UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Registration No. 239-1246. Captan.
Response to April 29, 1985 Data Call-In
Letter Requesting Large Animal Feeding Study.
Accession No. 258867, RCB No. 1273

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Chevron Chemical Company has submitted to the PM a dairy cow feeding study which was requested in the April 29, 1985 Data Call-In letter.

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This study was conducted in 1976-77 in conjunction with Stauffer Chemical Company, and was originally submitted (but somehow, never reviewed) in support of PP#7F1962. This same study will also be discussed in the Registration Standard Residue Chemistry chapter.

Chevron's cover letter indicates that this dairy cow study and an analytical method document contained in this same volume are also responsive to a request made by letter dated March 23, 1984 (which is not found in the registration jacket or special review files). Since we would need to discuss analytical methods along with the feeding study, we are combining the reviews and sending them to all persons concerned (DCI, Special Review, and PM).

Chevron has also included in this volume a goat metabolism study using carbonyl-labeled ^{14}C-captafol. This study was reviewed when originally submitted and again in the Registration Standard. It will not be discussed again here. The metabolism of captafol in ruminants is considered to be adequately understood; the residue of concern consists of captafol, tetrahydrophthalimide (THPI), 3-OH THPI, and 5-OH THPI.

Similarly, the metabolism of captan in ruminants has long been considered adequately understood, with the residue of concern considered to be captan, THPI, 3-OH THPI and 5-OH THPI. The (soon to be published) Captan Registration Standard will state that the metabolism of captan in ruminants is not understood, and that metabolism data reflecting the fate of the (thiophosgene) side chain moiety are required.

However, we are evaluating this feeding study under the assumption that the residue of concern has not changed. If the metabolism data being requested as result of the Standard suggest that the residue of concern needs redefinition we will have to revisit all residue chemistry issues and, at that time, another feeding study might be required. For the present (Special Review) purposes, we are operating with the best available information, and have discussed the interrelationship of the review processes in order to avoid confusion and misunderstanding.

and 3-OH THPI prior to acidification, and with parent (either captan or captafol) immediately after acidification.

The sample is allowed to settle and the extract filtered through anhydrous sodium sulfate. Extraction is repeated twice, extract combined and the solvent evaporated until about 10 mL remain or foaming begins. At that point, acetonitrile is added and the evaporation continued to dryness.

The residue is transferred to a separatory funnel with alternate portions of hexane and acetonitrile. The acetonitrile fraction is retained and washed twice with hexane, then evaporated to dryness. The residue is taken up in diethyl ether diluted with equal volume hexane and cleaned up on silica gel. The captan (or captafol) is eluted with 50 percent hexane:ether evaporated to dryness, then dissolved in hexane and cleaned up on Florisil by washing with hexane, then benzene, and eluting with 1 percent methanol in benzene. The eluate is evaporated to dryness, taken up in hexane and quantitated by GC. For captan, peak height is compared with the height of a reference standard peak. Interestingly, captafol is quantitated by comparing the square root of the peak height with the same measurement for a reference standard.

Metabolites (THPI and 3-OH THPI) are eluted from the silica gel column with acetone, and an aliquot of the eluate evaporated to dryness. For heart and muscle samples, the residue is dissolved in ether, subjected to a second silica gel column, eluted with acetone and vacuum evaporated to dryness. All other samples skip this second silica gel step.

The residue remaining after evaporation of acetone is taken up in 15 percent ethyl acetate in benzene and applied to a Nuchar-silica gel column. THPI is eluted with 15 percent acetone in benzene, dried under vacuum and derivatized (see below). The 3-OH THPI is eluted with 30 percent acetone in benzene, evaporated under vacuum and derivatized with alpha-bromo-2,3,4,5,6-pentafluorotoluene by standing overnight in a solution of K_2CO_3 in acetone. Reference standards are to be derivatized at the same time. Derivatives are evaporated to dryness under vacuum.

