casein was precipitated by adjusting the mixture to pH 4.5 with phosphoric acid. The precipitates were dried, resolubilized with 0.2 N sodium hydroxide and adjusted to pH 4.5 with acetic acid to reprecipitate [^{14}C]casein; the solubilization and precipitation procedure was repeated until a constant specific activity was obtained. Hexane-soluble, methanol-soluble, and unextractable residues accounted for 32.1%, 27.3%, and 40.2% of milk TRR, respectively; ca. 16% of milk TRR was putatively casein.

Liver and Kidney: Samples were lyophilized to a dry powder and sequentially extracted with hexane and methanol. Methanol-soluble residues were concentrated with a dry ice/acetone evaporator prior to analysis by HPLC. Hexane-soluble and unextracted residues were analyzed for total radioactivity. In liver, 96.6% of the TRR was recovered in hexane (6.1%), methanol (31.1%), and insoluble residue (59.4%); the 3.4% loss during extraction was unexplained. Other tissues were not further fractionated or characterized. In kidney, 89.9% of the TRR was recovered in hexane (1.4%), methanol (35.3%), and in insoluble residue (53.2%); the 10.1% loss during extraction was unexplained.

Metabolite Identification

Milk: The registrant tentatively characterized hexane-soluble residues as lipids, the methanol-soluble residues as lactose, and the precipitates isolated from the hexane fraction as casein. Quantitative data supporting the identification of ^{14}C-residues in milk were not presented.

Liver and Kidney: Methanol extracts were filtered and analyzed directly by reversed phase HPLC with a UV detector; eluted fractions were collected and analyzed by LSS. The registrant indicated that the only metabolites isolated by HPLC eluted with the solvent front, but they were not identified; representative UV chromatograms were not presented.

Excreta: Most of the recoverable radioactivity was in the feces (20.5% of the administered dose) but these residues were not fractionated or characterized. After filtering, residues in urine were analyzed directly by HPLC and metabolites were tentatively identified by comparison with standards having known retention times; eluted fractions were collected and analyzed for radioactivity by LSS. The metabolites thiazolidine-2-thione-4-carboxylic acid (TTC) and dithio-bis-methanesulfonic acid (DMS) were tentatively identified, and accounted for ca. 24% and 9% of urine TRR, respectively; an additional 22% of urine TRR eluted at the solvent front or was distributed unresolved throughout the chromatogram. Metabolites were not confirmed by a second analytical method, therefore the identifications were inconclusive. More than 55% of the urine TRR was not recovered by HPLC.

Laying Hens: Chevron Chemical Co. (1988; MRIDs 40658003 and 40658004) also submitted data pertaining to the metabolism of captan in laying hens. Twenty White Leghorn laying hens (10 hens per label position) were administered [TCM-^{14}C]captan (s.a. 18.8 and 2.64 mCi/mmole for low and high dose, respectively; radiochemical purity >99%) or [CHR-^{14}C]captan (s.a. 9.42 and 0.77 mCi/mmole for low and high dose, respectively; radiochemical purity >99%). The hens were dosed by oral capsule for five consecutive days according to the schedule presented in Table 10. The low-dose hens were used for TRR analysis and metabolite identification and the high-dose hens were used for structure confirmation; four additional hens per label position served as controls.

Table 10. Dietary exposure of White Leghorn laying hens receiving oral doses of [TCM-^{14}C] and [CHR-^{14}C]captan.

Parameter	[TCM-^{14}C]captan		[CHR-^{14}C]captan	
	Low dose	High dose	Low dose	High dose
Dose/hen	1.39 mg	9.32 mg	0.87 mg	8.52 mg
Average body weight	1.78 kg	1.77 kg	1.74 kg	1.70 kg
Daily feed consumption	0.131 kg	0.130 kg	0.148 kg	0.141 kg
Dietary exposure	10.6 ppm	71.7 ppm	5.87 ppm	60.4 ppm

Samples of eggs and excreta were collected twice daily and pooled; birds were sacrificed for tissue analysis 4 hours after administration of the last dose. Tissue samples were rinsed in tap water prior to storage, and excreta, egg, and tissue samples were stored at an unspecified temperature below 0 C before and after analysis. The rinse water was not analyzed for radioactivity, and the interval between sampling and storage was not reported.

