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

JUL 21 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: DDVP (dichlorvos); Goat Metabolism Study Following **Dermal** Application for 3 Consecutive Days.
[CB #: 11768; MRID: 42721601; DP Barcode:D190450]

FROM: Dennis McNeilly, Chemist Francis B Suhre for DM
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Amvac Chemical Co. has submitted a DDVP lactating goat metabolism study in response to the 9/87 DDVP Reg. Std. CBRS previously reviewed a protocol for this metabolism study (W. Hazel, 8/14/90).

CONCLUSIONS

1. Metabolism of dichlorvos (DDVP) in ruminants, following dermal exposure, is adequately understood. Data presented in MRID 42721601, indicates extensive metabolism of ^{14}C-DDVP following dermal exposure. No DDVP or primary metabolites of DDVP were found in milk or tissues of treated goats, furthermore, incorporation of ^{14}C into endogenous milk and tissue components of the treated goats was demonstrated.

2. CI mass spectra of water-soluble tissue extracts showed dechlorinated radioactive residues with molecular weights ranging from 147 to 243 m/e. Further identification was not provided.

3. ^{14}C-lactose was shown to be present in the aqueous extract of milk from goats treated with ^{14}C-DDVP.

4. Saponification of aqueous-soluble radioactive residues indicate the presence of radioactive lipids (complex and simple). ^{14}C-glycerol was shown to be present following hydrolysis of saponifiable-aqueous extracts of milk, liver, and fat.

5. TLC analysis of radioactive hydrolysates from previously extracted liver and milk samples showed co-elution of radioactivity with several components of a mixture of ^{14}C-amino acids reference standard. Identification of the co-eluting amino acids were not provided.

RECOMMENDATIONS

AMVAC Chemical Corp. should be notified that the data described in MRID 42721601 are adequate to fulfill the DDVP Residue Chemistry Data Requirement for a dermal goat metabolism study, 171-4(b).

DETAILED CONSIDERATIONS

The DDVP Guidance Document (9/87) concluded that the registrant must provide ruminant and poultry metabolism studies.

GOAT METABOLISM

TEST MATERIAL/DOSE PREPARATION

The following figure shows the structure of DDVP and the location of the radiolabeled carbon. A [vinyl-1-^{14}C] DDVP test substance was supplied by DuPont.

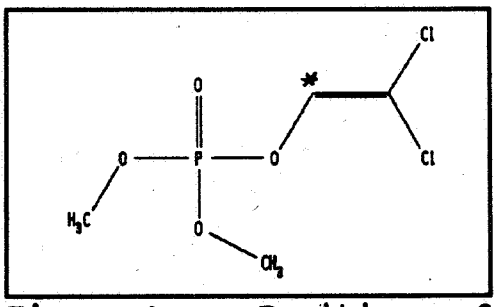


Figure 1. Position of the ^{14}C radiolabel.

Chemical identification of the test material was confirmed using TLC. Radiochemical purity of the test substance was determined using HPLC.

IN-LIFE PHASE

Three adult lactating cross-bred goats were acclimated for seven days prior to dosing. Animals were housed in stainless steel metabolism cages in a well ventilated room. The control goat was kept in an area "upstream" from the air-flow. Goats were fed a combination of alfalfa hay and Purina Goat Chow. Water was provided *ad libitum*.

Two lactating goats were dermally treated twice daily for three consecutive days with [vinyl-1-^{14}C] DDVP (14.2 uCi/mg) at ca. 10 mg/kg body weight/day. To dose at an exaggerated rate, the application site of one of the treated goats was shaved and the application site was occluded with a teflon patch immediately after each treatment to prevent volatilization of DDVP.

The occluded goat displayed signs of pesticide poisoning after the AM dose on the third day. Observations included listlessness, involuntary muscle twitching in the foreleg and thigh muscles, and diarrhea. Feed consumption by the occluded goat declined to ca. one-third acclimation levels by the third day of dosing.

DOSE ADMINISTRATION

Dosage calculations were based on the body weights of the two treated goats one day prior to the first dose preparation. The target rate was 10 mg/kg/day, to be administered in two equal daily doses.

Prior to dosing, an area approximately 18" X 24" was shaved along the mid-line of the back of one of the test goats. The second test goat was not shaven. Treatment soln' was applied dermally to both test animals twice daily, immediately after the AM and PM milkings for three consecutive days. Approximately 6 ml of treatment soln' was applied along a 3" wide band on either side of the midline of the back with a gavage needle attached to a disposable syringe. The soln' was spread onto the skin/hair with the side of the needle to prevent dripping. After air drying for ca. 15 minutes, the procedure was repeated with an additional 6 ml of soln'. Immediately after treatment, an occlusive teflon film patch was placed over the application site of the shaved test animal. Both test goats were stanchioned throughout the treatment period.

The "occluded" goat was demonstrating signs of pesticide poisoning and therefore the final evening dose on day three was not administered to the "occluded" goat.

SAMPLE COLLECTION

Urine and Feces: Urine samples were collected once daily (7-8 AM) beginning 24 hours prior to the first administered dose. Urinary output was determined gravimetrically and aliquots were stored frozen in plastic bottles.

Feces samples were also collected once a day (7:30-8:30 AM) beginning 24 hours prior to the first administered dose. Fecal output was determined gravimetrically and aliquots were stored frozen in plastic bottles. After animal sacrifice, cage solids, comprised of wetted fecal and feed matter remaining on the cage floor, were collected by sweeping the particulate matter up with a brush. Subsamples of cage solids were stored frozen until processed.

Milk: The goats were hand-milked twice daily (7-10 AM and 4-5 PM) throughout acclimation and treatment periods.

Tissues: The goats were sacrificed within 24 hours of the last dosing using a captive bolt pistol shot to the head followed by exsanguination. Immediately after sacrifice, the goats were necropsied and the following tissues removed: liver, kidneys, composite muscle, composite fat, skin, and hair. For the treated animals, separate muscle, fat, skin, and hair samples were taken from areas proximal and distal to the application site.

After necropsy, tissues (except skin and hair) were rinsed with tap water and placed in tared and labelled sample bags. Tissue samples were stored frozen at -20 C until processed.

SAMPLE PREPARATION AND EXTRACTION

Liver, kidney, muscle, and fat tissues were partially thawed, weighted and manually minced into small pieces using a serrated knife. Due to the potential for volatile residues, only a small portion of the entire sample was selected and homogenized. Subsamples were homogenized in a frozen state with dry ice in a small commercial food grinder.

To determine the residues remaining in/on the application site skin and hair samples, these samples were first extracted with an organic solvent prior to combustion analysis. Skin samples were partially thawed, weighted, and minced into small pieces. Hair samples were cut into small sections with scissors. The hair samples were placed into Nalgene bottles with a volume of toluene adequate to cover the sample. The bottles were sealed and left overnight. After 16 hours, the extracts were decanted through

glass wool into a second Nalgene bottle. The glass wool was rinsed with additional toluene which was added to the original extract. This extraction procedure was repeated twice more, leaving the samples to stand at room temperature for ca. 20-30 minutes for each extraction.

Daily feces samples and cage solids were homogenized to a smooth semi-solid consistency using tap water and/or wet ice. The registrant states that when water was added weight adjustment factors were made to adjust sample residue levels back to preprocessed levels. All processed samples were stored frozen until analysis.

CHARACTERIZATION OF RESIDUES

All samples were combusted to CO_2, H_2O, and inorganic ash in a Harvey Biological Oxidizer. Trapped CO_2 was mixed with a compatible scintillation cocktail for radioassay during this procedure. The TRR in selected tissues determined by this type of analysis are shown in Table 1.

Tissue	Occluded (ppm)	Non-occluded (ppm)
liver	36.1	9.1
kidney	13.4	3.2
distal muscle	2.3	0.6
proximal muscle	2.6	0.5
distal fat	0.7	0.1
proximal fat	0.6	8.4

Table 1. TRR in selected goat tissues after three consecutive days of dermal treatment with DDVP at a dose of 10 mg/kg/day.

Since tissue and milk samples from the occluded goat contained higher residues than the non-occluded goat, all samples extracted for characterization were taken from the occluded goat.

