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

MEMORANDUM FEB 28 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: **Thiodicarb.** Ruminant Metabolism Study (liver, kidney, muscle, milk, and fat)
DP Barcode: D195281; CBRS No. 12594; MRID No.: 42919601; Case No. 2675

FROM: David J. Miller, SA HSO, US Public Health Service
Special Review Section I
Chemistry Branch II--Reregistration Support
Health Effects Division (7509C)

THRU: Francis B. Suhre, Section Head
Special Review Section II
Chemistry Branch II--Reregistration Support
Health Effects Division (7509C)

TO: Bonnie Adler, PM Team 52
Accelerated Reregistration Branch
Special Review and Reregistration Division (7508W)

In response to the information contained in CBRS's Phase IV review, Rhone-Poulenc submitted data (1993; MRID 429196-01) concerning the metabolic fate of [^{14}C]thiodicarb in lactating goats. These data were submitted in order to satisfy the data requirements set forth in the EPA Pesticide Assessment Guidelines, Subdivision O, Series 171-4, "Nature of the Residue in Livestock". The data are reviewed here for their adequacy in fulfilling this outstanding residue chemistry data requirement.

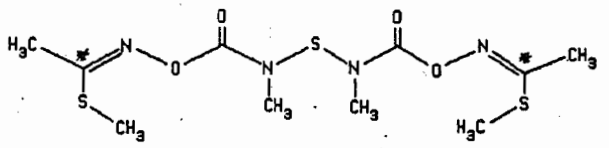
Tolerances for residues of thiodicarb [dimethyl N,N'-[thiobis[[(methylimino) carbonyl]oxy]bis[ethanimidothioate] in or on plant commodities are expressed in terms of residues of thiodicarb and its regulated metabolite methomyl (S-methyl N-[(methylcarbamoyl)oxy]-thioacetimadate) (40 CFR §180.407). Residues in animal commodities are not regulated. Codex MRLs for thiodicarb (and its methomyl and

methomyl oxime metabolites, expressed as thiodicarb) exist for meat and milk at 0.02 ppm, each.

The Phase IV CBRS review (dated 12/10/90) stated that the studies submitted in support of nature of the residue in plants do not completely satisfy the acceptance criteria.

The Pesticide Analytical Manual (PAM) Vol II lists gas chromatographic methods for the determination of thiodicarb in or on plant commodities only. Tolerances have not been established for residues of thiodicarb or any metabolites on any animal commodities. If metabolites which require regulation are found during the animal metabolism studies, the registrant will be required to develop an acceptable enforcement method for the determination of thiodicarb and its regulated metabolites in animal commodities. Any regulatory methods submitted will require an independent laboratory method validation as described in PR Notice 88-5 (July 15, 1988).

The structure of thiodicarb is presented below:



CONCLUSIONS

1. The preparation of the dosing capsules was adequately described. Thiodicarb radiolabeled in the 1-acetyl position (as shown above) was administered to two lactating goats at 208 and 292 ppm in the diet for seven consecutive days. This represents approximately 390-450x the maximum theoretical dietary burden.

2. Total radioactive residues (TRR) were determined in various tissue and organ matrices using HPLC with a radiocarbon detector. Total radioactive residues exceeded CBRS trigger values in all pertinent matrices (i.e., liver, kidney, muscle, fat, and milk), ranging from 0.450 ppm in fat to 25.2 ppm in liver.

3. Thiodicarb and its regulated methomyl metabolite were detected only in feces and stomach contents: no thiodicarb or any of its primary metabolites (i.e., methomyl, methomyl sulfoxide, methomyl sulfone, methomyl oxime, or methomyl oxime sulfoxide) was detected in the edible tissues. Identified metabolites which are not currently regulated, but were detected in edible tissues were acetamide, acetonitrile, acetic acid, and lactose.

4. The registrant identified approximately 19% of the TRR in liver, 24% of the TRR in kidney, 44% of the TRR in muscle, 16% of the TRR in milk, and 0% of the TRR in fat. In liver, an unknown was present which comprised up to 31% of the TRR (Unknown A1/A2), while in kidney an unknown was present which comprised approximately 12% of the TRR (Unknown C). The registrant should make further attempts to identify these unknowns.

5. Approximately 50-60% of the TRR remained unidentified in liver, kidney, and muscle, while approximately 62 to 93% of the TRR remained unidentified in milk and fat. The registrant is required to perform additional characterization/identification of the following fractions:

Tissue/Organ	Extract: Fraction Identification	Deficiency
Liver	Aqueous I: Unknown A1	Unknown A1 comprises approximately 24-31% of the TRR (ca. 6 ppm)
	Aqueous I: Column-retained material	Column-retained material comprises 16-17% of TRR (ca 4 ppm)
Kidney	Aqueous I: Unknown C	Unknown C comprises approximately 12% of the TRR (ca. 1.9 ppm)
	Aqueous I: Column-retained material	Column-retained material comprises approximately 26-27% of the TRR (ca. 3-4 ppm)
Milk	Hexane V: hexane soluble acids/alcohols	This fraction comprises approximately 18% of the TRR (ca. 2.6 ppm). While the registrant classified this fraction as "free fatty acids", CBRS does not believe that the saponification procedure followed permits this degree of characterization. CBRS will require that the registrant identify the constituents present in this fraction using more definitive methods.
	KOH III: non-ionizable acids/alcohols	This fraction comprises approximately 14% of the TRR (ca. 2.0 ppm). While the registrant classified this fraction as "other saponifiable lipids", CBRS does not believe that the saponification procedure followed permits this degree of characterization. CBRS will require that the registrant identify the constituents present in this fraction using more definitive methods.
Fat	Hexane IV: hexane soluble acids/alcohols	This fraction comprises approximately 40% of the TRR (ca. 0.6 ppm). While the registrant classified this fraction as "free fatty acids", CBRS does not believe that the saponification procedure followed permits this degree of characterization. CBRS will require that the registrant identify the constituents present in this fraction using more definitive methods.

Tissue/Organ	Extract: Fraction Identification	Deficiency
Fat (Cont'd)	KOH III: non-ionizable acids/alcohols	This fraction comprises approximately 40% of the TRR (ca. 0.6 ppm). While the registrant classified this fraction as "free fatty acids", CBRS does not believe that the saponification procedure followed permits this degree of characterization. CBRS will require that the registrant identify the constituents present in this fraction using more definitive methods.

6. In general reasonable mass balances/mass closures were achieved for the pertinent matrices. Except for milk (with losses of 22%) losses in no case exceeded 10% and mass was accounted for in all cases to within 7%.

7. Samples were stored frozen until analysis at a nominal -10°C temperature. Sufficient storage stability information was provided for muscle, kidney and liver. The registrant, however, did not include any storage stability data for milk and fat, nor was any information provides as to the storage interval associated with milk and fat. The registrant should submit adequate storage stability data for these matrices with the additional required information.

8. Based on the evidence presented, the registrant proposed a metabolic pathway for thiodicarb in lactating goats. The pathway involves the cleavage of the sulfur bridge in the thiodicarb bridge to yield two molecules of methomyl which are oxidized to the oxime. Subsequent metabolic processes degrade the methomyl oxime to acetonitrile, acetamide, acetic acid, and carbon dioxide, with incorporation via natural product pathways into amino acids, lipids, and carbohydrates.

RECOMMENDATIONS

The present "Nature of the Residue in Ruminants" study does not meet EPA Guideline requirements and is not acceptable: the registrant did not perform adequate characterization/identification of the radioactive residue present in kidney, liver, milk, and fat from lactating goats. Characterization/identification of the TRR in ruminant muscle was, however, adequate. SRRD should require the registrant to perform additional characterization/identification procedures on the specific tissue extracts identified in Conclusion #5.

DETAILED CONSIDERATIONS

Rhone-Poulenc submitted data (1993; MRID 429196-01) pertaining to a ruminant metabolism study conducted with radio-labeled thiodicarb. This study was submitted in order to comply with EPA Pesticide Residue Guidelines regarding the nature of the residue in livestock.

Two non-pregnant lactating goats were dosed by oral capsule twice daily for seven consecutive days at 208.3- and 291.6 ppm in the diet; the dose was administered using a balling gun after each morning and evening milking. Since soybeans, soybean hulls, cottonseed, and cottonseed hulls are the only dairy and beef feed items for which thiodicarb is registered, the maximum theoretical dose can be calculated and compared to the actual dosing levels used in the study: this calculation reveals that the feeding levels used in the study represent approximately 390 to 450 times the maximum theoretical dose.

Test Material

The radiolabeled thiodicarb was supplied by New England Nuclear. The [^{14}C]thiodicarb test material had a radiochemical purity of 97.6%. The treatment solution had a specific activity of 22,837 dpm/μg.

Radioanalytical Methods and Radiovalidation Studies

Liquid Scintillation Counting (LSC) was performed with a Beckman LS 9800 Liquid Scintillation System. Quench correction was performed automatically. Counting was for a minimum of ten minutes.

The twice background detection limit for combustion samples was calculated as 0.01 ppm. The twice-background detection limit for LSC was calculated as 0.002 μg. These detection limits were calculated by using the average of six representative control samples.

Chromatographic Techniques

The registrant used thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC) to identify residues in the organ/tissue matrices examined. Each of these techniques is summarized below:

> TLC: TLC analyses used pre-coated silica gel plates that were solvent developed in one of several different solvent systems. After solvent development, radiolabeled material was detected and quantitated by an Ambis Radioanalytic Imaging System®. Unlabeled standards were visualized by viewing the plates under UV light.

HPLC: The HPLC system employed during this study included two Shimadzu LC-600 pumps, a Rheodyne 7125 injector, an LDC 3100X UV variable wavelength detector, and a Berthold LB 506 radioactivity detector. The various columns used included a Zorbax C8 column, an Alltech OA-1000 organic acid column, an Alltech OA 1793 column, and a Biorad HPX-72S organic base column.