Total Radioactive Residue (TRR)

Samples were analyzed for total radioactivity by combustion/LSS; all solid samples were homogenized prior to analysis. The registrant listed the limit of detection as 0.001-0.009 ppm for the TCM label and 0.002-0.03 ppm for the CHR label. In hens administered TCM-labeled captan, ca. 46% of the administered dose was recovered in excreta (44%), tissues and blood (ca. 1%), and eggs (ca. 0.2%); the missing 54% of administered radioactivity was not adequately explained. In hens administered CHR-labeled captan, ca. 72% of the administered dose was recovered in excreta (ca. 66%), tissues and blood (ca. 4%), egg yolk (0.3%), and egg white (ca. 0.7%); the missing 28% of administered radioactivity was not adequately explained. The TRR in eggs and poultry tissues is presented in Table 11.

Table 11. Total radioactive residue in eggs and tissues from laying hens respectively administered [TCM-^{14}C]captan and [CHR-^{14}C]captan in the diet for five consecutive days.

Sample	TRR (ppm captan equivalents)	
	[TCM-^{14}C]captan	[CHR-^{14}C]captan
Egg yolk	0.04-0.22	0.14-0.28
Egg white	0.01-0.07	0.22-0.3
Blood	0.17	0.4
Kidney	0.82	0.69
Liver	0.41	0.56
Breast Muscle	0.05	0.46
Thigh Muscle	0.06	0.47
Fat (abdominal)	0.03	0.11
Skin	0.06	0.28
Gizzard	0.18	0.43
Ovaries	0.37	0.38
Oviducts	0.22	0.42
Heart	0.13	0.5

Extraction and Characterization

Extraction and characterization procedures for egg, liver, and kidney samples from hens dosed with [TCM-^{14}C]captan are described below for each matrix.

Eggs: Egg yolks from hens dosed with [TCM-^{14}C]captan were sequentially extracted with hexane and methanol. The hexane extracts were evaporated to dryness over dry ice/acetone, residues were partitioned with acetonitrile and hexane, and both phases analyzed by LSS. Hexane-soluble, methanol-soluble, and unextractable residues accounted for 5.4%, 44.4%, and 64.1% of yolk TRR, respectively; total recovery was ca. 114%. Pooled methanol extracts were concentrated and filtered prior to HPLC analysis. Enzymatic hydrolysis of the insoluble residues with protease in 0.05 M phosphate buffer (37 C for 18 hours) yielded three phases: oily, aqueous, and solid. Hexane extraction of the incubation mixture released an additional 20.3% of the TRR; 20.3% was aqueous-soluble and 24.4% of the TRR remained insoluble. Hexane extracts were combined, evaporated, and partitioned as previously described.

Liver: Samples of liver from hens dosed with [TCM-^{14}C]captan were extracted with methanol, evaporated to dryness, then resolubilized and partitioned with acetonitrile and hexane; that part of the methanol-soluble residue not resolubilized in acetonitrile or hexane, was solubilized in methanol and analyzed

by HPLC. Unextractable residues were hydrolyzed with protease at 37 C for 18 hours; half of the aqueous hydrolysate was extracted with ethyl acetate, and the other half was acidified to pH 2 prior to ethyl acetate extraction. All aqueous and organic phases were analyzed by LSS. An aliquot of the aqueous extract from the hydrolysate was filtered prior to analysis by HPLC. Methanol extracted 68.2% of liver TRR; after evaporation and partitioning, hexane-soluble and acetonitrile-soluble residues accounted for 10.6% and 3.3% of TRR, respectively. Proteolysis of unextractable residues released 23.5% of bound liver residues into the aqueous phase; ca. 0.04% was extracted into ethyl acetate.