The highest levels of TRR were detected in the excretory organs, i.e., the liver and kidneys of both animals. Comparison of TRR levels in proximal and distal muscle and fat samples from both treated animals are inconclusive, but suggest residues will be nearly equivalent. Registrant speculates contamination of non-occluded proximal fat tissue based on the lack of difference between distal and proximal muscle tissue for both goats and the distal and proximal fat tissue from the occluded goat.

The TRR found in animal tissues accounts for 6.7 and 1.3% of the administered dose, for the occluded and non-occluded goats, respectively. TRR in milk from the occluded goat ranged from 6.1 to 10.8 ppm and accounted for 3.5% of the administered dose. TRR levels in the non-occluded goat ranged from 0.6 to 1.8 ppm and accounted for 0.6% of the administered dose.

EXCRETA

TRR excreted in the urine accounted for 17.5 and 2.4% of the administered dose in the occluded and non-occluded goats, respectively. The higher levels of residue in excreta indicate that teflon occlusion patch resulted in exposing the goat to a higher effective treatment rate.

Fecal excretion was determined to be a minor route of elimination. Radioactivity recovered in the feces ranged from 0.4-0.5% in the occluded goat, and was consistently 0.1% in the non-occluded goat.

Characterization of the TRR

EXCRETA

No effort was reported to characterize the residues in excreta.

MILK

Residue levels in milk ("occluded goat") were 6.1, 10.8, and 8.4 ppm on days 1, 2, and 3 of treatment. Due to adverse reaction to the dosing the "occluded" goat did not receive the final dose on the third day. Residues in milk from the non-occluded goat were 0.6, 1.3, and 1.8 ppm on treatment days 1, 2, and 3. These data indicate residue levels increase in milk during the treatment period.

Whole milk was extracted and analyzed according to the protocols outlined in Appendices 1 and 2.

Milk subsamples were extracted with chloroform for ca. 2 minutes using a polytron homogenizer. The mixture was centrifuged to separate the liquid and solid fractions. The solids were further extracted with chloroform and acetone. Post-extracted solids were hydrolyzed by refluxing with 1N HCl for approximately 18 hours. The acid hydrolysate was separated from the post-extracted solids by centrifugation and the solids were further hydrolyzed by refluxing with 1N NaOH for ca. 18 hours.

Extraction efficiency was 70.5% (7.6 ppm) with 7.3% (0.8 ppm) accounted for in the organic fraction and 63.2% (6.8 ppm) in the aqueous fraction. Radioactivity remaining bound to solids accounted for 29.5% of the TRR.

An attempt was made to characterize bound radioactivity using acid and base hydrolysis. Radioactivity in the aqueous acid and base fractions accounted for 21.0% (2.3 ppm) and 8.5% (0.9 ppm) of the TRR, respectively. No radioactivity was detected in the organic fractions. Radioactivity remaining bound to solids accounted for less than 0.01 ppm (<0.1%) of the TRR.

Milk organic extract was further characterized by saponification and analysis of the resulting fractions. 4.1% of the TRR partitioned into the non-saponifiable" (lipid) fraction and 3.0% of the TRR partitioned into the saponified-aqueous" (glycerol) fraction. Virtually none of the TRR was distributed into the "saponifiable" acid organic fraction (Appendix 2).

AQUEOUS FRACTION

The aqueous extract of milk (63.2% TRR) was analyzed by HPLC Systems 1 and 3. These analyses indicated a single radioactive peak which was not DDVP. Another aliquot was analyzed after fortification with [^{14}C]-lactose. This analysis reflected coelution of the unknown metabolite with the radiolabeled lactose standard. Identification of this metabolite as lactose was confirmed using TLC System 2.

ORGANIC FRACTION

HPLC analysis of the organo-soluble residues indicated that the majority of the TRR eluted as broad peaks between 9 and 16 minutes. Analysis of samples spiked with DDVP verifies that parent DDVP was not present in any of the organic extracts.

The registrant states that given the condition of the chromatographic system, only very apolar compounds would be expected to be retained. They suspect that the organo-soluble residues reflect incorporation of the radiolabelled moiety into endogenous fats.

Milk organic extracts were saponified and the resulting fractions were characterized. 4.1% (0.44 ppm) of the TRR partitioned into the non-saponifiable (simple lipids) fraction and 3.0% (0.32 ppm) of the TRR partitioned into the "saponified-aqueous" (complex lipids, e.g., glycerol) fraction. Less than 0.01 ppm of TRR was detected in the "saponifiable" acidified organic fraction.

HPLC analysis of the saponified-aqueous fraction from milk indicated co-chromatography with [^{14}C]-glycerol. The identification was confirmed using TLC, which also indicated co-chromatography with [^{14}C]-glycerol standard.

Therefore, 66.2% (63.2 + 3.0) of the TRR in milk was characterized as lactose and glycerol. Another 4.1% of the TRR is suspected to be incorporated into nonsaponifiable lipids.

MUSCLE

Muscle was extracted and analyzed according to the procedures outlined in Appendices 3 and 4.

Extraction efficiency was 51.3% (1.3 ppm) of the TRR with 2.7% (0.07 ppm) accounted for in the organic fraction and 48.5% (1.3 ppm) in the aqueous fraction. Bound radioactivity accounted for 48.8% (1.3 ppm) of the TRR.

The extracted tissues were hydrolyzed with acid and base and partitioned with chloroform in an attempt to release bound residues. Radioactivity in the aqueous acid and base hydrolysates accounted for 20.5% (0.5 ppm) and 28.4% (0.7 ppm) of the TRR, respectively. No radioactivity was detected in the organic fractions of these samples. Radioactivity remaining bound to muscle tissue accounted for 0.4% (0.01 ppm) of the TRR.

AQUEOUS FRACTION

Water-soluble residues accounted for 48.5% of the TRR in muscle. HPLC (system 1) analysis of an aliquot of this extract indicated one metabolite (figure 19, MRID 42721601). However, it must be noted that recoveries of radioactivity spiked with DDVP in muscle tissue were 18.4% after 10 months. The registrant analyzed the extract using a different HPLC system (3) and demonstrated that no DDVP or primary metabolites of DDVP (except possibly 2,2-dichloroacetic acid) were present. However, analysis of liver tissue with a peak at this same retention time indicates that the peak was not 2,2-dichloroacetic acid. Based on this information it was concluded that no known primary metabolites of DDVP were present in aqueous extracts.

No further effort was made to characterize these residues. However, further efforts were made with liver aqueous residues. See that section for additional information.

ORGANIC FRACTION

Organo-soluble residues accounted for 2.7% of the TRR in muscle tissue. HPLC analysis of organo-soluble fractions of the TRR in goat tissue (muscle, liver, kidney, fat) result in broad peaks eluting between 9 and 16 minutes. Analysis of samples spiked with DDVP verifies that parent DDVP was not present in the organic extracts.

The registrant also states that given the condition of the chromatographic system, only very apolar compounds would be

expected to be retained. They suspect that the organo-soluble residues reflect incorporation of the radiolabelled moiety into endogenous fats.

Muscle (organic extracts) were saponified and the resulting fractions were characterized. 0.8% (0.02 ppm) of the TRR partitioned into the non-saponifiable (lipid) fraction and 1.9% (0.05 ppm) of the TRR partitioned into the "saponified-aqueous" (glycerol) fraction. Less than 0.01 ppm of TRR was detected in the "saponifiable" (acidified organic) fraction.

The residue detected in muscle saponified-aqueous fraction chromatographed as a single radioactive peak, with a retention time of ca. 10 minutes. Since the unidentified metabolite was formed during saponification and was relatively more polar than glycerol, it probably reflects a product of endogenous fat.

BOUND RESIDUES

Mild acid hydrolysis of post-extracted solids solubilized 20.0% of the TRR in muscle tissue. Sequential mild base hydrolysis solubilized an additional 28.4% of the TRR in muscle tissue. HPLC analysis of the saponified acidified aqueous fraction of muscle organic fractions indicates one or possibly two metabolites.

Acid hydrolysis conducted under severe conditions solubilized ca. 46.7% of the TRR. This percent recovery is very close to the summation of the mild acid and base hydrolysis. The registrant states that this suggests the residues are associated with protein.

LIVER

Liver was extracted and analyzed following the procedures depicted in Appendices 5 and 6.