The following radiolabeled compounds were used as reference standards:

Thiodicarb	Acetic Acid
Methomyl	D-glucose
Methomyl Sulfoxide	Acetamide
Methomyl Oxime	Acetonitrile
Lactose	

Storage Conditions and Storage Stability Data

All samples were stored at the laboratory at a nominal -10°C temperature. No information was provided with respect to the length of time over which samples were stored. Nevertheless, a storage stability test was conducted by comparing the chromatographic profiles from the initial Bligh-Dyer extractions with Bligh Dyer analysis repeated near the end of the study (on samples which were store frozen from study initiation). This comparison was performed in the following way: the liver, kidney, and muscle were initially extracted using a modified Bligh-Dyer extraction method with the aqueous and organic fractions from this extraction analyzed by HPLC. Approximately one year later, the stored tissues were re-extracted by the same method and again subject to HPLC analysis. Comparison of the initial chromatographic profiles with those done at the end of the study showed no significant differences in the metabolic profiles of the liver, kidney, and muscle extracts.

No information is available regarding the storage stability of the metabolites in milk and fat matrices.

Sampling Procedures

Antemortem Sample Collection: In order to account for 100% of the administered radioactive dose, excreta, milk, and volatiles (from respiration) were collected during the in-life phase of the study prior to sacrifice.

The animals were milked every morning and evening during the acclimation period and through the completion of the in-life phase. After every collection, each sample was thoroughly mixed and its volume recorded. The samples were transferred to plastic

bottles and stored frozen. Urine and feces were also collected and weighed twice each day during the morning and evening dosings. The total volume of each urinary collection was recorded, and the feces and urine samples placed into the freezer.

Once during the acclimation phase and once on Day 6 of the treatment phase, each of the treated goats was placed in a respiration chamber to monitor the production of volatiles. Each collection was for approximately 10 hours. A total of four gas collection traps were used: the first trap was a dry cold trap for moisture collection which was maintained in an ice-salt bath at approximately -10°C. The second trap contained 2-butoxyethanol for the collection of volatile organics, and was maintained similarly in an ice-salt bath. The third and fourth traps each contained Harvey Carbon 14 Cocktail at room temperature and were used to collect carbon dioxide.

Sacrifice and Post-Mortem Tissue Collection Approximately 16-18 hours after the last dose, each of the animals was sacrificed using a captive bolt pistol and exsanguination. The following samples were collected for subsequent determination of radiolabel levels:

- Muscle
- Liver
- Kidney
- Composite fat (subcutaneous, renal, and visceral)
- Stomach and Gastrointestinal (GI) tract contents
- Blood

The samples were placed in insulated boxes with dry ice for transport to the ADC. At ADC, the samples were stored frozen until analysis.

Sample Processing

All tissues were partially thawed prior to processing. Muscle, fat, liver, and kidney were cleaned of extraneous material prior to homogenization. The liver and kidneys were processed in a food processor, while the fat and muscle were ground in a meat grinder. After thawing, the gut contents were mixed well by stirring. Blood samples were mixed on a vortex mixer. Subsamples were removed from each sample for combustion analysis. All samples were returned to the freezer after processing.

Radiolabel Recovery and Distribution by Organ/Tissue

Following sacrifice and sample storage and processing, the registrant measured the radiolabel levels in the sampled goat matrices by LSC. These determinations served to a) account for the majority of the administered dose, thereby verifying acceptable recovery of the administered radiolabel; and b) permit evaluation of the distribution of the thiodicarb among the various target organs/tissues and allow the estimation of resultant radiolabel concentrations in those organ tissues.

Table 2 shows these distributions and the resultant concentrations for the milk commodity for each of the two dosed goats.

Table 2. Percent of Radioactive Dose Recovered in Milk of Lactating Goats Administered [^{14}C]Thiodicarb at 208 and 292 ppm Concentrations in the Diet of 7 Days.

Collection Time	[^{14}C]Thiodicarb Concentration			
	Goat #4		Goat #5	
	TRR, % Radioactive Dose	TRR, ppm [^{14}C]thiodicarb equivalents	TRR, % Radioactive Dose	TRR, ppm [^{14}C]thiodicarb equivalents
Day 1 p.m.	0.09	2.64	0.10	2.25
Day 2 a.m.	0.26	5.27	0.44	6.44
Day 2 p.m.	0.37	10.5	0.26	6.19
Day 3 a.m.	0.65	12.3	0.46	6.93
Day 3 p.m.	0.48	13.3	0.25	6.92
Day 4 a.m.	0.63	11.9	0.38	7.17
Day 4 p.m.	0.52	14.5	0.19	7.65
Day 5 a.m.	0.61	13.3	0.30	8.71
Day 5 p.m.	0.37	11.8	0.17	9.82
Day 6 a.m.	0.43	11.3	0.21	12.2
Day 6 p.m.	0.45	13.4	0.14	12.0
Day 7 a.m.	0.47	13.5	0.11	15.1
Day 7 p.m.	0.45	14.8	0.05	19.9
Day 8 a.m.	0.60	11.4	--[a]	--[a]
Total Recovered Dose	6.38		3.06	

[a] Goat #5 did not produce any milk during this time latest period. At necropsy, a contusion in the back of the oral cavity was noted which the pathology report indicated was probably due to balling gun trauma. Feed consumption and milk production in Goat #5 decreased throughout the study culminating in zero milk production on Day 8--a.m.

As can be seen from the Table, total radioactive residues found in milk accounted for approximately 3-6 percent of the administered dose. Total radioactive residue concentrations ranged up to approximately 20 ppm.

Radiolabel recovery information was also collected for additional matrices (organs, tissues, and excreta) in order to account for the majority of the administered dose and determine the TRR present in each edible tissue. These data are presented in Table 3.

Table 3. Estimated Recoveries of Total Radioactive Residues (TRR) in Matrices of Lactating Goats Administered [^{14}C]Thiodicarb at 208 and 292 ppm Concentrations in the Diet for 7 Days.

Matrix	[^{14}C]Thiodicarb Concentration			
	Goat #4		Goat #5	
	TRR, % Radioactive Dose	TRR, ppm [^{14}C]thiodicarb equivalents	TRR, % Radioactive Dose	TRR, ppm [^{14}C]thiodicarb equivalents
Liver	0.93	25.2	1.06	23.0
Kidney	0.078	12.6	0.11	13.9
Muscle	3.80[c]	4.34	4.39[c]	4.24
Fat	0.066	1.37	0.026	0.450
Blood	0.83[c]	10.5	1.00[c]	10.7
Stomach Contents	1.62	--	5.13	--
Small Intestine Contents	0.29	--	0.38	--
Large Intestine Contents	0.61	--	1.44	--
Milk	6.38	14.9[b]	3.06	19.9[b]
Urine	8.86	--	5.78	--
Feces	7.07	--	3.51	--
Volatiles[a]	20.95	--	23.21	
Total Recovered Dose	51.5%	--	50.0%	--

[a] Volatiles were only collected for 10 hours during Day 6 of the treatment. The registrant estimated volatile production by extrapolation over the whole dose period to represent 20.95% of the total dose. For Goat #4, this 20.95% consisted of 0.11% detected in the dry trap, 9.54% detected in the butoxyethanol trap, and 11.30% in the Harvey Carbon-14 trap. For goat 5, volatile production was similarly estimated.

[b] These concentrations represent the highest concentrations found in the milk samples over the 7-day post -treatment period during which milk was collected.

[c] These figures differ slightly from those calculated by the registrant. The registrant assumed that muscle and blood represented 40% and 8% of the body weight of the goat, respectively. CBRS assumed these values to be 45.5% and 4.1%, respectively (see Davis *et al.* Am. J. Vet. Res. 36(3):309-311, 1975)

Table 3 shows that the total recovery from all matrices (including blood) was approximately 50% of the administered dose in both goats. The registrant notes that the largest percent of the dose was respired as volatiles, and states that since volatile production was monitored for only 10 hours during the treatment phase and these values were extrapolated to cover the entire treatment phase, it is likely that the remaining 50% of the unaccounted for radioactivity is attributable to volatiles.

As also can be seen from the Table, roughly 90% of the doses is expected to be either respired as volatiles or excreted in the urine or feces[1] . Of the edible tissues (i.e., muscle, liver, kidney, fat, and milk), radioactivity was found to be most concentrated in the liver with TRR reaching 25.2 ppm at the 390x dosing level. Concentrations is milk averaged approximately 10-15 ppm. Since total ^{14}C residue concentrations in all edible tissues were greater than Chemistry Branches' 10 ppb (0.010 ppm) trigger value, the registrant was thus required to perform further characterization/identification of the metabolites in these tissues.

Extraction of Radiolabeled Residues and Characterization/Identification of Extract Residues in Organs/Tissues

As discussed in the previous section, all edible tissues (including milk) require further metabolite characterization/identification since they were all found to contain TRR concentrations greater than 0.01 ppm. The registrant was thus required to further characterize/identify the extractable and non-extractable residues appearing in these matrices, as per the Chemistry Branches' Metabolism Guidance Document.

The following paragraphs deal with specific extraction and TLC/HPLC characterization procedures used for each analyzed tissue (e.g., liver, muscle, milk, excreta, etc.). For each matrix, a flow diagram and summary table are provided.

Liver: A 5 g liver sample was extracted by blending the sample with 5 mL of water, homogenizing the sample for 1 minute with a Polytron® homogenizer, and centrifuging the sample to separate the water and solids. This resulted in Aqueous Fraction I (70.1% TRR, 17.67 ppm), and Solid Fraction I[2] (see Figure 1). Results are summarized in Table 4. The Aqueous I fraction was analyzed by HPLC using a Biorad HDX 72S organic base column; recovery from the column was only 76.9%, or 54.2% TRR (the registrant contends that the material retained in the column consists of radiolabeled proteins--see discussion of this contention in the following paragraph). The HPLC analysis of the material which eluted from the column revealed four major peaks, identified as Unknown A1 (24.1% TRR, 6.07 ppm), acetamide (4.0% TRR, 1.00 ppm), acetonitrile (10.1% TRR, 2.54 ppm), and acetic acid (4.0% TRR, 1.00 ppm). The identity of Unknown A1 seen in this analysis was investigated by enzymatic treatment of an aliquot of that portion of the Aqueous I water extract which eluted from the column: although no sample chromatograms were provided, the registrant stated that enzymatic treatment of an aliquot of this aqueous fraction with glusulase, sulfatase, β-glucosidase, and β-glucuronidase showed no chromatographic profile changes,

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[1] This is true if one assumes that the missing 50% of TRR is respired as volatiles.