The THPI derivative is taken up in benzene and transferred to a prewashed, deactivated Florisil column. The column is washed with benzene, then with 2 percent ethyl acetate in benzene; the derivatized THPI is eluted with 5 percent ethyl acetate in benzene. After evaporation to dryness under vacuum, the residue is taken up in benzene and subjected to GC. Quantitation is by comparison of peak heights with the derivatized standard N-pentafluorobenzyl THPI.

The 3-OH THPI derivation is dissolved in 25 percent acetone in benzene and similarly applied to a prewashed deactivated Florisil column. After washing with 25 percent acetone in

benzene and then 50 percent acetone in benzene, the 3-OH THPI derivative is eluted with 50 percent acetone in benzene. The eluate is vacuum evaporated to dryness, taken up in benzene, and subjected to GC. Quantitation is by comparison of peak heights with the derivatized standard N-pentafluorobenzyl 3-OH THPI.

Recovery data from tissues fortified at 0.05 ppm are given with the feeding study. Although some of the fortified samples were stored frozen (-20 °C) for a year, no differences in recovery appear related to loss during storage. The storage stability over 15 months was confirmed by repeat analysis of control muscle and liver samples from the animal slaughtered the day after cessation of dosage at 1200 ppm.

Recovery of parent captan was 54-56% in fat, 80-97% in heart, 73-85% in kidney, 61-68% in liver, and 93-110% in muscle. For THPI, recoveries were 50-51% in fat, 85-94% in heart, 73-104% in kidney, 69-109% in liver, and 79-90% in muscle. For 3-OH THPI, recoveries were 39-52 in fat, 46-67% in heart, 50-57% in kidney, 62-71% in liver, and 59-71% in muscle.

Recoveries from fat were consistently lower than other tissues, and were particularly low for the 3-OH THPI metabolite. Since THPI and 3-OH THPI do not selectively partition into fat, and 3-OH THPI is a minor portion of the residue, these recoveries are acceptable.

Sample chromotograms of recovery studies are only available at the 0.05 ppm levels. Since this level approximates the residue levels expected to occur from dietary consumption (i.e., the 1X or 100 ppm feeding level), these limited recovery data are sufficient.

The analytical method given for eggs and tissues is adequately validated and acceptable for the purposes of this feeding study.

Analytical Method for Milk and Cream

For milk and cream, captan residues are extracted into hexane and then partitioned into acetonitrile. The acetonitrile is evaporated, the residue taken up in benzene and quantitated using GC/ECD. Preconditioning of the GC column is necessary.

Metabolites in milk and cream are extracted with acetone--cream samples have an extra portion of water added at the outset. Residues are then partitioned into methylene chloride and dried by passing over Na_2SO_4 (3 times, fractions combined), and the solvent evaporated. The residue is taken up in 5 percent ethyl acetate in benzene, with Na_2SO_4 added. This solution is cleaned up on a column of silicic acid with anhydrous Na_2SO_4, and eluted with 30 percent acetone in benzene. Solvent is again evaporated, the residue dissolved in benzene and quantitated using GC/ECD as for captan.

According to the write-up, attempts to synthesize 5-OH THPI were unsuccessful. The compound was identified by MS after isolation and purification on LC; the 3-OH THPI standard was used for quantitation purposes. Recovery data for milk and cream are summarized in the following table.

Recovery Percentages

Fortification Level, ppm	Spike Material	Substrate Milk	Cream
0.05	Captan	104-110	80
	THPI	94-114	78-88
	3-OH	92-100	80
0.1	Captan	100	
	THPI	92-113	
	3-OH	90-140	
0.2	THPI		93
	3-OH		100
0.4	THPI	95-107	
	3-OH	87-105	
0.5	3-OH		80
1.0	THPI		70
	3-OH		79
2.0	THPI	94-103	
	3-OH	79-96	
4.0	THPI	105	
	3-OH	97	
10	THPI	105	
	3-OH	93	

The analytical method presented for milk and cream is satisfactorily validated and is considered acceptable for the purposes of this study.