Extraction efficiency was 30.7% (11.3 ppm) of the TRR with 5.2% (1.9 PPM) accounted for in the organic fraction and 25.5% (9.4 ppm in the aqueous fraction. Radioactivity remaining bound to the liver tissue accounted for 69.3% of the TRR.

AQUEOUS FRACTION

The aqueous fraction comprised 25.5% of the TRR in liver. HPLC analysis indicated at least three metabolites. No parent compound was detected. Further HPLC analysis using a different system (3) indicated no parent compound and none of the suspected primary metabolites () were present. Additional HPLC analysis (System 5) indicated at least three, possibly five, metabolites.

An attempt was made to identify the metabolites that chromatographed in HPLC system 5. The isolate was trimethylsilated in acetonitrile and the TRR partitioned into n-hexane after derivitization. GC-RAM (Radiomatic radioactivity monitor) and GC/MS characterization was conducted using positive and negative chemical ionization (CI) modes (scan 35-350 amu). GC/MS analysis indicated that molecular ions for five of the derivitized metabolites ranged between 219 - 336 m/e. The compounds did not contain Cl atoms. They state that a sixth metabolite co-eluted with the solvent and could not be detected.

The registrant states that based on the mass spectra, they conclude that incorporation of the dechlorinated vinyl portion of DDVP into relatively high-molecular weight natural products.

ORGANIC FRACTION

Organo-soluble residues accounted for 5.2% of the TRR in liver. HPLC analysis (System 2) showed that the radioactivity eluted as broad peaks between 9 and 16 minutes, which appear to consist of three major components. HPLC analysis using a different system shows (3) that the parent compound was not present in the organic fraction.

The registrant also states that given the condition of the chromatographic system, only very apolar compounds would be expected to be retained. They suspect that the organo-soluble residues reflect incorporation of the radiolabelled moiety into endogenous fats.

Liver organic extracts were saponified and the resulting fractions were characterized. 1.4% (0.5 ppm) of the TRR partitioned into the non-saponifiable (lipid) fraction and 3.8% (1.4 ppm) of the TRR partitioned into the "saponified-aqueous" (glycerol) fraction. Less than 0.01 ppm of TRR was detected in the "saponifiable" (fatty acid) fraction.

HPLC System 4 analysis of saponified-aqueous fraction from liver indicated that 28.6% coeluted with radiolabeled glycerol. This identification was confirmed with TLC. the remaining residue found in liver tissue eluted as a single peak. This compound was relatively more polar than glycerol and most likely reflects an endogenous fat.

BOUND RESIDUES

Liver bound residues were hydrolyzed with acid and base and partitioned with chloroform. Radioactivity in the aqueous acid and base hydrolysates accounted for 41.5% (15.2 ppm) and 27.1% (10.0 ppm) of the TRR, respectively. No radioactivity partitioned into the organic fractions for either the acidic or

base hydrolysis. Radioactivity remaining bound to liver tissue after hydrolysis accounted for 0.7% (0.27 ppm) of the TRR.

Acid hydrolysis conducted under severe conditions solubilized ca. 68.8% of the TRR. This percent recovery is very close to the summation of the mild acid and base hydrolysis. After partitioning with n-hexane, >97% of the radiocarbon remained in the aqueous fraction.

HPLC profile of the severe acid liver hydrolysate fortified with a mixture of radiolabeled amino acids demonstrated that the solubilized residues eluted in the same region as some of the amino acids.

The registrant attempted to chromatographically resolve the hydrolysate by derivatization with o-phthaldialedyhyde. A reagent that reacts with primary and secondary amines. The derivitization reaction was found to be quantitative. As much as 90% of the hydrolysate radiocarbon could be reacted, as shown by partitioning of the radiocarbon into the organo-soluble fraction. They state that direct analysis of the sample was not feasible due to the large amount of reagent in the sample. Other hydrolysate samples were prepared using a minimum amount of reagent. TLC analysis of derivatized liver and milk hydrolystates resolved 1 or 2 major peaks. These peaks co-migrated with amino acid standards.

Based on this information the registrant suggests that the residues are associated with protein.

KIDNEY

Kidney was extracted and analyzed following the procedure depicted in Appendices 7 and 8.

Extraction efficiency was 37.2% (5.1 ppm) with 4.7% (0.7 ppm) accounted for in the organic fraction and 32.5% (4.4 ppm) in the aqueous fraction. Radioactivity remaining unextracted or bound to the kidney tissue accounted for 62.8% (8.6 ppm) of the TRR.

AQUEOUS FRACTION

The aqueous fraction comprised 32.5% (4.4 ppm) of the TRR in kidney tissue. HPLC analysis of kidney aqueous extract indicates that no parent DDVP were present. HPLC analysis (System 5) indicated the presence of at least four metabolites. No additional effort was made to characterize kidney residue. The registrant concentrated their efforts on liver tissue when the residues were higher.

ORGANIC FRACTION

Organo-soluble residues accounted for 4.7% of the TRR in kidney tissue. HPLC analysis verified that no parent DDVP was present in the organic fraction.

The registrant also states that given the condition of the chromatographic system, only very apolar compounds would be expected to be retained. They suspect that the organo-soluble residues reflect incorporation of the radiolabelled moiety into endogenous fats.

Kidney organic extracts were saponified and the resulting fractions were characterized. 1.7% of the TRR partitioned into the non-saponifiable (lipid) fraction and 2.9% (0.4 ppm) of the TRR partitioned into the "saponified-aqueous" (glycerol) fraction. Less than 0.01 ppm of TRR was detected in the "saponifiable" (fatty acid) fraction.

BOUND RESIDUES

The kidney bound residues were then base and acid hydrolyzed and partitioned with chloroform. Radioactivity in the aqueous acid and base hydrolysates accounted for 49.8% (6.8 ppm) and 11.9% (1.6 ppm) of the TRR, respectively. No radioactivity was detected in the organic fractions of these samples. Radioactivity remaining in the kidney tissue after hydrolysis accounted for 1.1% (0.15 ppm) of the TRR.

Acid hydrolysis conducted under severe conditions solubilized ca. 62.3% of the TRR. This percent recovery is very close to the summation of the mild acid and base hydrolysis. After partitioning with n-hexane, >97% of the radiocarbon remained in the aqueous fraction.

HPLC analysis (System 1) of the mild acid and base hydrolysates indicated no parent DDVP was present. HPLC profile of the severe acid liver hydrolysate fortified with a mixture of radiolabeled amino acids demonstrated that the solubilized residues eluted in the same region as some of the amino acids.

FAT

Fat was extracted and analyzed as specified in Appendices 9 and 10.

Extraction efficiency was 67.2% (0.5 ppm) of the TRR with 60.0% (0.4 ppm) accounted for in the organic fraction and 7.2% (0.05 ppm) in the aqueous fraction. Radioactivity remaining bound to the tissue accounted for 32.8% (0.2 ppm) of the TRR.

AQUEOUS FRACTION

Water-soluble residues accounted for 7.2% of the TRR in fat. HPLC analysis of fat aqueous extract indicates that no parent DDVP were present. No additional effort was made to characterize kidney residue. The registrant concentrated their efforts on liver tissue were the residues were higher.

ORGANIC FRACTION

Organo-soluble residues accounted for 60.0% of the TRR in fat. HPLC analysis verified that no parent DDVP was present in the organic fraction.

The registrant also states that given the condition of the chromatographic system, only very apolar compounds would be expected to be retained. They suspect that the organo-soluble residues reflect incorporation of the radiolabelled moiety into endogenous fats.

The fat bound residues were then base and acid hydrolyzed and partitioned with chloroform. Radioactivity in the aqueous acid and base hydrolysates accounted for 32.8% (0.2 ppm) and 0.0% of the TRR, respectively. No radioactivity was detected in the organic fractions of these samples, nor was any radioactivity remaining in fat tissue after hydrolysis.

Fat organic extracts were saponified and the resulting fractions were characterized. 23.6% (0.2 ppm) of the TRR partitioned into the non-saponifiable (lipid) fraction and 36.4% (0.3 ppm) of the TRR partitioned into the "saponified-aqueous" (glycerol) fraction. Less than 0.01 ppm of TRR was detected in the "saponifiable" (fatty acid) fraction.