[2] A total of two goats were used in the study. For simplicity and ease of review, the text of this review reports only the concentrations and percent TRRs for Goat #4. Results for the second goat (Goat #5) are generally comparable and are presented in the tables instead.

indicating that the unknown is not a conjugate that these enzymes would cleave. The identity of the unknown was also investigated by base hydrolysis of the Aqueous I extract: aliquots of the Aqueous I extract were concentrated to dryness by rotoevaporation, with the residues reconstituted in 1 mL of deionized water and sonicated. Addition of 0.5 mL of a 50% NaOH solution to this reconstituted aliquot from the Aqueous I extract converted the unknown to acetic acid and acetonitrile *[the registrant used the results from this base hydrolysis to classify the unknown as a mixture of acetic acid and acetonitrile. In general, hydrolysis with highly concentrated acid or base solutions can release moieties as their final hydrolysis products which may have only a minor relationship to the conjugated form of radioactivity. CBRS believes that the extreme nature of this hydrolysis reaction does not permit this assertion, and CBRS will consider the unknown to be unidentified].*

Since it was suspected that protein binding to the column was the reason for the significant amount of radioactivity bound to the column (see above), proteins were precipitated out of an aliquot of the original aqueous extract by the addition of ethanol. After vortexing and chilling to facilitate protein precipitation, the extracts were centrifuged, with the resulting supernatants analyzed by HPLC with recovery of radioactivity measured as 111%: the registrant contends that since recovery from the HPLC column <u>following</u> protein precipitation was significantly greater than the recovery seen in the extract <u>prior</u> to protein precipitation (111% vs. 76.9%--see above), this is evidence of binding of radiolabeled protein material to the column, which in turn indicates that thiodicarb in rapidly metabolized and that the metabolism proceeds via natural product pathways incorporating radiolabel into proteins. CBRS does <u>not</u> accept the registrant's contention that poor recovery from an HPLC column is evidence of radiolabeled proteins, and will not attribute this portion of radioactivity to protein incorporated residue. As indicated in Table 4, CBRS will instead classify the material which remained bound in the HPLC column following the original aqueous extract as "column-retained material." *[CBRS notes that even if this were in actuality a protein fraction, the radioactivity does not necessarily consist of radioactive amino acids. The radioactivity may consist of biological macromolecules having radioactive portions of the pesticide chemically conjugated <u>onto</u> them which is distinct from having the macromolecules constructed <u>from</u> them]*

The solids from the above-described protein precipitation were sonicated and hydrolyzed with concentrated sodium hydroxide for 21 hours at room temperature; HPLC analysis of the extract following neutralization showed two radioactive regions of interest, acetonitrile and acetic acid. The registrant thus classified these proteins as a mixture of acetonitrile and acetic acid *[CBRS is unsure of the reason for the registrant's reclassification of these precipitated proteins (?) to acetic acid and acetonitrile. If the precipitate is indeed composed of proteins (of which CBRS remains unconvinced), then base hydrolysis to acetonitrile and acetic acid would be unnecessary].* Given the extreme conditions under which base hydrolysis was performed, CBRS does not believe the registrant's contention that the acetonitrile and

acetic acid detected in the base-hydrolyzed protein extracts can be attributed to *in-vivo* metabolites, but instead believes that the detected acetonitrile and acetic acid may be due to *in vitro* reactions with the concentrated sodium hydroxide solution. CBRS will not consider the results of this base-hydrolyzed (protein?) precipitate analysis as sufficient evidence for the presence of acetonitrile and acetic acid metabolites.

The original solid fraction (Solid I) was blended with 5 mL MeOH, homogenized, and centrifuged in order to separate a methanol fraction (MeOH I: 6.0% TRR, 1.52 ppm) and a solid fraction (Solid II). Direct analysis of MeOH I was not possible due to the low levels of radioactivity and incompatibility of the organic solvent with the organic base column. Rotoevaporation of this MeOH extract to dryness resulted in an average loss of 38.9% of the radioactivity associated with the MeOH fraction, or 2.3% of the TRR (0.58 ppm). The registrant attributed this loss to acetonitrile *[CBRS does not believe that the registrant has provided sufficient evidence to accurately attribute this lost radioactivity to acetonitrile for the following reasons: (i) the registrant cannot assign a component an acetonitrile identity based solely on its volatilization during rotary evaporation; and (ii) any acetonitrile would be expected to have been partitioned previously into the water fraction following the initial fractionation into aqueous and solid fractions, i.e., no acetonitrile should have been present in the Solid I fraction from which the MeOH I fraction was originally derived).* The dried methanol extract was reconstituted in $(NH_4)_2SO_4$ and HPLC analysis of this reconstituted fraction revealed 3 radioactive regions of interest: the major region was Unknown A2 (identified by the registrant as the same unknown seen in the water extracts (i.e., A1)), with acetamide and acetic acid also detected. The registrant did not provide any information regarding the ppm or % TRR values associated with these three regions of interest, nor were any chromatograms provided. Instead, the registrant treated the reconstituted MeOH extracts with concentrated base, and only then used HPLC to identify and measure the base-hydrolyzed products (identified by the registrant as acetic acid and acetamide). As stated earlier, CBRS will not accept this identification since the extreme nature of the hydrolysis reaction is of questionable validity. Instead, CBRS has classified the non-volatilized component of the original MeOH I fraction (i.e., that fraction which the registrant reconstituted with ammonium sulfate, treated with concentrated base, and analyzed by HPLC) as "non-volatiles" (3.7% TRR, 0.92 ppm)(see Table 4).

The Solid II fraction was washed with water (resulting water wash: 0.4% TRR, 0.11 ppm)[3] resulting in a Solid III fraction (20.8% TRR, 5.24 ppm)[4].

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[3] To maintain mass balance, these water wash results were incorporated into the calculations for the Aqueous I fraction.

[4] In all cases, the registrant did not directly measure the TRR of the Solid III fraction. Instead, these values were obtained by the reviewer by summing the contributions from the subsequent fractionation into protease (supernatant) and Solids.

Unextracted residues remaining in the Solids III were solubilized by subjecting this fraction to protease enzymatic treatment. Two fractions, a protease supernatant (Protease I: 15.6% TRR, 3.94 ppm) and a solid fraction (Solids IV: 5.2% TRR, 1.30 ppm) resulted. HPLC analysis of the protease supernatant showed the majority of radioactivity eluting from the column (Unknown A2: 6.9% TRR, 1.7 ppm) at the same retention time as the unknown previously seen in the water extracts. A second unknown (Unknown B: 0.9% TRR, 0.23 ppm) as well as acetic acid (0.5% TRR, 0.18 ppm) were also detected. Base hydrolysis of this protease extract with concentrated NaOH resulted in an average loss of 19.5% of the radioactivity associated with the protease fractions which the registrant attributed to acetonitrile (as stated earlier, CBRS does not believe that volatilization of radioactive residues following treatment with concentrated base is a valid means of identification, and will not attribute this loss to acetonitrile). HPLC analysis of the base-hydrolyzed extract showed hydrolysis of the above unknown to acetic acid. As described earlier, CBRS does not believe that the extreme conditions under which base hydrolysis was performed are appropriate for identification purposes.

A summary of the results of this extraction scheme are presented in Table 4.

CBRS will require that the registrant perform additional analysis to further characterize/identify Unknown A1 and the Column-Retained Material, both of which were present in the original Aqueous I fraction. Unknown A1 comprises approximately 24-31% of the TRR (ca. 6 ppm) associated with the liver fraction, while the Column-Retained Material comprises approximately 16-17% of the TRR (ca. 4 ppm).

Kidney: A 5 g kidney sample was treated in a manner similar to that of the liver sample described above (see Figure 1). The results are summarized in Table 5. The sample was extracted with water, resulting in an Aqueous I Fraction (81.2% TRR, 10.23 ppm), and a Solid I Fraction. The Aqueous I fraction was analyzed by HPLC using an organic base column which revealed four major peaks; these were identified as Unknown C (12.2% TRR, 1.54 ppm), acetamide (6.7% TRR, 0.85 ppm), acetonitrile (10.8% TRR, 1.36 ppm), and acetic acid (6.3% TRR, 0.80 ppm) Recovery from the column was only 68.0% (or 55.5% of TRR), with the registrant contending that the material retaining in the column consisted of radiolabeled proteins (see further discussion of this contention in the following paragraph). The identity of the unknown seen in the analysis performed above was investigated by enzymatic treatment of an aliquot of the Aqueous I water extract: although no sample chromatograms were provided, the registrant stated that enzymatic treatment of an aliquot of this aqueous fraction with glusulase, sulfatase, β-glucosidase, and β-glucuronidase showed no chromatographic profile changes, indicating that the unknown is not a conjugate that these enzymes would cleave. The identity of the unknown was also investigated by base hydrolysis of a second aliquot of the Aqueous I extract: addition of a concentrated NaOH solution to an aliquot of the Aqueous I extract converted the unknown to acetic acid *[as with the unknown from the liver extract, the registrant used the results from*

this base hydrolysis to classify the unknown as acetic acid: CBRS believes that the extreme nature of this hydrolysis reaction does not permit this assertion, and CBRS will consider the unknown to be unidentified].