Feeding Study

Five lactating cows were used for each captan feeding level: 100, 600, and 1200 ppm in the total diet. Dosage was administered as a feed concentrate (mixed daily) at morning and evening milkings, for 28 consecutive days. Tissue samples were sent to Chevron, and milk samples sent to Stauffer for analysis. Tissue samples are discussed here.

Control animals are not mentioned and no values are given for analysis of controls, although copies of chromatograms of control samples and fortified controls are included. We presume that the unfortified samples reported in the recovery study are from control samples. The control chromatograms, when compared with fortified controls (0.05 ppm), appear to have captan and THPI peaks (but not 3-OH THPI) at levels 10 to 30 percent of the fortified samples (< 0.05 ppm, which is considered the limit of detection).

Since control animals are discussed in the Stauffer portion of this same study, and captan levels in control diet are reported to be zero ppm, we conclude that the lack of specific reference to controls must be an oversight due to the sharing of analyses and write-ups. We are satisfied that control animals were maintained during the study, and that control samples were analyzed and did, indeed, have no residues above the limit of detection.

The 5-OH THPI metabolite was neither mentioned nor measured. We understand that synthesis of this isomer has proven "difficult." In the (captafol) goat metabolism study (Cheng 1979) reviewed in the Registration Standard and earlier RCB reviews, 5-OH THPI is consistently less than 3-OH THPI (up to 50 percent less). We, therefore, consider that the absence of 5-OH residue levels is not crucial--we have adequate information to extrapolate tissue residue levels for the purpose of dietary exposure calculations. (We understand from a meeting with Stauffer on October 8, 1985 that the 5-OH THPI has been made for use as a reference standard, and will be used in the poultry feeding study which is being planned. If, as discussed above, new components are added in the residue definition and if another feeding study must be done, the 5-OH THPI should be measured.)

One animal from each treatment group was slaughtered on day 21; additional animals were slaughtered on days 29 and 32 (1 and 4 days after dosing ceased). Samples of fat, heart, kidney, liver, and "muscle" tissue were collected, frozen, and shipped to Chevron labs. All tissues except fat were extracted upon receipt, and the otherwise unmentioned control/untreated samples fortified and extracted. The 21-day samples were analyzed "within a short period of time," while other extracts (experimental and fortified control) were stored for over a year.

Chevron's write-up notes that stability of metabolites in frozen muscle and liver was confirmed by comparing results of analysis of fresh extracts with the stored extract. No details are given, so this exercise has very limited value as a formal storage stability study. If anything, this study indicates THPI in the stored liver extract might be unstable, since THPI levels were considerably higher in the freshly extracted previously frozen samples of liver, but not significantly so for muscle the only other tissue so compared. Since the reanalysis of fortified samples showed no loss of analyte (see discussion under Analytical Methods above) we consider that the storage of extracts prior to quantitation does not affect the reliability of the data.

The following table contains the residue data reported for this study. Results shown are not corrected for recoveries or controls.