HPLC System 4 analyses of the saponified-aqueous fractions from fat demonstrated that 23.1% of the fraction radiocarbon coeluted with authentic [^{14}C]-glycerol. Confirmation was made using TLC System 4. The remainder if the TRR chromatographed as a single radioactive peak. The registrant states: "Since the unidentified metabolite was formed during the saponification process and was relatively more polar than glycerol, it was most likely a polar hydrolytic product of endogenous fat."

BOUND RESIDUES

Residues bound to fat were then base and acid hydrolyzed and partitioned with chloroform. Radioactivity in the aqueous acid and accounted for 32.8% of the TRR. Fat tissue was not hydrolyzed using a base.

Acid hydrolysis conducted under severe conditions solubilized ca. 46.7% of the TRR. Percent recovery after severe acid hydrolysis was very close to the summation of the mild acid and base

hydrolysis in the other tissues. After partitioning with n-hexane, >97% of the radiocarbon remained in the aqueous fraction.

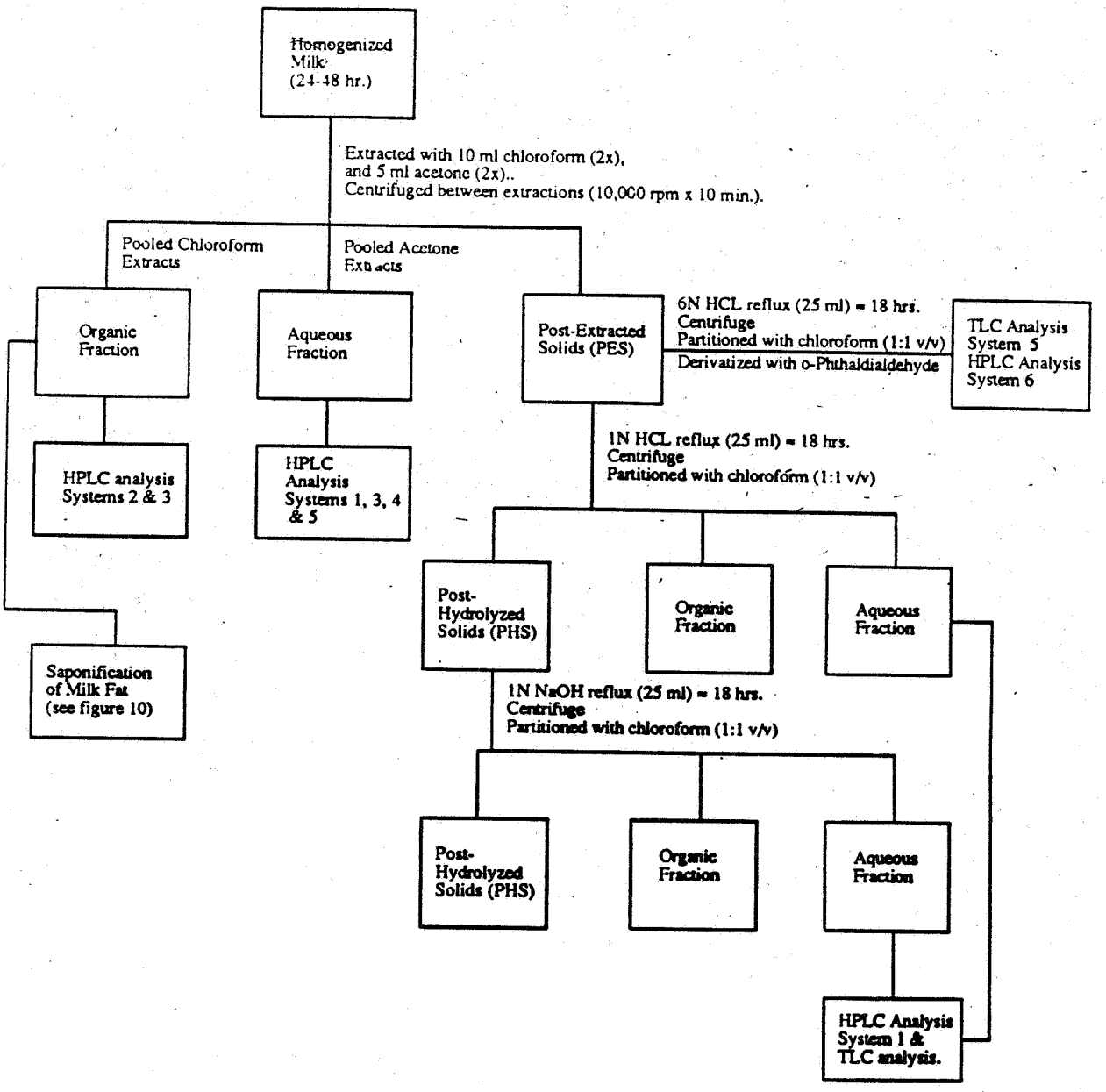
HPLC analysis (System 1) of the mild acid and base hydrolysates indicated no parent DDVP was present. HPLC profile of the severe acid liver hydrolysate fortified with a mixture of radiolabeled amino acids demonstrated that the solubilized residues eluted in the same region as some of the amino acids.

Based on the analysis of liver and milk acid hydrolysates, the registrant suggests that the residues are associated with amino acids.

Attachments: Extraction/Analysis Schematics from MRID 42721601.
Appendix 1-10

CC: D. McNeilly;RF;SF;DDVP List A File;Circulation.
RDI: FBS, 7/13/93;MSM 7/19/93;EZ,7/19/93.
H7509C: DMM;dmm;CM-2;Rm804F;X305-5934;7/21/93

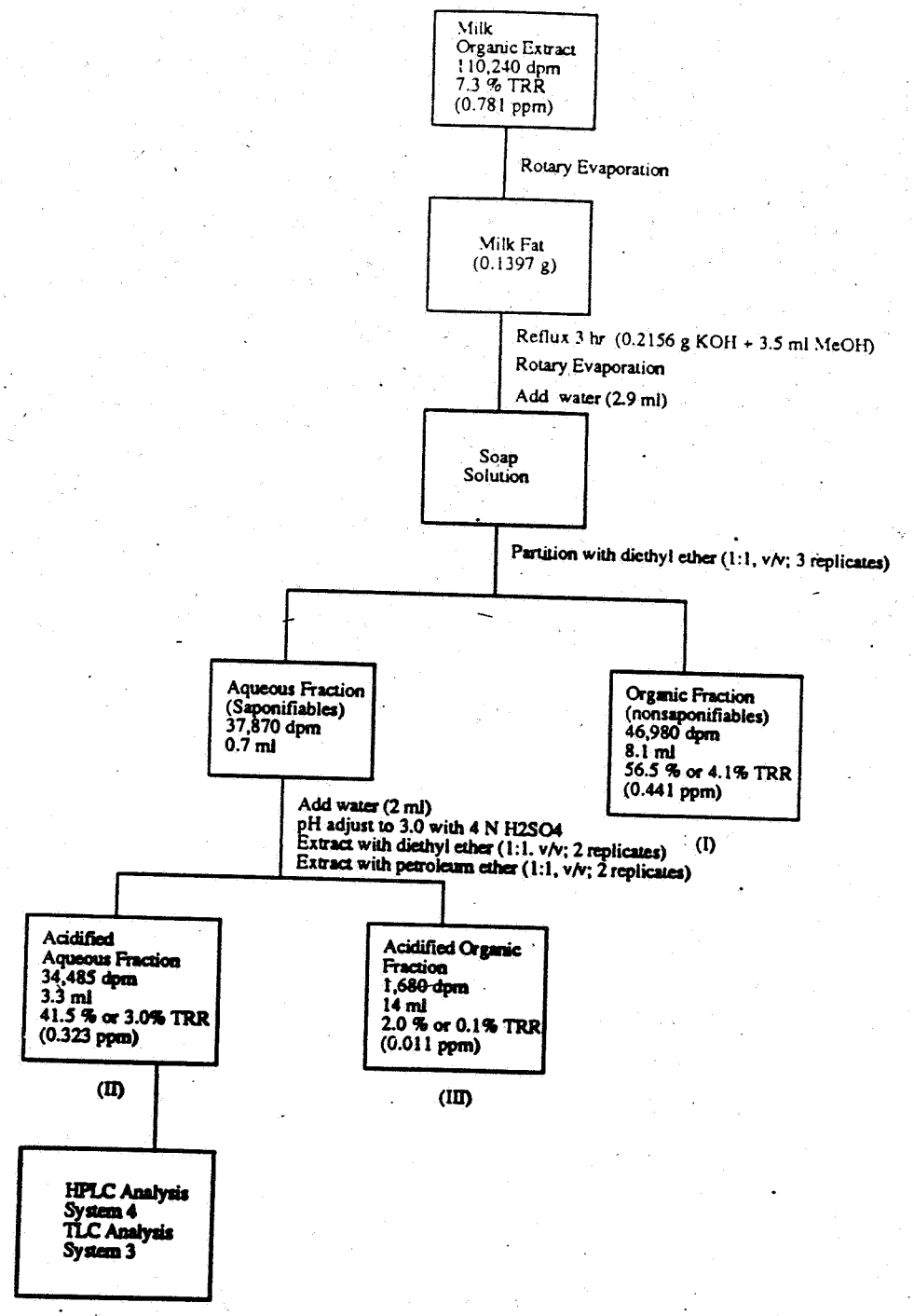
Amount Extracted: 9.741 g



Extraction and Analysis Schematic for Milk.