Since, as in the case of liver, it was suspected that protein binding to the column was the reason for the significant amount of radioactivity remaining in the column, proteins were precipitated out of the water extracts by the addition of ethanol in the manner described above for liver with the resulting extract analyzed by HPLC: the registrant contends that since recovery from the HPLC column following protein precipitation was significantly greater than the recovery seen in the extract prior to protein precipitation (81.7% vs. 68.0%), this is evidence of protein or peptide binding to the column and indicates metabolism via natural product pathways and incorporation of the radiolabel into proteins. As in the case for liver, CBRS does not accept the registrant's contention that poor recovery from an HPLC column is evidence of radiolabeled proteins, and will not attribute this portion of radioactivity to protein-incorporated residues. As indicated in Table 5, CBRS will classify the material which remained bound to the HPLC column following the original aqueous extraction as "column-retained material".

The solids from the above-described protein precipitation were hydrolyzed with concentrated sodium hydroxide as was done with the liver extracts; HPLC analysis of the extract showed two radioactive regions of interest, corresponding to acetonitrile and acetic acid. The registrant thus classified these proteins as acetonitrile and acetic acid. Given the extreme conditions under which base hydrolysis was performed, CBRS does not believe that characterization/ identification procedure followed for identification of acetonitrile and acetic acid following protein precipitation is appropriate (see discussion under liver), and will not consider the results of the base-hydrolyzed (protein?) precipitation analysis as sufficient evidence for the presence of acetonitrile and acetic acid metabolites.

The original Solid I fraction was blended with 5 mL MeOH, homogenized, and centrifuged in order to separate a MeOH fraction (MeOH I: 3.3% TRR, 0.42 ppm) and a solid fraction (Solid II). Direct analysis of the MeOH I fraction was not possible for the reasons stated earlier. Rotoevaporation to dryness of aliquots from this MeOH extract resulted in an average loss of 29.8% of the radioactivity associated with the MeOH fraction, or 1.0% of the TRR (0.13 ppm). The registrant attributed this loss to acetonitrile. For the reasons stated above, CBRS does not believe that this loss can be attributed to acetonitrile, and will instead attribute this loss to "volatile components" (see Table 5). The dried MeOH extract was reconstituted in ammonium sulfate and analyzed by HPLC which, according to the registrant, showed no conclusive results due to low levels of radioactivity. After concentrated base treatment and neutralization of the extract, HPLC analysis showed two regions of interest, an unknown and acetic acid. As stated earlier, CBRS will not accept this identification due to the extreme

nature of the hydrolysis. Instead, CBRS has classified the non-volatile components of the original MeOH I fraction as "non-volatiles" (2.3% TRR, 0.29 ppm) (see Table 5).

The Solid II fraction was washed with water (resulting water wash: 0.4% TRR, 0.05 ppm) resulting in a Solid III fraction (7.5% TRR, 0.93 ppm).

Unextracted residues remaining in Solid III were further solubilized by subjecting this fraction to protease enzymatic treatment. Two fractions, a protease supernatant (Protease I: 6.6% TRR, 0.84 ppm) and a Solid IV fraction (0.9% TRR, 0.11 ppm) resulted. HPLC analysis of the protease supernatant showed the majority of radioactivity eluting as an unknown from the column although no chromatogram or other ancillary data was supplied. Base hydrolysis of this protease extract resulted in an average loss of 12.7% of the radioactivity associated with the protease fractions (or 0.8% of the TRR) which the registrant attributed to acetonitrile. As stated earlier, CBRS does not believe this to be a valid assignment given the extreme nature of the base hydrolysis. HPLC analysis of the base-hydrolyzed protease extract showed that the concentrated base solution had hydrolyzed the above unknown to acetic acid. CBRS does not believe that the extreme conditions under which base hydrolysis was performed are valid for characterization/identification purposes. In accordance with this belief, CBRS will not attribute any of the registrant designations to the Solids III fraction.

A summary of the results of this extraction scheme are presented in Table 5.

CBRS will require that the registrant perform additional analysis to further characterize/identify Unknown C and the Column-Retained Material, both of which were present in the original Aqueous I fraction. Unknown C comprises approximately 12% of the TRR (ca. 1.9 ppm) associated with the kidney fraction, while the Column-Retained Material comprises approximately 26-27% of the TRR (ca. 3-4 ppm).

Muscle: A 5 g muscle sample was treated in a manner similar to that of the liver and kidney sample described above (see Figure 1). Results are summarized in Table 6. The sample was extracted with water, resulting in an Aqueous I Fraction (91.6% TRR, 3.97 ppm) and a solid fraction (Solid I). The Aqueous I Fraction was analyzed by HPLC which revealed three major peaks; these were identified as Unknown D (6.1% TRR, 0.27 ppm), acetamide (7.51% TRR, 0.33 ppm), and acetonitrile (36.0% TRR, 1.56 ppm). No acetic acid was detected. Because recovery from the column was 104.2%, protein binding to the column was not considered a problem and the protein precipitation procedure carried out for liver and kidney was not deemed necessary. The identity of Unknown D was investigated by base hydrolysis of an aliquot of the water extract with a concentrated NaOH solution: this showed conversion of the unknown to acetic acid *[As before, CBRS believes that the extreme nature of this hydrolysis*

reaction, does not permit this assertion, and CBRS will consider the identity of Unknown D to remain unknown].

The remaining solid fraction (Solid I) was blended with 5 mL MeOH, homogenized, and centrifuged in order to separate a MeOH I fraction (5.3% TRR, 0.23 ppm) and a Solid II fraction. Direct analysis of the MeOH fraction was not possible for the reasons stated earlier. Rotoevaporation of aliquots from this MeOH extract resulted in an average loss of 59.5% of the radioactivity associated with the MeOH fraction (or 3.2% of the TRR). The registrant attributed this loss to acetonitrile. For the reasons stated above, CBRS will not attribute this loss to acetonitrile. HPLC analysis of the reconstituted ^{14}C residue from the rotary-evaporated MeOH extract showed four major peaks including two unknowns (0.1% TRR and 0.3% TRR, ca. 0.01 ppm each), acetonitrile (0.3% TRR, 0.01 ppm) and acetic acid (0.3% TRR, 0.01 ppm).

The Solid II fraction was washed with water (resulting water wash: 0.6% TRR, 0.03 ppm) resulting in a solid fraction which was designated as Solids III (7.5% TRR, 0.93 ppm).

Unextracted residues remaining in the Solids III fraction were further solubilized by subjecting this fraction to protease enzymatic treatment. Two fractions, a protease supernatant (10.8% TRR, 0.47 ppm) and a Solid IV fraction (1.2% TRR, 0.05 ppm) resulted. Direct HPLC analysis of the protease supernatant extract did not yield any conclusive data due to low levels of radioactivity. Base hydrolysis of this protease extract resulted in an average loss of 17.6% of the radioactivity associated with the protease fractions which the registrant attributed to acetonitrile. As stated earlier, CBRS does not believe this to be a valid assignment and will designate the fractions simply as Protease I and Solids IV. According to the registrant, HPLC analysis of the base-hydrolyzed protease extract did not yield any conclusive data due to low levels of radioactivity.

A summary of the results of this extraction scheme are presented in Table 6.

CBRS judges that the nature of the residue in ruminant muscle is adequately characterized/identified, and will not require further analytical work on this matrix.

Milk: Approximately 5 g aliquots of milk (from the 8MK milk sample containing 14.5 ppm thiodicarb equivalents) were blended with acetonitrile, shaken gently, and centrifuged resulting in an ACN I (27.0% TRR, 3.9 ppm) and a Solid I fraction (see Figure 2). Results are summarized in Table 7. The ACN I supernatant fraction was then partitioned with hexane and centrifuged in order to separate the non-aqueous soluble compounds (e.g., fats) in the resulting Hexane I fraction from the aqueous-soluble compounds in the resulting ACN II fraction. The ACN II fraction was then analyzed by HPLC: only ACN (10.2% TRR, 1.48 ppm) was detected.[5]

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[5] This is a reviewer-calculated value from the HPLC data. The registrant chose not to calculate the ACN concentration from the HPLC data, but instead chose to calculate the concentration by performing rotary evaporation: the acetonitrile fraction was concentrated to

The Solids I fraction from the original centrifugation was re-extracted with hexane, with the resulting Hexane II fraction centrifuged and subsequently combined with the hexane fractions from the original hexane/acetonitrile partition (i.e., Hexane I). The combined Hexane III fraction represented 37.5% (5.4 ppm) of the milk TRR. This fraction was evaporated to dryness by rotoevaporation and the residues saponified (i.e., reconstituted in 75 mL of methanolic potassium hydroxide solution which was refluxed for approximately 4 hours). After cooling to room temperature, the resulting KOH I fraction was partitioned with hexane, yielding Hexane IV (0.3% TRR, 0.04 ppm) and KOH II. The KOH II fraction was acidified and again partitioned with hexane. Aliquots of the resulting Hexane V fraction (18.0% TRR, 2.61 ppm) and post-acidification KOH III fraction (14.4% TRR, 2.09 ppm) were removed for radioassay and analysis. The registrant characterized the Hexane IV, Hexane V, and KOH III fractions as containing non-saponifiable lipids, free fatty acids, and other saponifiable lipids, respectively. While CBRS agrees that non-saponifiable lipids and saponifiable lipids would partition in accordance with the registrant's classification scheme (i.e., into the Hexane IV and Hexane V/KOH III fractions, respectively), CBRS does not consider this to be adequate identification.

The Solids III fraction was rinsed with deionized water to remove any organics that might affect enzyme activity and treated with protease to liberate bound ^{14}C residues. These water wash fractions were analyzed by TLC and HPLC: HPLC revealed only one radioactive region of interest which had the retention time of lactose and TLC analysis showed the region to co-migrate with a radiolabeled lactose standard confirming the presence of lactose at 2.3% TRR (0.34 ppm). Protease treatment of Solids III liberated 7.3% of the TRR (1.04 ppm), leaving 1% (0.4 ppm) bound to the solids in the Solid IV fraction. HPLC analysis of the Protease I supernatant identified lactose (2.7% TRR, 0.40 ppm) and ACN (0.8% TRR, 0.11 ppm).

A summary of the results from this extraction scheme are presented in Table 7.