Kidney: Samples of kidneys from hens dosed with [TCM-^{14}C]captan were sequentially extracted with acetone, methanol:water (1:1, v/v), and water; unextractable residues were re-extracted in the same manner described for the initial sample, and the respective phases were combined with the initial extracts. Acetone extracts were evaporated to dryness over dry ice/acetone, and partitioned with acetonitrile and hexane; the acetonitrile phase was concentrated for HPLC analysis. Combined, extractable residues accounted for 52.9% of kidney TRR from TCM-treated hens.

All egg and tissue samples from CHR-treated hens were sequentially extracted with acetone and methanol:water (1:1, v/v); different phases were separated by centrifugation and analyzed for radioactivity by LSS. Unextractable residues were analyzed by combustion/LSS. Radioactive residue recoveries from eggs and tissues of CHR-treated hens are presented in Table 12.

Table 12. Recoveries of radioactive residues from eggs and tissues from hens administered [CHR-^{14}C]captan in the diet for five consecutive days.

	% of Total Radioactive Residue			
Sample	Acetone	Methanol:water	Insoluble	Total
Egg yolk	98.0	2.9	4.9	106
Egg white	86.1	5.4	10.1	102
Liver	88.0	8.8	2.3	99.1
Kidney	75.0	13.7	4.8	93.5
Thigh Muscle	90.0	1.5	1.1	98.4
Breast Muscle	99.2	4.5	3.9	102
Fat	78.9	ND[a]	0.5	79.4

[a]Not detectable

The acetone extracts from the matrices listed in Table 12, and the methanol:water extract from kidney, were evaporated to

dryness, then resolubilized and partitioned with hexane and acetonitrile; each phase was analyzed by LSS. Approximately 92-104% of extractable residues were partitioned into acetonitrile, except for egg white (30%); hexane-soluble residues accounted for <0.1-3% of the initially extracted residues. Partitioning data are presented in Table 13. Acetonitrile extracts were concentrated, filtered, and analyzed for captan metabolites by reversed phase HPLC. Representative radiochromatograms were presented, but not UV chromatograms.

Table 13. Distribution of radioactivity in extracts of egg and tissue samples from hens administered [CHR-^{14}C]captan in the diet for five consecutive days.

Sample	% of Total Radioactive Residue	
	Acetonitrile	Hexane
Egg yolk	90.2	1.6
Egg white	26.4	0.3
Liver	82.6	0.5
Kidney	69.6	2.3
Thigh Muscle	83.3	<0.1
Breast Muscle	93.8	0.6
Fat[a]	82.1	0.6

[a] Note that 78.9% of the TRR was acetone soluble; the registrant reports 104% recovery of acetone-soluble ^{14}C-activity in the acetonitrile fraction.

Metabolite Identification

Metabolites isolated from eggs, liver, and kidney samples from TCM-treated hens are described below:

Eggs: Concentrated methanol extracts were filtered and analyzed by reversed phase HPLC with a UV detector; eluted fractions also were collected and the radioactivity was determined by LSS. Radiochromatograms were presented, but not UV chromatograms. No metabolites co-eluted at the retention times of known standards, but one large, unidentified peak accounted for 17.1% of yolk TRR in TCM-treated hens; unidentified metabolites were not further characterized.

Liver: Methanol-solubilized precipitates were concentrated, filtered, and analyzed by reversed phase HPLC. Radiochromatograms were presented, but not UV chromatograms. Radioactivity that eluted at the same retention time as the TTC standard accounted for 10.6% of liver TRR in TCM-treated hens; unidentified metabolites were not further characterized.

Aqueous-soluble residues released by proteolysis also were analyzed by HPLC, but no known metabolites were isolated.

Kidney: Acetonitrile extracts (5% of kidney TRR) were concentrated, filtered, and analyzed by reversed phase HPLC. Radiochromatograms were presented, but not UV chromatograms. Radioactivity co-eluting at the same time as the TTC standard accounted for 0.5% of kidney TRR in TCM-treated hens; 0.25% of the TRR was tentatively identified as DMS and its monosulfoxide (DMS-O), based on TLC R_f values from rat metabolism studies. Authentic standards of DMS-O were not available.