APPENDIX 1

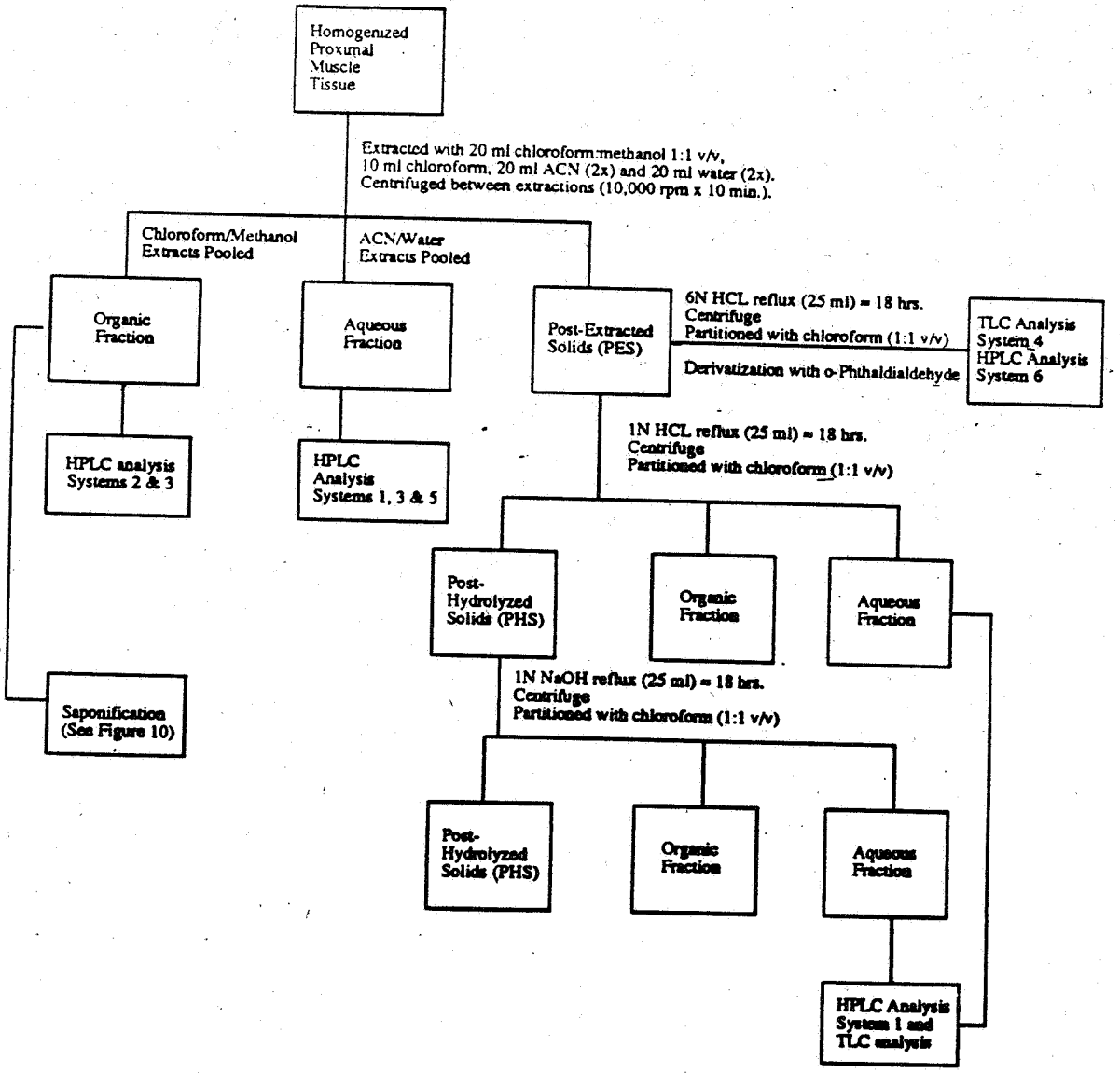
Amount Extracted: 1.3 ml



Saponification and Analysis Schematic of Milk Fat.

APPENDIX 2

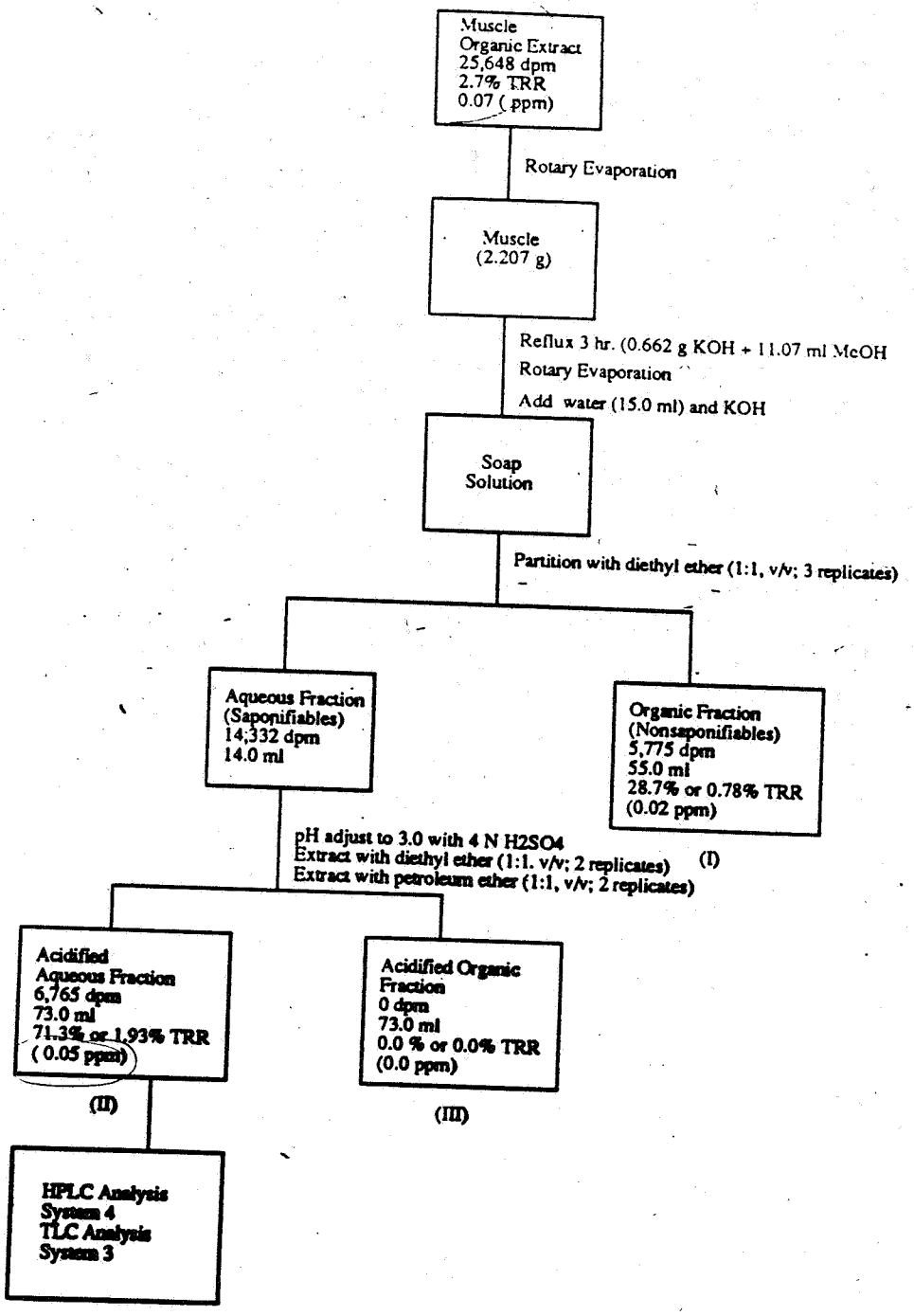
Amount Extracted: 7.800 g



Extraction and Analysis Schematic for Muscle.