The registrant did not perform adequate characterization of the Hexane V (loosely considered to contain fatty acids and other hexane soluble acids/alcohols) and KOH III fractions (loosely considered to contain non-ionizable acids/alcohols or "other saponifiable lipids"). These fractions contain approximately 18% (ca. 2.6 ppm) and 14% (ca. 2.0 ppm) of the TRR associated with milk. CBRS does not believe the saponification procedure followed provides sufficient evidence for characterization of the residues as saponifiable lipids and will require the registrant to perform additional confirmatory characterization/identification procedures.

Fat: Approximately 10 g of fat (containing ca. 1.37 ppm TRR) were blended with hexane and homogenized with a Polytron® homogenizer (see Figure 3). The results of

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near dryness by rotoevaporation, with 75.9% of the radioactivity associated with this fraction lost. This loss was attributed by the registrant to acetonitrile. It is unclear to CBRS why the registrant chose to attribute only this (volatilized) fraction to acetonitrile when HPLC analysis performed prior to rotary evaporation revealed by direct measurement an ACN concentration at 10.2% TRR (1.48 ppm).

these extractions are shown in Table 8. The samples were centrifuged, with the hexane supernatants (Hexane I) removed and partitioned with acetonitrile (ACN I).

The Hexane I fraction was reduced to dryness by rotoevaporation (yielding Hexane II) with the residues reconstituted in methanolic KOH and refluxed for four hours (yielding KOH I). The KOH I fraction was then saponified as done with the milk sample. This resulted in Hexane III (1.1% TRR, 0.01 ppm), Hexane IV (40.7% TRR, 0.56 ppm), and KOH III (17.8% TRR, 0.24 ppm). The registrant characterized these three latter fractions as non-saponifiable lipids, free fatty acids, and other saponifiable lipids, respectively. As in the case for milk, CBRS does not believe that the procedural methodology followed (i.e., saponification followed by repeated sequential partitioning) permits this degree of characterization, and will require that additional characterization/identification be performed with the Hexane IV (40.7% TRR, 0.56 ppm) and KOH III (17.8% TRR, 0.24 ppm) fractions.

After the hexane extraction, acetonitrile was added to the milk solids from the original centrifugation (i.e., Solids I). The samples were polytroned, centrifuged, and combined with ACN from the original ACN partitions (i.e., ACN I) to yield an ACN III fraction. Direct HPLC analysis of this combined acetonitrile extract was not possible due to the low levels of radioactivity. It was thus evaporated to dryness, and reconstituted in ACN to be analyzed by TLC/HPLC. Average losses upon concentration were 62.1% of the radioactivity associated with this fraction (or 9.9% of the TRR) which the registrant attributed to acetonitrile. For the reasons explained previously, CBRS will attribute this loss simply to "volatile components" (see Table 8). HPLC analysis of the concentrated ACN extract showed only acetamide, which analysis by TLC confirmed; the registrant acknowledges that this may be due to hydrolysis of acetonitrile to acetamide. CBRS will classify that portion of the original ACN fraction which did not evaporate (i.e, 37.9% of the radioactivity associated with the fraction, or 6.0% of the TRR) as "non-volatile components" (see Table 8).

The post extracted fat solids were taken through a saponification procedure identical to that followed for the hexane extracts (i.e., reflux with KOH, partition with hexane, acidify, etc.). The Hexane VI fraction represented 5.0% TRR (0.07 ppm), KOH VI represented 9.3% TRR (0.13 ppm), and Hexane V represented 0.2% (<0.01 ppm). The registrant characterized these three latter fractions as free fatty acids, other saponifiable lipids, and non-saponifiable lipids, respectively. Although CBRS does not believe that the procedural methodology followed (i.e., saponification followed by repeated sequential partitioning) permits this degree of characterization or is satisfactory evidence for incorporation into lipids, CBRS will not require that additional characterization/identification be performed since radioactive residue levels represent less than 10% of the TRR.

A summary of the results from this extraction scheme are presented in Table 7.

The registrant did not perform adequate characterization of the Hexane IV (loosely considered to contain fatty acids and other hexane soluble acids/alcohols) and KOH III fractions (loosely considered to contain non-ionizable acids/alcohols or "other saponifiable lipids"). These fractions contain approximately 40% (ca. 0.6 ppm) and

40% (ca. 0.6 ppm) of the TRR associated with fat. CBRS does not believe the saponification procedure followed provides sufficient evidence for characterization of the residues as saponifiable lipids and will require the registrant to perform additional confirmatory characterization/identification procedures.

Feces: Extraction of the feces from the day 3 a.m. sampling interval with methanol released 81.1% of the TRR. HPLC analyses of these methanol extracts showed the presence of three radioactive regions of interest: an unknown with a retention time of 2.5 minutes (3.1% TRR, 0.47 ppm), methomyl (8.64% TRR, 1.31 ppm), and thiodicarb (2.7% TRR, 0.33 ppm). TLC analysis confirmed the presence of thiodicarb and methomyl. Extraction of feces from the day 8 a.m. sampling interval showed similar results, with the presence of an unknown with the same retention time, as well as methomyl and thiodicarb also confirmed by TLC.

Urine: Urine samples from two different time points (Day 4 p.m and Day 7 a.m.) were analyzed directly by TLC and HPLC. HPLC analysis of urine from the Day 7 a.m. sampling interval showed all off the radioactivity eluting off the column in the void volume of a Zorbax C8 column, thereby suggesting that thiodicarb and related primary metabolites were not present since they would have been retained on the column. The same urine sample was analyzed by HPLC using an organic acid column which showed the presence of an unknown with a retention time of 4m36s, acetonitrile, and acetamide. These findings were confirmed by TLC analysis of this extract.

Stomach Contents: The stomach contents contained 21.5 ppm thiodicarb equivalents. Methanol extraction of the stomach contents released 62.0% of the TRR. Thiodicarb, methomyl, and acetamide were detected.

Summary of Mass Balance and Identified Thiodicarb Residue in Tissues

Tables 9 and 10 provide a summary of the identified, unknown, unidentified, lost, and unaccounted thiodicarb residues. While for each organ/tissue, it can be seen that an acceptable percentage of the mass was accounted for, insufficient characterization/identification of residues in liver, kidney, milk, and fat was performed. These tissues had unacceptably high concentrations of either specific unknowns which were identified by HPLC or unidentified residues which were insufficiently characterized.

RDI: FSuhre:2/9/94;MMetzger:2/16/94;EZager:2/17/94.
cc: RF, SF, List B File, Circ., DJM.
EF: 2/28/94.

Table 4. Summary of Distribution and Identity of Thiodicarb Metabolites in Liver from Lactating Goats Dosed with Radiolabeled Thiodicarb at ca. 208 and 292 ppm in the Diet for Seven Days.

A. Distribution of Radioactivity Into Extractable and Non-Extractable Fractions in the Liver (TRR=25.2 and 23.0 ppm for Goat #4 and Goat #5, respectively)

Extract	[^{14}C]Thiodicarb Concentration			
	Extractable		Non-extractable	
	TRR, % Radioactive Dose	TRR, ppm ^{14}C thiodicarb equivalents	TRR, % Radioactive Dose	TRR, ppm ^{14}C thiodicarb equivalents
Aqueous[a]	70.5% (73.6%)	17.78 (16.94)	--	--
Organic	6.0% (6.0%)	1.52 (1.37)	--	--
Post Extraction Solids	--	--	20.8% (19.8%)	5.24 (4.55)

B. Summary of Distribution of Radiolabeled Thiodicarb Among Extracts and Resulting TLC/HPLC Identification of Constituents

Extract (% TRR)[c]	Radiolabeled Thiodicarb			
	HPLC/TLC Analysis		Fraction ^{14}C in Sample	
	Metabolite	% of Run	%	ppm
Aqueous I (70.5%/73.6%)[b]				
Column-Eluted (54.2%/56.6%)	Acetamide	7.3% (11.1%)	4.0% (6.3%)	1.00 (1.44)
	ACN	18.6% (19.2%)	10.1% (10.9%)	2.54 (2.50)
	HOAc	7.3% (6.4%)	4.0% (3.6%)	1.00 (0.83)
	Unknown A1[e]	44.4% (35.4%)	24.1% (26.1%)	6.07 (5.99)
Column-Retained (16.3%/17.0%)	Column-Retained Material[j]	--	16.3% (17.0%)	4.08 (3.90)
MeOH I (6.0%/6.0%)				
Volatilized Components (2.3%)	Volatiles[g]	--	2.3% (--)[i]	0.58 (--)[i]
Non-Volatilized Components (3.7%)	Non-volatiles[d]	--	3.7% (--)[i]	0.93 (--)[i]
Solids III (20.8%/19.8%)				
Protease I[h] (15.6%/15.3%)	Unknown A2	44.2% (50.9%)	6.9% (7.79%)	1.74 (1.79)
	Unknown B	5.8% (5.0%)	0.90% (0.76%)	0.23 (0.18)
	Acetic Acid	3.0% (4.6%)	0.47% (0.70%)	0.18 (0.16)

Table 4.	Summary of Distribution and Identity of Thiodicarb Metabolites in Liver from Lactating Goats Dosed with Radiolabeled Thiodicarb at ca. 208 and 292 ppm in the Diet for Seven Days.			
Solids IV (5.2%/4.5%)		not further analyzed	5.2% (4.5%)	1.30 (1.04)

[a] The registrant attempted to identify this unknown by enzyme hydrolysis which did not cleave the molecule. The identity of the unknown was also investigated by base hydrolysis: the registrant hydrolyzed this unknown to HOAc and ACN and classified the unknown as a mixture of acetic acid and acetonitrile. As described in the text of this review, CBRS does not feel this identification technique is appropriate and has classified this component as an unknown.

[b] While 70.1% of the TRR was present in the Aqueous I fraction, column recovery was only 76.9% (meaning that only 54.2% of the TRR was eluted from the column). The calculations presented in this table adjust for this recovery and include the unrecovered material under the category "Column-retained material."