Feeding Study Results

Tissue	Captan Level	Day of Slaughter	Residue Found, ppm		
			Captan	THPI	3-OH THPI
FAT	100 ppm	21	0.00	0.03	0.01
		29	0.00	0.02-0.03	0.01-0.01
		32(odd)	0.00	0.10-0.12	0.04
	600 ppm	21	0.00	0.93-1.0	0.06-0.09
		29	0.00	0.36-0.41	0.13
		32	0.00	0.01-0.02	0.00
	1200 ppm	21	0.00	3.9-4.0	0.09-0.14
		29	0.00	1.1	0.22-0.25
		32	0.00	0.01	0.00-0.01
HEART	100 ppm	21	0.00	0.11-0.12	0.02
		29	0.00	0.01	0.00
		32	0.00	0.00	0.00
	600 ppm	21	0.00	2.5-2.9	0.15-0.16
		29	0.00	0.65	0.01
		32	0.00	0.0	0.00
	1200 ppm	21	0.00-0.03	13	0.12-0.19
		29	0.00	2.8-3.1	0.11-0.04
		32	0.00	0.00	0.00
KIDNEY	100 ppm	21	0.00	0.04	0.02
		29	0.00	0.01-0.02	0.00
		32	0.00	0.02-0.04	0.00
	600 ppm	21	0.00	1.6-2.0	0.04-0.06
		29	0.00	0.30-0.74	0.17-0.19
		32	0.00	0.01	0.00
	1200 ppm	21	0.00	6.6-8.4	0.06-0.29
		29	0.00	3.9-4.3	0.49-0.67
		32	0.00-0.01	0.01-0.02	0.00
LIVER	100 ppm	21	0.00	0.04-0.07	0.00-0.01
		29	0.00	0.01	0.00
		32	0.00	0.01	0.00
	600 ppm	21	0.00	0.29-1.37	0.01-0.02
		29	0.00	0.84-0.86	0.05-0.06
		32	0.00	0.01	0.00
	1200 ppm	21	0.00	5.8-10.5	0.05
		29	0.00	2.9-3.2	0.24-0.30
		32	0.00	0.01-0.02	0.00
MUSCLE	100 ppm	21	0.00	0.08-0.09	0.01
		29	0.00	0.01	0.00
		32	0.00-0.01	0.01	0.00
	600 ppm	21	0.00	2.7-2.9	0.06
		29	0.00-0.01	0.78	0.04-0.05
		32	0.00	0.00	0.00
	1200 ppm	21	0.00	12	0.18-0.20
		29	0.00	3.2-3.8	0.26-0.32
		32	0.00	0.00	0.00

This portion of the feeding study (cattle tissue residues) is sufficient to enable us to determine residue levels which are likely to occur in meat as a result of animals consuming captan-treated feed items.

We, therefore, consider the study acceptable. As discussed above, the lack of 5-OH THPI residue data is not considered a problem. We note that another feeding study (steers) is available for use as supporting data. The IBT feeding study on captan was reevaluated and is considered valid (memorandum of E. Zager April 13, 1981).

Milk Residue Study

Milk and cream samples were collected from animals in the same study discussed above. Morning and evening milkings were composited for individual animals on days 0, 1, 3, 7, 14, 21, 28, 29, 31, and 35 (28 days on treatment, 7 days withdrawal). Samples were frozen, shipped in dry ice, and maintained in -10 °C frozen storage until analyzed by the method discussed above. The 3-OH THPI metabolite was used as reference standard for both 3- and 5-OH THPI, since the 5-OH THPI could not be synthesized. Since no residues of parent captan were found in any samples (milk or cream) from high dose (1200 ppm) animals, the lower dose (100 and 600 ppm) samples were not analyzed for captan. This is acceptable to RCB.

Maximum residue levels found in milk are given below. Residues in cream were analyzed for high-dose animals only, and were consistently lower than milk residues (only 2 samples of roughly 50 showed slightly higher metabolite concentration in cream--20 percent more 3-OH THPI). Thus, captan and its metabolites do not concentrate in cream, and any milk tolerance should be expresed in terms of whole milk.

Maximum Milk Residues

Metabolite	Nominal Dose Level, ppm		
	100	600	1200
THPI	0.40	7.50	31.60
3-OH THPI	0.26	2.10	3.50
5-OH THPI	0.59	2.30	2.70

During the 7-day withdrawal period, residues of THPI and 3-OH THPI declined to nondetectable in all groups (0.00 ppm). Residues of 5-OH THPI declined but were still present (0.01 to 0.02 ppm) in all groups after 7 days withdrawal.

This portion of the feeding study (milk and cream) is well done, and provides sufficient data to allow determination of dietary exposure, levels likely to occur as a result of animals consuming captan-treated feed items.

Conclusion

This feeding study in combination with other available data is adequate to allow determination of residue levels (dietary exposure analysis) expected to occur in meat and milk as a result of animals consuming captan-treated feed items.

The requirement for a large animal feeding study, as requested in the April 29, 1985 DCI letter, is satisfied.

cc: Captan S.F., R.F., PP#7F1962, Reviewer, Circu, PMSD/ISB

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