APPENDIX 3

Amount Extracted: 23.184 grams

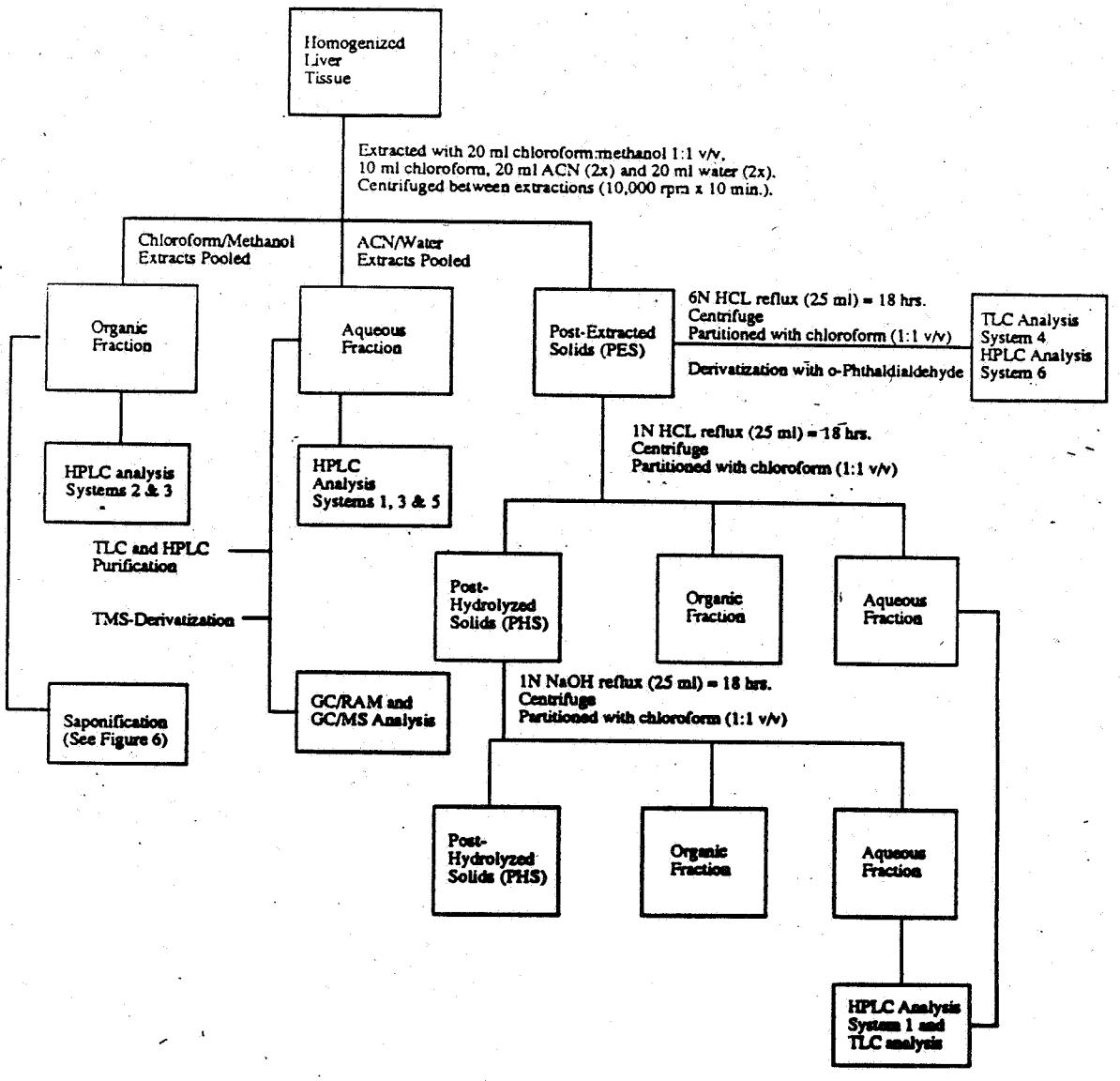


Total Radiocarbon Recovery = I + II + III = 48.9 %

Saponification and Analysis Schematic of Proximal Muscle.

APPENDIX 4

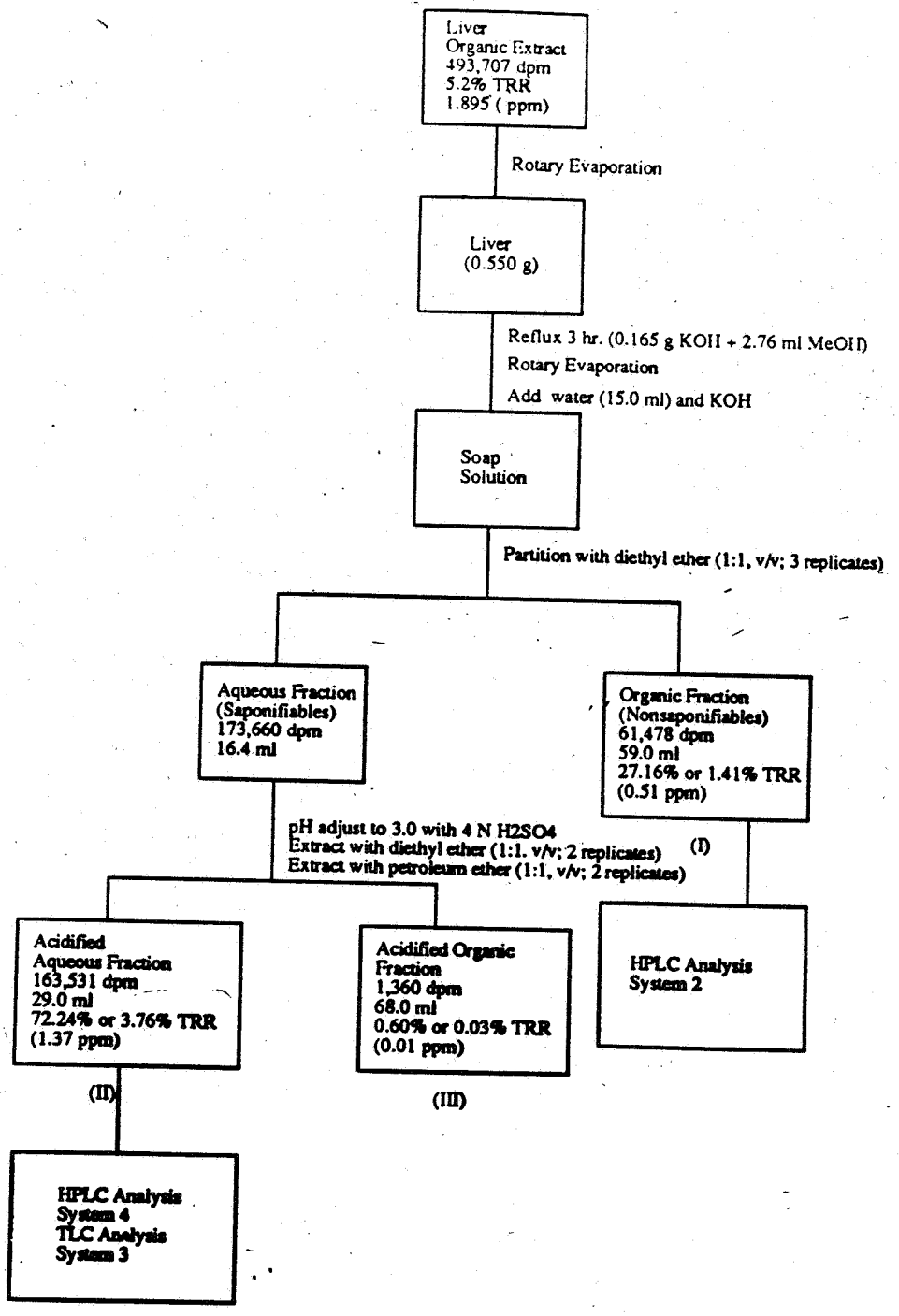
Amount Extracted: 12.371 g



Extraction and Analysis Schematic for Liver.