[c] These are provided for both goats in the form of Goat#4/Goat#5

[d] According to the registrant, HPLC analysis of the reconstituted non-volatile components revealed the presence of three radioactive regions of interest: the major region was an unknown (same unknown as seen as Aqueous I column-eluted material), with acetamide and acetic acid also detected at presumably lower levels. The registrant did not provide any data or chromatographs from which concentrations of these three compounds could be calculated. Instead, the registrant performed a concentrated base hydrolysis on this extract and identified acetamide (0.8% TRR, 0.20 ppm) and acetic acid (2.9% TRR, 0.73 ppm). As discussed in the text, CBRS does not feel that this is an appropriate means of identification, and is thus forced to simply classify the material as "non-volatile".

[e] The TRR values include the TRR from the water wash of solids (0.4% TRR and 0.6% TRR for Goat #4 and Goat #5, respectively).

[f] The registrant attributed this column-bound material to proteins, concluding that thiodicarb is rapidly metabolized and that metabolism proceeds via natural product pathways incorporating radiolabel into proteins. CBRS chooses instead to classify this as "column-retained material"

[g] The registrant attributed this to ACN, but provided no data in support of this assignment.

[h] Subsequent to the HPLC analysis of the protease extract in which acetic acid and two unknowns were identified, the registrant performed a base hydrolysis of the fraction, resulting in a loss of 19.5% of the radioactivity associated with the protease fraction. The registrant attributed this to ACN, but provided no additional information or support. HPLC analysis of the resulting extract showed that the unknowns were hydrolyzed to acetic acid, from which the registrant concluded that acetic acid represented 12.6% (3.18 ppm) of the TRR. As described earlier, CBRS does not believe that the extreme conditions under which base hydrolysis was performed are valid for identification purposes.

[i] This was not performed with the extracts from Goat #5.

[j] The registrant attributed this bound material to proteins, concluding that thiodicarb is rapidly metabolized via natural product pathways, with thiodicarb incorporated into proteins. CBRS chooses instead to classify this material as "column-retained."

Table 5. Summary of Distribution and Identity of Thiodicarb Metabolites in Kidney from Lactating Goats Dosed with Radiolabeled Thiodicarb at ca. 208 and 292 ppm in the Diet for Seven Days.

A. Distribution of Radioactivity Into Extractable and Non-Extractable Fractions in the Kidney (TRR= 12.6 ppm and 13.9 ppm for Goat #4 and Goat #5, respectively)

Extract	[^{14}C]Thiodicarb Concentration			
	Extractable		Non-extractable	
	TRR, % Radioactive Dose	TRR, ppm ^{14}C thiodicarb equivalents	TRR, % Radioactive Dose	TRR, ppm ^{14}C thiodicarb equivalents
Aqueous[a]	81.6% (84.1%)	10.23 (11.69)	--	--
Organic	3.3% (4.9%)	0.42 (0.68)	--	--
Post Extraction Solids	--	--	7.5% (9.2%)	0.93 (1.28)

B. Summary of Distribution of Radiolabeled Thiodicarb Among Extracts and Resulting TLC/HPLC Identification of Constituents

Extract (% TRR)[a]	Radiolabeled Thiodicarb			
	HPLC/TLC Analysis		Fraction ^{14}C in Sample	
	Metabolite	% of Run	%	ppm
Aqueous I (81.6%/84.1%)[d]				
Column Eluted (55.5%/57.2%)	Unknown C[b]	22.0% (24.1%)	12.2% (13.8%)	1.54 (1.92)
	Acetamide	12.1% (14.8%)	6.7% (8.5%)	0.85 (1.18)
	ACN	19.5% (25.0%)	10.8% (14.3%)	1.36 (1.99)
	HOAc	11.4% (6.2%)	6.3% (3.6%)	0.80 (0.49)
Column-Retained (26.1%/26.9%)	Column-Retained Material[h]		26.1% (26.9%)	3.29 (3.73)
MeOH I (3.3%/4.9%)				
Volatilized Components (1.0%)	Volatiles[g]	--	1.0% (--)[f]	0.13 (--)[f]
Non-Volatilized Components (2.3%)	Non-volatiles	--	2.3% (--)[f]	0.29 (--)[f]
Solids III (7.5%/9.2%)				
Protease I (6.6%/7.5%)			6.6% (7.5%)[e]	0.83 (1.04)[e]
Solids IV (0.9%/1.7%)			0.9% (1.7%)	0.11 (0.24)

[a] Includes TRR from water wash of solids

[b] The registrant attempted to identify this unknown by enzyme hydrolysis, which did not cleave the molecule. The identity of the unknown was also investigated by base hydrolysis with concentrated NaOH solution, resulting in conversion of the unknown to acetic acid. Given the extreme nature of the hydrolysis reaction, CBRS will continue to consider the identity of Unknown C to remain unknown.

[c] The registrant indicated that an unknown accounted for "the majority" of radioactivity eluting from the column, but no HPLC chromatograms were provided.

[d] While 81.2% of the TRR was present in the Aqueous I fraction, only 68.0% was recovered from the column. The calculations presented in this table adjust for this recovery and include the unrecovered material under the category "Column-retained material."

[e] These are provided for both goats in the form of Goat#4/Goat#5

[f] This was not performed with the extract from Goat #5.

[g] The registrant attributed this loss to ACN. CBRS will attribute this loss instead to "volatile components".

[h] The registrant attributed this bound material to proteins, concluding that thiodicarb is rapidly metabolized via natural product pathways, with thiodicarb incorporated into proteins. CBRS chooses instead to classify this material as "column-retained" material.

Table 6. Summary of Distribution and Identity of Thiodicarb Metabolites in Muscle from Lactating Goats Dosed with Radiolabeled Thiodicarb at ca. 208 and 292 ppm in the Diet for Seven Days.

A. Distribution of Radioactivity Into Extractable and Non-Extractable Fractions in Muscle (TRR= 4.34 ppm and 4.24 ppm for Goat #4 and Goat #5, respectively)

Extract	[^{14}C]Thiodicarb Concentration			
	Extractable		Non-extractable	
	TRR, % Radioactive Dose	TRR, ppm ^{14}C thiodicarb equivalents	TRR, % Radioactive Dose	TRR, ppm ^{14}C thiodicarb equivalents
Aqueous[a]	91.6% (77.3%)	3.97 (3.28)	--	--
Organic	5.3% (4.2%)	0.23 (0.18)	--	--
Post Extraction Solids	--	--	12.0% (7.4%)	0.52 (0.31)

B. Summary of Distribution of Radiolabeled Thiodicarb Among Extracts and Resulting TLC/HPLC Identification of Constituents

Extract (% TRR)[c]	Radiolabeled Thiodicarb			
	HPLC/TLC Analysis		Fraction ^{14}C in Sample	
	Metabolite	% of Run	%	ppm
Aqueous I (91.6%/77.3%)	Unknown D[b]	6.7% (7.8%)	6.1% (6.0%)	0.27 (0.26)
	Acetamide	8.2% (17.6%)	7.5% (13.6%)	0.32 (0.58)
	ACN	39.3% (36.4%)	36.0% (28.1%)	1.56 (1.19)
MeOH I (5.3%/4.2%)				
Volatilized Components (3.2%)	Volatiles[d]	--	3.2% (--%)[e]	0.14 (--)[e]
Non-Volatilized Components (2.1%)	Unknown E	6.4% (--%)[e]	0.1% (--%)[e]	0.006 (--)[e]
	Acetamide	5.9% (--%)[e]	0.1% (--%)[e]	0.005 (--)[e]
	ACN	12.5% (--%)[e]	0.3% (--%)[e]	0.011 (--)[e]
	Unknown F	12.1% (--)[e]	0.3% (--)[e]	0.011 (--)[e]
Solids III (12.0%/7.1%)				
Protease I (10.8%/6.2%)	--	--	10.8% (6.2%)	0.47 (0.26)
Solids IV (1.2%/1.2%)	--	--	1.2% (1.2%)	0.05 (0.05)

[a] Includes TRR from water wash of solids

[b] The registrant attempted to identify this unknown by enzyme hydrolysis, which did not cleave the molecule. The identity of the unknown was also investigated by base hydrolysis with concentrated NaOH solution, resulting in

conversion of the unknown to acetic acid. Given the extreme nature of the hydrolysis reaction, CBRS will continue to consider the identity of Unknown D to remain unknown.

[c] These are provided for both goats in the form of Goat#4/Goat#5

[d] The registrant attributed this loss to ACN

[e] These analysis were not performed for Goat #5.

Table 7. Summary of Distribution and Identity of Thiodicarb Metabolites in Milk from Lactating Goats Dosed with Radiolabeled Thiodicarb at ca. 208 and 292 ppm in the Diet for Seven Days.