The metabolites THPI, 3-OH-THPI, and 5-OH-THPI were tentatively identified in samples of egg, liver, and kidney from hens dosed with [CHR-^{14}C]captan; the hydroxylated metabolites could not be resolved in the HPLC systems tested. Distribution of metabolites isolated from eggs and tissues of CHR-treated hens is presented in Table 14. Metabolite identification using a second, confirmatory method was not performed.

Table 14. Metabolites isolated from eggs and tissues of hens administered [CHR-^{14}C]captan in the diet for five consecutive days.

Sample	% of Total Radioactive Residue		
	3/5-OH-THPI	THPI	Total
Egg yolk	26.0	59.0	84
Egg White	2.4	16.1	18.5
Liver	21.1	43.6	64.7
Kidney	21.7	37.7	59.4
Thigh Muscle	16.8	59.7	76.5
Breast Muscle	18.1	67.6	85.7
Fat	3.8	68.9	72.7

In summary, the qualitative nature of the residue in ruminants and poultry is not adequately understood. The submitted goat metabolism study did not sufficiently characterize or identify the TRR present in milk, and in muscle, fat, kidney, and liver tissues. The registrant indicates that additional work to characterize these residues is in progress. The poultry metabolism studies are insufficient because virtually none of the terminal ^{14}C-residues in eggs, liver, and kidney from hens dosed with [TCM-^{14}C]captan were identified.

Residue Analytical Methods

An Addendum to the Captan Registration Standard (DEB No. 2317, dated 4/22/88 by N. Gray) concludes that, although the nature of the residue in animals is not completely understood, significant residues in animal commodities are known to be captan and its metabolites THPI, 3-OH-THPI, and 5-OH-THPI. The Addendum also concludes that the compilation analytical method (Chevron RM-6G-2 and RM-1G-1 and Stauffer RRC-75-32) for residues of captan, THPI, 3-OH-THPI, and 5-OH-THPI in meat, milk, poultry, and eggs is not acceptable for enforcement purposes but is acceptable for collection of data. The current ICI submissions (1989; MRIDs 41386501 and 41406901) include two GC/mass-selective detector (MSD) methods for determining residues of the captan metabolites THPI, 3-OH-THPI, and 5-OH-THPI in eggs (Method No. 152) and in milk and animal tissues (Method No. 166). Independent validation of Method No. 152, conducted by Battelle Labs, is presented in MRID 41393001.

Adequate methodology is available for the enforcement of existing tolerances for residues of captan per se in or on crop commodities. A GLC/electron capture detector (ECD) method, a colorimetric method, and a thin-layer chromatography (TLC) method are listed in Pesticide Analytical Manual (PAM) Vol. II as Methods I, II, and A, respectively (Pesticide Reg. Sec. 180.103), for a variety of raw agricultural commodities.

The Pestrak data base dated 12/13/89 indicates that complete recovery (>80%) of captan may be expected using PAM Vol. I FDA Multiresidue Protocol E, Section 211.1 (for fatty and non-fatty foods); partial recovery (50-80%) may be expected using Multiresidue Protocol E, Section 212.1 (for fatty and non-fatty foods); and complete recovery also may be expected using Multiresidue Protocol D.

It should be noted that the plant and animal metabolism studies reviewed here do not adequately describe the qualitative nature of the residue in plants and animals. Thus, additional methodology for tolerance enforcement and data collection may be needed if additional data pertaining to plant or animal metabolism reveal metabolites of concern in addition to captan, THPI, 3-OH-THPI, and 5-OH-THPI.