APPENDIX 5

Amount Extracted: 10.133 grams

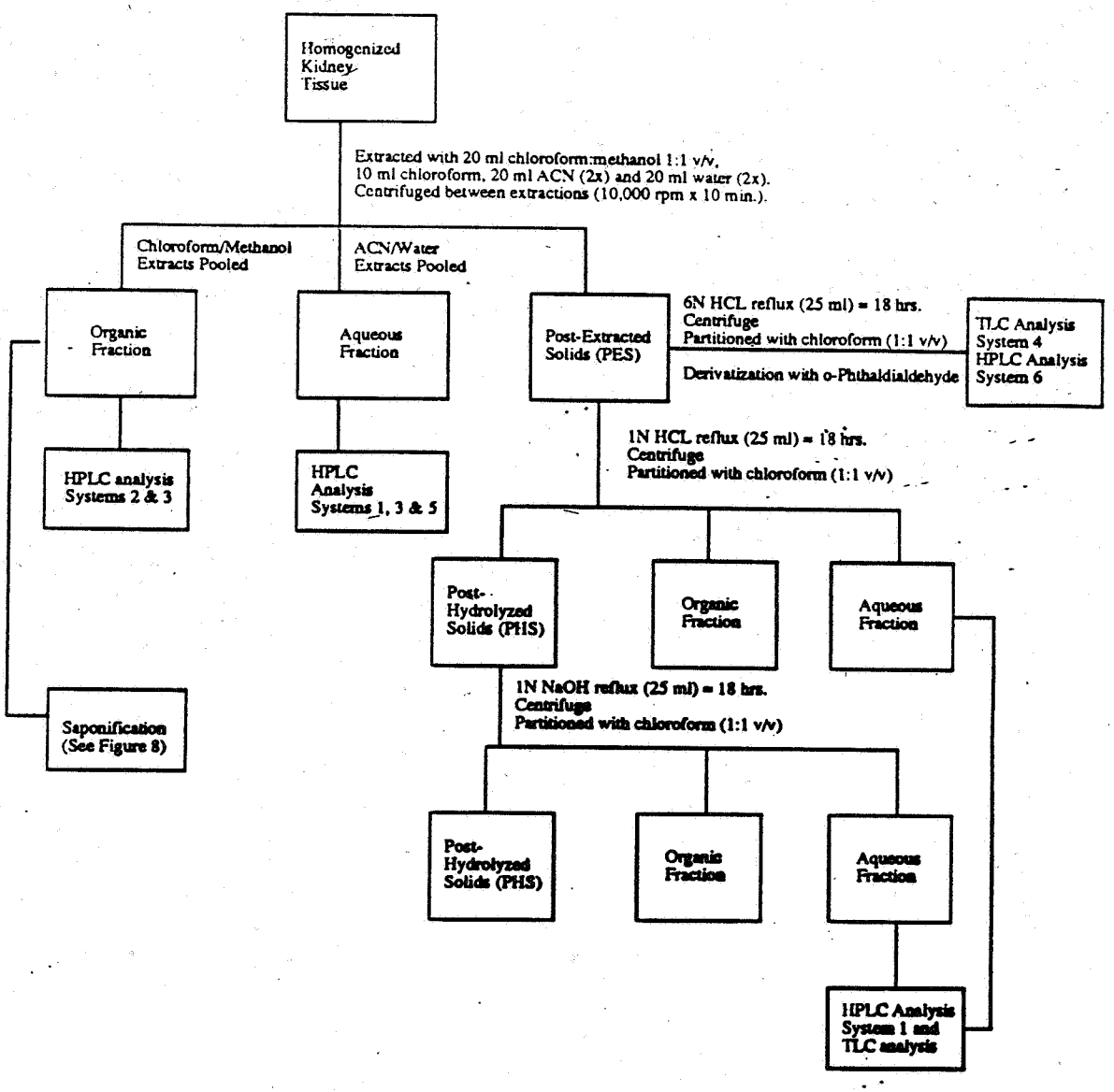


Total Radiocarbon Recovery = I + II + III = 45.9 %

Saponification and Analysis Schematic of Liver.

APPENDIX 6

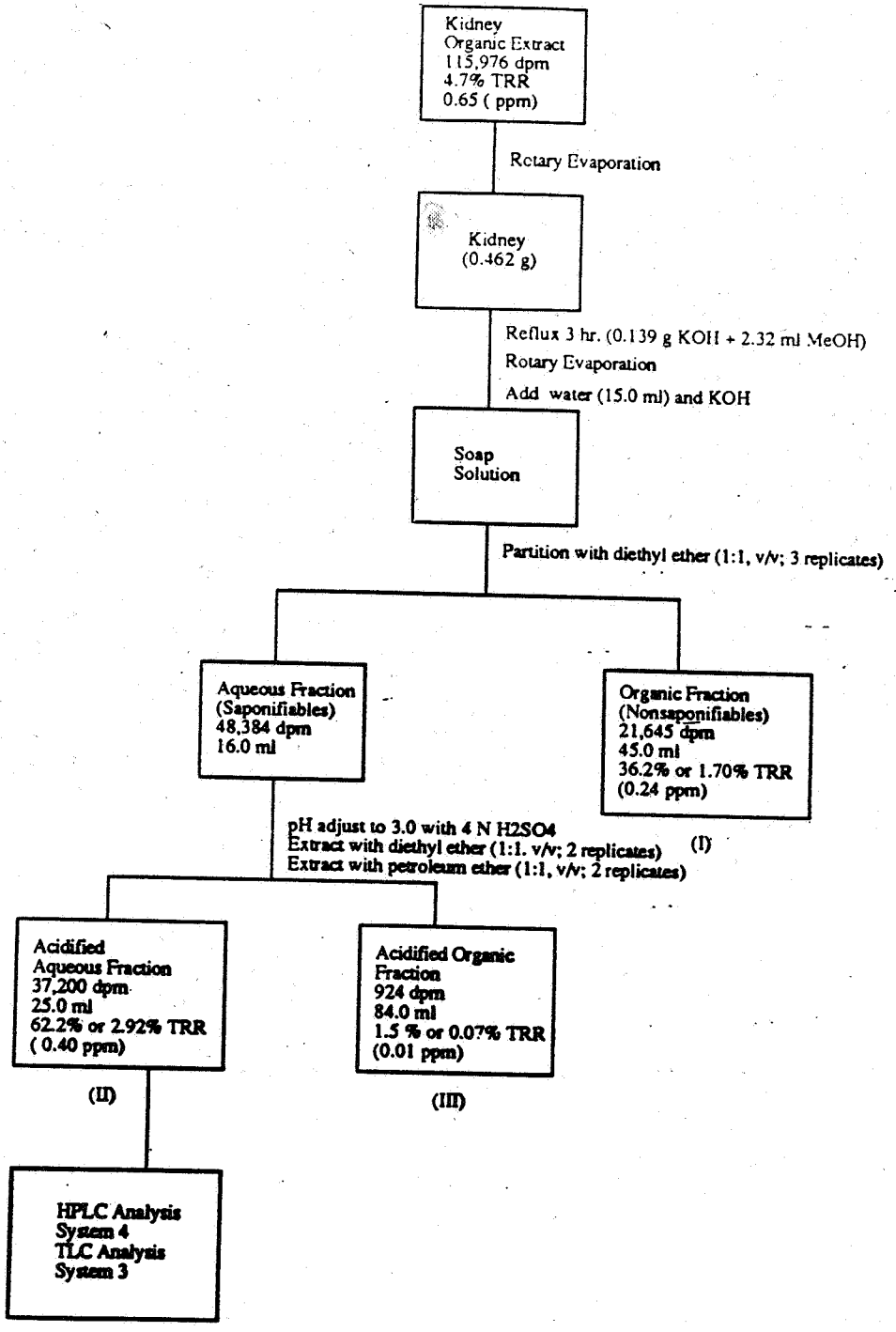
Amount Extracted: 6.310 g



Extraction and Analysis Schematic for Kidney.