A. Distribution of Radioactivity Into Extractable and Non-Extractable Fractions in Milk (TRR= 14.5 ppm and 9.8 ppm for Goat #4 and Goat #5, respectively)

Extract	[^{14}C]Thiodicarb Concentration			
	Extractable		Non-extractable	
	TRR, % Radioactive Dose	TRR, ppm ^{14}C thiodicarb equivalents	TRR, % Radioactive Dose	TRR, ppm ^{14}C thiodicarb equivalents
ACN	32.6% (32.4%)	4.65 (3.17)	--	--
Organic	37.5% (20.2%)	5.44 (1.98)	--	--
Post Extraction Solids	--	--	8.3% (18.4%)	1.18 (1.80)

B. Summary of Distribution of Radiolabeled Thiodicarb Among Extracts and Resulting TLC/HPLC Identification of Constituents

Extract (% TRR)	Radiolabeled Thiodicarb			
	HPLC/TLC Analysis		Fraction ^{14}C in Sample	
	Metabolite	% of Run	%	ppm
ACN (27.0%/25.5%)	ACN	37.9% (28.8%)	10.2%(7.3%)	1.48 (0.72)
	Lactose	ND (9.1%)	ND (2.3%)	ND(0.23)
	Acetamide	ND (8.5%)	ND (2.2%)	ND (0.21)
	HOAc	ND (8.5%)	ND (2.2%)	ND (0.21)
Waterwash (5.6%/6.9%)	Lactose	41.5% (30.6%)	2.3% (2.1%)	0.34 (0.21)
Hexane III (37.5%/20.2%)				
Hexane V (18.0%/8.2%)	hexane-soluble acids/alcohols[a]	--	18.0% (8.2%)	2.61 (0.80)
KOH III (14.4%/9.7%)	hexane insoluble acids/alcohols[a]	--	14.4% (9.7%)	2.09 (0.95)
Hexane IV (0.3%/0.3%)	non-ionizable acids/alcohols[a]	--	0.3% (0.3%)	0.04 (0.03)
Solids III (8.3%/18.4)				
Protease I (7.3%/15.9%)	lactose	37.9% (38.6%)	2.7% (6.1%)	0.40 (0.60)
	ACN	10.9% (14.4%)	0.80% (2.3%)	0.11 (0.22)
Solids IV (1.0%/2.5%)	--	--	1.0% (2.5%)	0.14 (0.24)

[a] The registrant classified hexane soluble acids/alcohols, hexane insoluble acids/alcohols, and non-ionizable acids/alcohols as fatty acids, other saponifiable lipids, and non-saponifiable lipids, respectively. As discussed in the text, CBRS does not believe that the saponification procedure provides adequate evidence of exclusive incorporation of radioactive residues into these biological molecules. Thus, CBRS prefers to operationally define these fractions.

Table 8. Summary of Distribution and Identity of Thiodicarb Metabolites in Fat from Lactating Goats Dosed with Radiolabeled Thiodicarb at ca. 208 and 292 ppm in the Diet for Seven Days.

A. Distribution of Radioactivity Into Extractable and Non-Extractable Fractions in Fat (TRR= 1.37 ppm and 0.45 ppm for Goat #4 and Goat #5, respectively)

Extract	[^{14}C]Thiodicarb Concentration			
	Extractable		Non-extractable	
	TRR, % Radioactive Dose	TRR, ppm ^{14}C thiodicarb equivalents	TRR, % Radioactive Dose	TRR, ppm ^{14}C thiodicarb equivalents
ACN	15.9% (36.5%)	0.22 (0.16)	–	–
Hexane	59.6% (23.7%)	0.81 (0.11)	–	–
Post Extraction Solids	–	–	14.5% (18.2%)	0.20 (0.08)

B. Summary of Distribution of Radiolabeled Thiodicarb Among Extracts and Resulting TLC/HPLC Identification of Constituents

Extract (% TRR)	Radiolabeled Thiodicarb			
	HPLC/TLC Analysis		Fraction ^{14}C in Sample	
	Metabolite	% of Run	%	ppm
ACN I (15.9%/36.5%)				
Volatile Components (9.9%/11.1%)	Volatiles	–	9.9% (11.1%)	0.14(0.05)
Non-volatile Components (6.0%/25.4%)	Non-volatiles	–	6.0% (25.4%)	0.12 (0.11)
Hexane II (59.6%/23.7%)				
Hexane IV (40.7%/12.8%)	hexane soluble acids/ alcohols[a]	–	40.9% (12.8%)	0.56 (0.06)
KOH III (17.8%/10.2%)	hexane insoluble acids/ alcohols[a]	–	17.5% (10.2%)	0.24 (0.05)
Hexane III (1.1%/0.7%)	non-ionizable acids/ alcohols[a]	–	0.7% (0.7%)	0.01 (<0.01)
Non-extractable (14.5%/18.7%)				

Table 8. Summary of Distribution and Identity of Thiodicarb Metabolites in Fat from Lactating Goats Dosed with Radiolabeled Thiodicarb at ca. 208 and 292 ppm in the Diet for Seven Days.				
Hexane VI (9.3%/16.0%)	hexane soluble acids/ alcohols[a]	--	9.3% (16.0%)	0.13 (0.07)
KOH VI (5.0%/2.4%)	hexane insoluble acids/ alcohols[a]	--	5.0% (2.4%)	0.07 (0.01)
Hexane V (0.2%/0.3%)	non-ionizable acids/ alcohols[a]	--	0.2% (0.3%)	<0.01 (<0.01)

[a] The registrant classified hexane soluble acids/alcohols, hexane insoluble acids/alcohols, and non-ionizable acids/alcohols as fatty acids, other saponifiable lipids, and non-saponifiable lipids, respectively. As discussed in the text, CBRS does not believe that the saponification procedure provides adequate evidence of exclusive incorporation of radioactive residues into these biological molecules. Thus, CBRS prefers to operationally define these fractions.

Table 9. Summary of Identified Residues in Liver, Kidney, and Muscle Tissues Following Oral Administration of Radiolabeled Thiodicarb to Lactating Goat #4 at 208 ppm in the Diet for Seven Days.

	Liver		Kidney		Muscle	
Metabolite/Fraction	% TRR	ppm	%TRR	ppm	% TRR	ppm
Identified						
Acetamide	4.0%	1.00	6.7%	0.85	7.6%	0.32
ACN	10.1%	2.54	10.8%	1.36	36.3%	1.57
HOAc	4.5%	1.18	6.3%	0.80	ND	--
Total Identified	18.7	4.72	23.8%	3.01	43.5%	1.89
Unknown						
Unknown A1	24.1%	6.07				
Unknown A2	6.9%	1.74				
Unknown B	0.9%	0.23				
Unknown C	--		12.2%	1.54		
Unknown D	--				6.1%	0.27
Unknown E	--				0.1%	0.006
Unknown F	--				0.3%	0.011
Total Unknown	31.9%	9.72	12.2%	1.54	6.6%	0.29
Unidentified						
Aqueous					42.0%	1.82
Column-retained	16.2%	4.08	26.1%	3.29		
Not Otherwise Classified	22.4%	5.64	19.2%	2.43		
Total Aqueous	38.6%	9.72	45.4%	5.72		
Organic						
Volatile	2.3%	0.58	1.0%	0.13	3.2%	0.14
Non-volatile	3.7%	0.93	2.3%	0.29	1.3%	0.06
Total Organic	6.0%	1.50	3.3%	0.42	4.6%	0.20
Non-extractable	12.5%	2.22	7.5%	0.94	12.0%	0.52
Total Unidentified	53.4%	13.45	59.5%	7.50	58.5%	2.54
Loss (Gain)[a]	2.6%	0.66	7.6%	0.96	(8.8%)	0.38
Total	106.6%	26.87	99.9%	12.59	100.0%	4.34
Unaccounted[b]	(6.6%)	(1.67)	0.1%	0.01	--	--
GRAND TOTAL	100.0%	25.2	100.0%	12.6	100.0%	4.34

[a] Calculated from LSC disintegration counts as difference between original (intact) tissue and the sum of disintegration counts from the various tissue extracts

[b] This value represents the mass which was unaccounted for (i.e., the difference between the concentration in the original tissue and the sum of the identified, unknown, unidentified, and lost (gained) masses.

Table 10. Summary of Identified Thiodicarb Residues in Milk and Fat Tissues Following Oral Administration of Radiolabeled Thiodicarb to Lactating Goat #4 at 208 ppm in the Diet for Seven Days.

	Milk		Fat	
Metabolite/Fraction	% TRR	ppm	%TRR	ppm
Identified				
Acetamide				
ACN	11.0%	1.59		
HOAc				
Lactose	5.0%	0.74		
Total Identified	16.1%	2.33		
Unidentified				
Aqueous/ACN	20.1%	2.91		
Volatiles			10.2%	0.14
Non-volatiles			8.8%	0.12
Total Aqueous/ACN	20.1%	2.91	19.0%	0.26
Organic				
Hexane Soluble Acids/Alc.	18.0%	2.61	40.9%	0.56
Non-ionic Acids/Alc.	14.4%	2.09	17.5%	0.24
Hexane Insoluble Acids/Alc.	0.3%	0.04	0.7%	0.01
Not Otherwise Classified	4.8%	0.70		
Total Organic	37.5%	5.44	59.1%	0.81
Non-extractable	4.8%	0.68		
Hexane Soluble Acids/Alc.			9.5%	0.13
Non-ionic Acids/Alc.			5.1%	0.07
Hexane Insoluble Acids/Alc.			<0.7%	[<0.01]
Not Otherwise Classified				
Total Unidentified	62.2%	9.03	92.7%	1.27
Loss (Gain)[a]	22.3%%	3.23	10.0%	0.14
Total	100.6%	14.59	102.9%	1.41
Unaccounted[b]	(0.6%)	(0.09)	(2.9%)	(0.04)
GRAND TOTAL	100.0%	14.5	100.0%	1.37

[a] Calculated from LSC disintegration counts as difference between original (intact) tissue and the sum of disintegration counts from the various tissue extracts

[b] This value represents the mass which was unaccounted for (i.e., the difference between the concentration in the original tissue and the sum of the identified, unidentified, and lost (gained) masses.