ICI Americas, Inc. (1989; MRID 41406901) submitted a GC/MSD method for determining residues of THPI, 3-OH-THPI, and two isomers of 5-OH-THPI in eggs (ICI Agrochemicals Residue Analytical Method No. 152). Samples are prepared by removing the egg shell, then blending the egg yolk and white (either together or separately). A portion of the sample is mixed with Celite 545 at a ratio of 5:1 (w/w) sample:Celite, to a homogeneous paste and lyophilized. After lyophilization, samples are Soxhlet-extracted

with acetone, the extract is mixed with hexane, and the mixture is evaporated to dryness. The residues are resolubilized in acetonitrile, partitioned twice with hexane, and the hexane extracts are discarded. Acetonitrile-soluble residues are evaporated to dryness, resolubilized in toluene:ethyl acetate (95:5, v/v) applied to a silica column, and eluted with ethyl acetate. The eluates are dried under an N_2 stream, solubilized in acetonitrile, mixed with the derivatizing agent N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) and 10% trichloro-methylsilane (TCMS), and heated at 100 C for 10 min. Trimethylsilyl derivatives of THPI, 3-OH-THPI, and 5-OH-THPI are then determined by GC/MSD with the detector in the selective ion mode; the stated limit of detection is 0.01 ppm. The registrant indicated that residue determinations should be made within 24 hours of derivatization. Recovery data submitted by ICI are presented in Table 15 along with data from Battelle Labs' independent validation trial.

Table 15. Comparison of method recoveries obtained by ICI and Battelle Labs using GC/MSD Method No. 152 for determination of THPI, 3-OH-THPI, and 5-OH-THPI in eggs.

Fortification Level (ppm)	Recovery (%) THPI	3-OH-THPI	5-OH-THPI(1)	5-OH-THPI(2)
	ICI Americas validation			
0.01	91	136	109	113
0.01	101	138	106	92
0.035	102	127	96	94
0.05	108	127	96	94
0.1	105	130	92	97
0.15	74	108	81	86
0.2	100	109	88	87
Average	97	125	96	94
	Independent validation - Battelle Laboratories			
0.03[a]	114, 131	101, 103	108, 230[b]	--
0.03[c]	125, 137	59, 118	95, 110	--
Average	127	95	136	--
0.15[a]	66, 81	60, 105	75, 127	--
0.15[c]	74, 97	77, 86	65, 82	--
Average	80	82	87	--

[a] Data set 1.
[b] The validating laboratory indicated that the 230% recovery may have resulted from a spiking error.
[c] Data set 2.

A validation trial of ICI's Method No. 152 conducted by an independent laboratory (Battelle Labs, PR 88-5 Analytical Validation; 1989) was submitted by ICI Americas (MRID 41393001). The validating lab reported the following problems using Method 152 to determine captan metabolites in eggs: (i) difficulty in derivatizing the residues (this difficulty was eliminated by increasing the amount of derivatizing agent [BSTFA plus 10% TCMS], and by increasing the derivatizing time to 20-30 minutes); (ii) it was difficult to obtain reproducible GC/MSD responses for consecutive sample injections (this problem was alleviated by injecting acetonitrile blanks between sample injections); (iii) certain (unspecified) details pertaining to GC/MSD operating conditions were not completely described; (iv) chromatographic peak splitting was reported, and probably resulted from using acetonitrile as a solvent (raising the column temperature to 110 C and decreasing the programmed temperature increase rate to 15 degrees per minute, achieved proper analyte separation); and (v) the GC/MSD calibration level of 1 ppm was probably too high; this may have resulted in inaccurate residue level estimates. All reported validation data (Table 15) were collected prior to any suggested methodology changes. The method tended to recover excessively high residue levels at the 0.03 ppm fortification level and recoveries were extremely variable regardless of the metabolite used or the fortification level. The validating laboratory concluded that accuracy may be close to acceptable but precision may be a problem.

ICI Americas, Inc. (1989; MRID 41386501) also submitted a GC/MSD method for determining THPI, 3-OH-THPI, and two isomers of 5-OH-THPI in milk and animal tissues (ICI Agrochemicals Residue Analytical Method No. 166). Method 166 is similar to the method described for residues in eggs (Method 152; MRID 41406901), except that the samples are not lyophilized prior to the initial extraction. The registrant listed the limits of detection as 0.05 ppm for tissues from unspecified animals and 0.01 ppm for milk. Recoveries of THPI, 3-OH-THPI, and 5-OH-THPI are detailed in Table 16.