APPENDIX 7

Amount Extracted: 11.746 grams

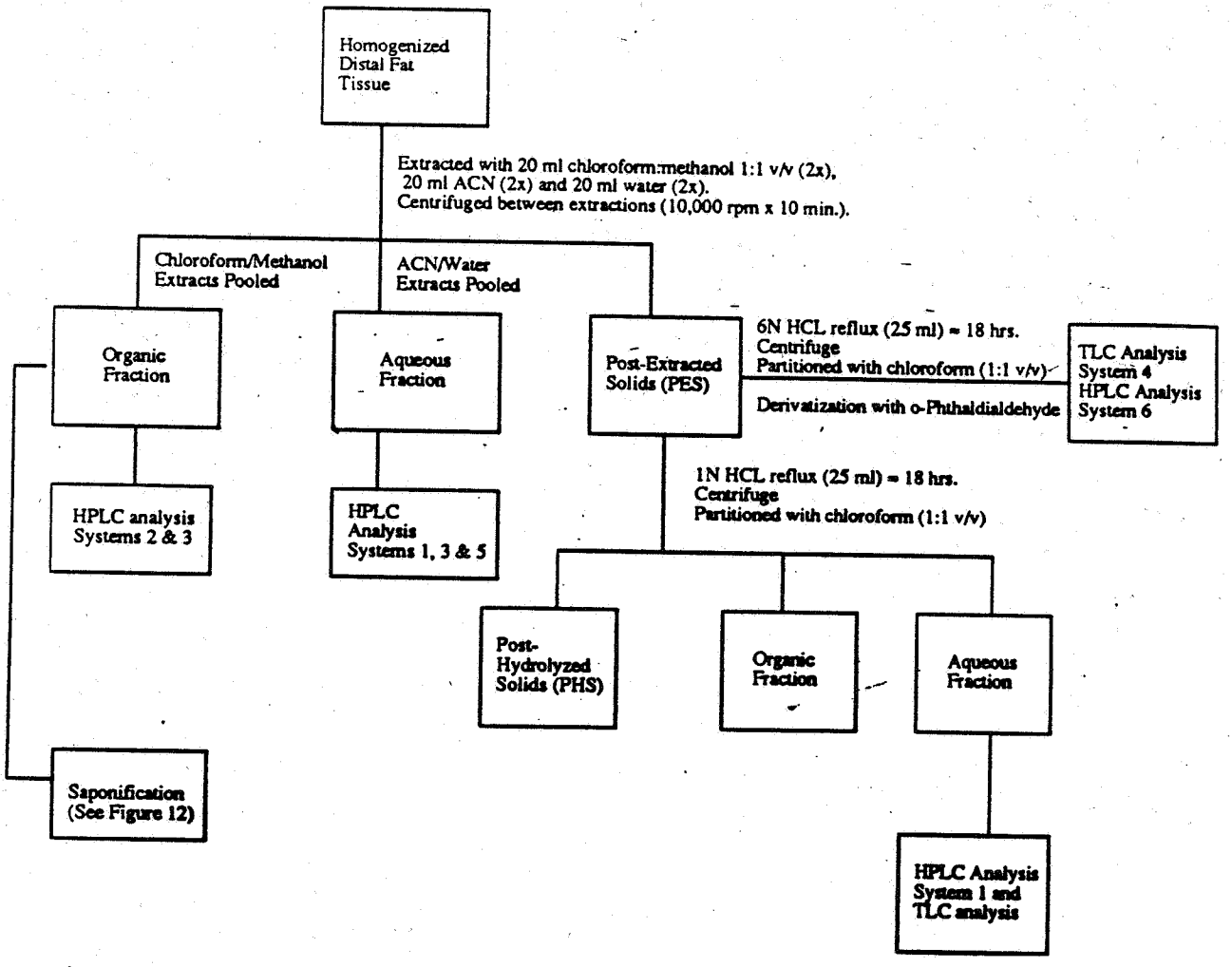


Total Radiocarbon Recovery = I + II + III = 51.5 %

Saponification and Analysis Schematic of Kidney.

APPENDIX 8

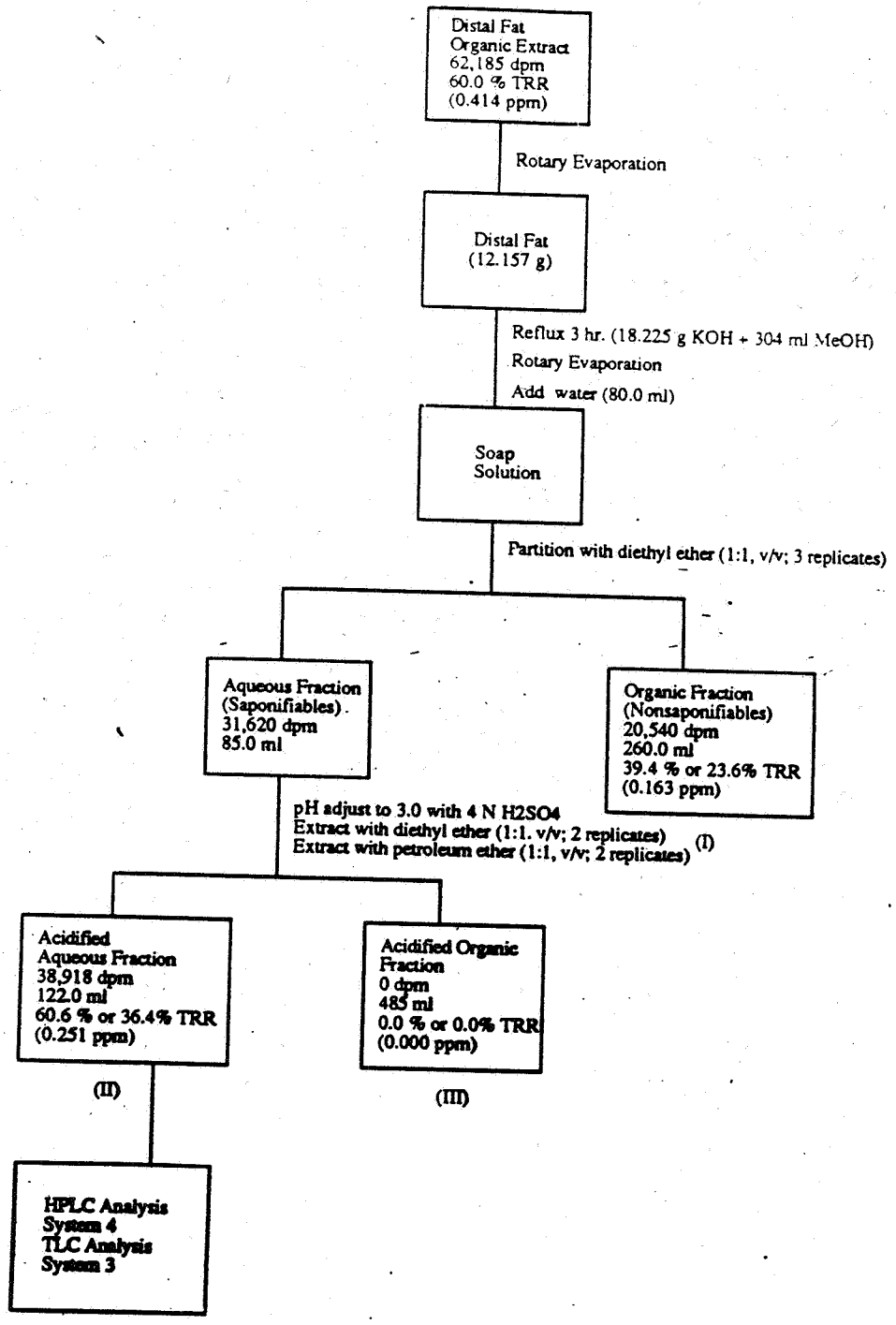
Amount Extracted: 5.040 g



Extraction and Analysis Schematic for Distal Fat.

APPENDIX 9

Amount Extracted: 30.787 grams



Total Radiocarbon Recovery = I + II + III = 95.6%

Saponification and Analysis Schematic of Distal Fat.

APPENDIX 10