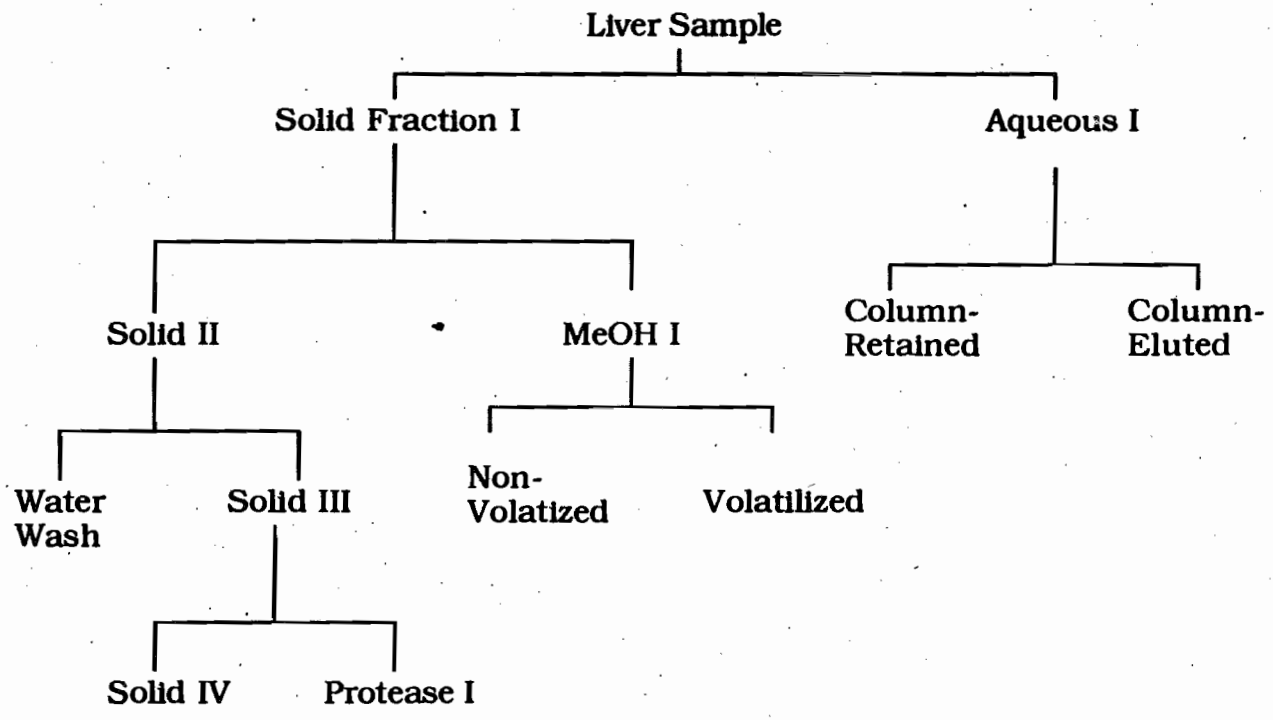
Liver Sample
Solid Fraction I
Aqueous I
Solid II
MeOH I
Column-
Retained
Column-
Eluted
Water
Wash
Solid III
Non-
Volatized
Volatilized
Solid IV
Protease I

Figure 1

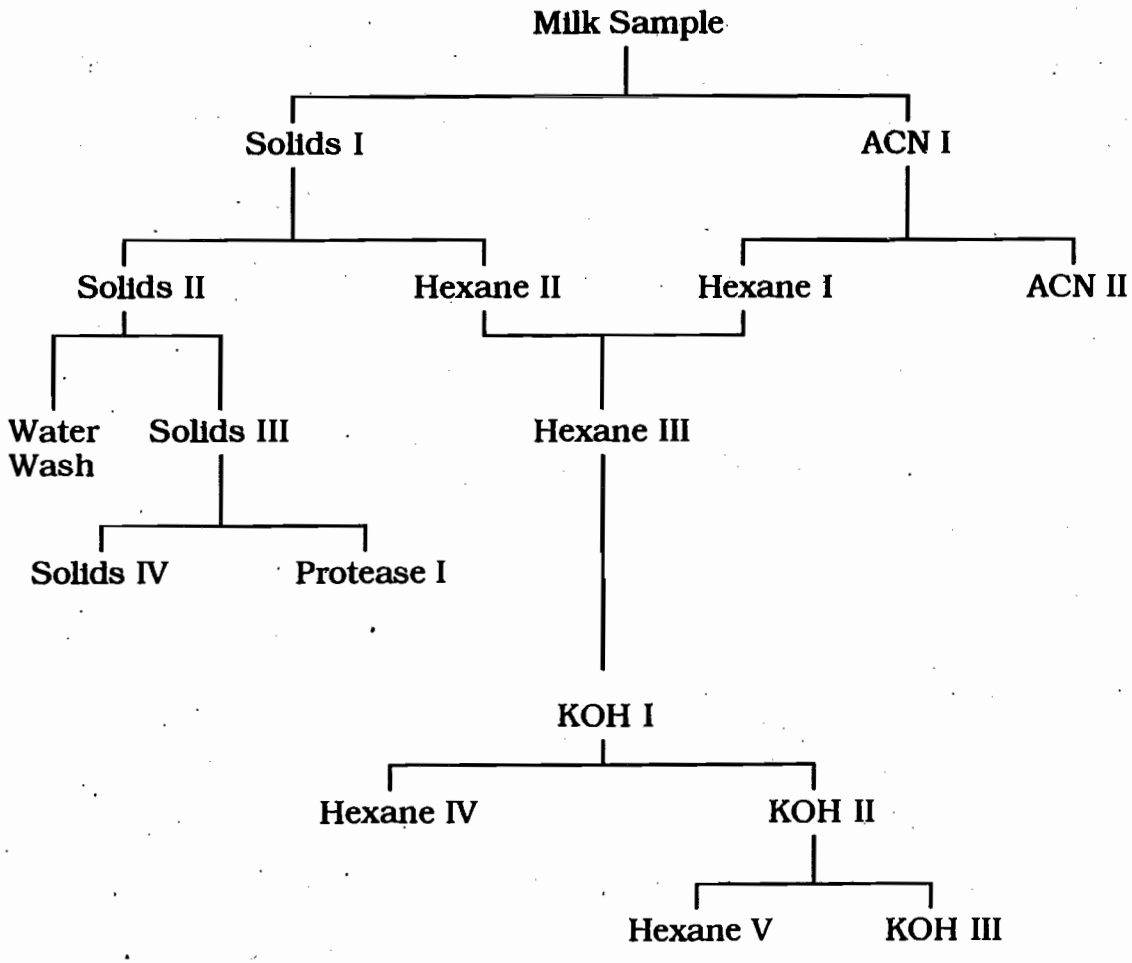
Milk Sample
Solids I
ACN I
Solids II
Hexane II
Hexane I
ACN II
Water Wash
Solids III
Hexane III
Solids IV
Protease I
KOH I
Hexane IV
KOH II
Hexane V
KOH III

Figure 2

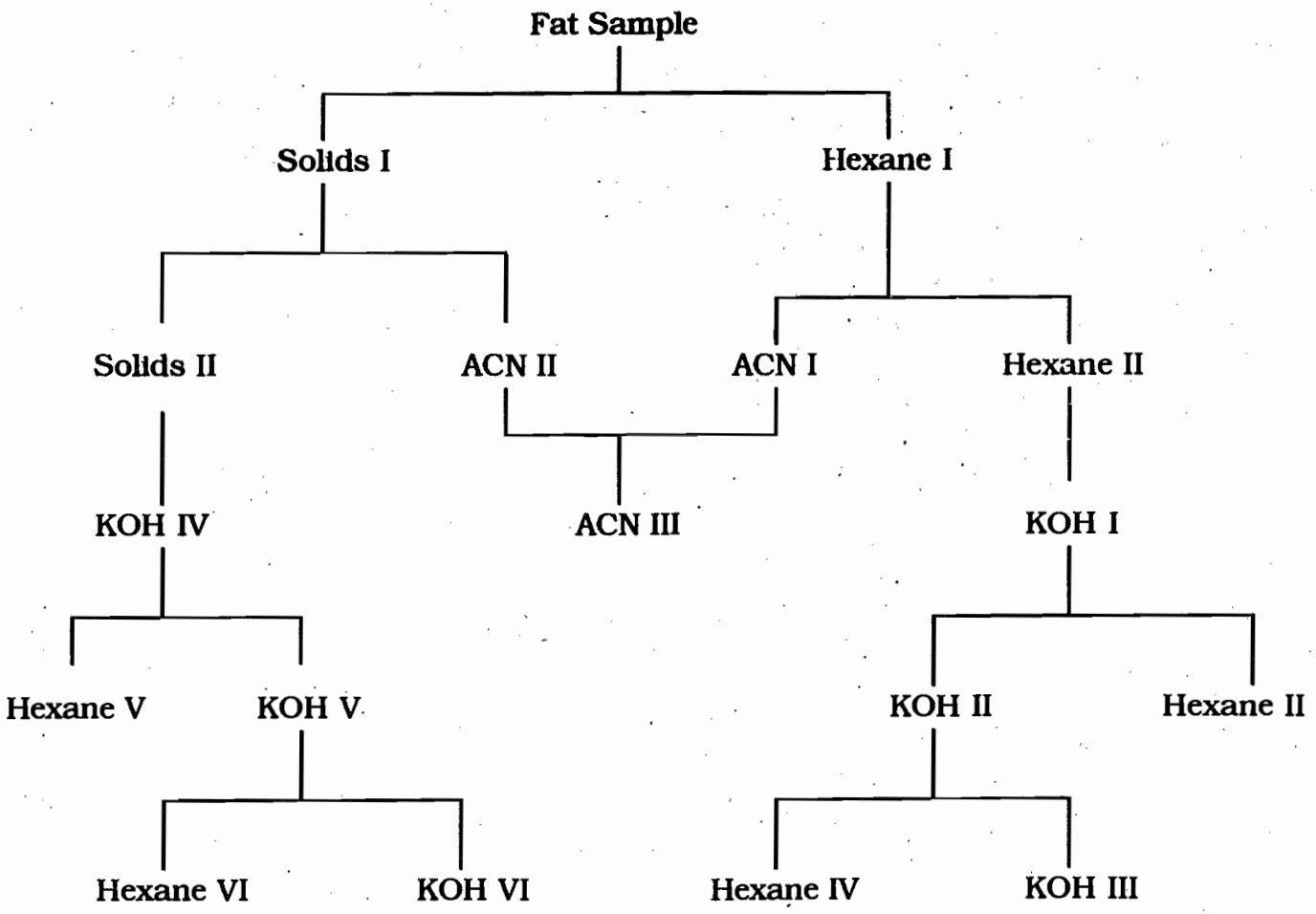
Fat Sample
Solids I
Hexane I
Solids II
ACN II
ACN I
Hexane II
KOH IV
ACN III
KOH I
Hexane V
KOH V
KOH II
Hexane II
Hexane VI
KOH VI
Hexane IV
KOH III

Figure 3