Table 16. Recoveries of THPI, 3-OH-THPI, 5-OH-THPI from animal tissues and milk fortified separately with each metabolite at 0.05-0.5 ppm (MRID 41386501).

Sample	Fortification level (ppm)	Recovery (%) THPI	3-OH-THPI	5-OH-THPI[a]
Muscle	0.05	116	146	116, 130
Muscle	0.3	107	123	108, 112
Muscle	0.5	95	104	103, 113
Liver	0.1	121	137	114, 137
Kidney	0.2	111	111	107, 115
Fat	0.4	88	100	101, 102
Milk	0.05	100	148	107, 113
	0.1	115	132	111, 112
	0.2	104	128	112, 116
	0.5	107	112	97, 100
	0.01	156	182	114, 124

[a] Isomers 1 and 2.

In summary, independent validation of ICI's Method No. 152 by Battelle Labs was unsuccessful. The method tended to recover excessively high residue levels at the 0.03 ppm fortification level and recoveries were extremely variable regardless of the metabolite used or the fortification level. We recommend that the registrant revise Method No. 152 for determination of THPI, 3-OH-THPI, and 5-OH-THPI in milk and animal tissues and run a second confirmatory trial using a different laboratory.

Bibliographic Citations

40658002 Daun, R. (1988) [Trichloromethyl-[Carbon 14 Captan: Nature of the Residue in Livestock--Lactating Goats: Laboratory Project ID: HLA 6183-105. Unpublished study prepared by Hazleton Laboratories, Inc. 67 p.

40658003 Daun, R. (1988) [Trichloromethyl-[Carbon 14 Captan: Nature of the Residue in Livestock--Laying Hens: Laboratory Project ID: HLA 6183-106. Unpublished study prepared by Hazleton Laboratories America, Inc. 62 p.

40658004 Daun, R. (1988) [Cyclohexene-1,2-[Carbon 14 Captan: Nature of the Residue in Livestock--Laying Hens: Laboratory Project ID: HLA 6183-104. Unpublished study prepared by Hazleton Laboratories America, Inc. 79 p.

40658005 Chen, Y. (1988) Plant Metabolism Study of [Trichloromethyl-[Carbon 14-Captan: Laboratory Project ID: MEF-0009/8808900. Unpublished study prepared by Chevron Chemical Co. 37 p.

40658006 Chen, Y. (1988) Plant Metabolism Study of [Cyclohexene-1,2-[Carbon 14-Captan: Laboratory Project ID: MEF-0010/8805420. Unpublished study prepared by Chevron Chemical Co. 38 p.

41386501 Davy, G. (1989) ICI Agrochemicals Residue Analytical Method No. 166: The Determination of Residues of Tetrahydrophthalimide, 3-Hydroxy-Tetrahydrophthalimide and 5-Hydroxy-Tetrahydrophthalimide in Milk and Animal Tissues: A Gas Liquid Chromtaographic: Mass-selective Detector Method Using External Standardization. Unpublished study prepared by ICI Agrochemicals. 38 p.

41393001 Yancey, M. (1989) Final Report on PR 88-5 Analytical Validation for ICI Agrochemical Residue Analytical Method 152 for Residues of THPI, 3-Hydroxy THPI and 5-Hydroxy THPI in Eggs: Lab Project Number: NO967-0701: ICI Protocol NO. CAPT-CL-AM-01. Unpublished study prepared by Battelle. 27 p.

41406901 Davy, G.; Atreya, N. (1989) The Determination of Residues of Tetrahydrophthalimide, 3-Hydroxy Tetrahydrophthalimide and 5-Hydroxy-Tetrahydrophthalimide in Eggs: A Gas Liquid Chromatographic Mass-selective Detector Method Using External Standardisation. Method No. 152. Unpublished study prepared by ICI Agrochemicals, Jealotts Hill Research Station, Bracknell, UK. 30 p.