Characterization of Malathion Residues in Dairy Goats and Poultry

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The chemical nature and magnitude of [^{14}C]malathion residues in the milk, eggs (whites and yolks), fat, liver, kidney, and muscle tissues in dairy goats and laying hens were examined after repeated oral exposure. The level and duration of the dose given to goats were equivalent to 86 ppm on feed for 5 days. Chickens received the equivalent of 28 ppm on feed for 4 days. Radioactive residues were characterized and identified using solvent extraction and chromatography. The parent chemical was not detectable, and immediate metabolites of malathion were not present in any tissues except traces of the mono- and dicarboxylic acid metabolites observed in goat kidney. This observation was consistent with urinary excretion as the major elimination pathway of malathion and immediate metabolites in poultry, laboratory animals, and humans. Extensive degradation and metabolism resulted in the reincorporation of the radiolabel into normal biogenic chemicals in all investigated samples. The products were typical of those derived from the tricarboxylic acid cycle. The products included pyruvate, lactate, lactose, oleic acid, stearic acid, and glycerol. Reincorporation of radiolabel into protein was also demonstrated by the isolation and hydrolysis of casein from milk. It was concluded that ingested malathion was converted to acetate or other volatile fatty acids, metabolized to acetyl-CoA, and then incorporated into the carbon pool.

Keywords: *Malathion; tissue residue study; reincorporation; metabolism*

INTRODUCTION

Malathion (121-75-5) is widely used for numerous insect infestations. Potential contamination of food crops and livestock has generated a concern regarding the potential toxicity to humans following ingestion of residues.

Malathion is generally acknowledged to be nontoxic to mammalian species. Rapid and extensive metabolism by carboxyesterases convert malathion to water-soluble metabolites. These water-soluble products are then rapidly excreted in urine and feces. Although urinary and fecal excretion represent near quantitative elimination pathways for the administered dose, residues may persist for significant periods of time.

Malathion residue disposition in a variety of animal and plant species has been reviewed by Mulla et al. (*IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*). Following administration of ^{32}P-labeled malathion spray to heifer calves, unchanged parent and chloroform-soluble metabolites were absent in tissues (March et al., 1956a). Water-soluble metabolites were present in many tissues; bone, liver, and kidney contained the highest levels of radioactivity. These workers also investigated the excretion in calves and laying hens after administration via feed, spray, and intraperitoneal injection (March et al., 1956b). The excretion products were typical of those expected from phosphatase and carboxyesterase activity; however, no characterization or identification studies were reported for any residues. Claborn and workers (1960a,b) used a colorimetric malathion assay and reported 0.08 ppm in cow milk within 5 h of spraying with 0.5% emulsion or suspension. No traces of malathion were evident after 3 days, and deposition in fat was not observed. Gupta and Paul (1977) also investigated [^{32}P]malathion residues in hens. Following a single oral dose of 394 mg/kg, liver and kidney radioactivity was significantly decreased after 36 h and virtually absent after 48 h. The residues present in these tissues were characterized from partition experiments as chloroform-soluble (i.e., malathion and maloxon) and water-soluble (i.e., desmethylmalathion and the mono- and dicarboxylic acids of malathion) products. Specific identification of these species was not reported. Although this and other studies reported the disposition of unchanged parent and/or its chloroform-soluble products, no characterization of the water-soluble residues has been reported.

To evaluate the potential toxicity resulting from ingestion of food products (meat, milk, and eggs) containing malathion residues, the metabolic fate of malathion in lactating goats and white leghorn chickens was investigated. The residues contained in edible tissues were characterized using radiometric, solvent extraction/hydrolysis, and chromatographic experiments. High-performance liquid chromatographic analysis was used to evaluate the presence of malathion and its immediate metabolites and to identify specific reincorporation products of malathion. The study was performed in accordance with subdivision O guidelines of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA).

MATERIALS AND METHODS

Test Materials. Radiolabeled and unlabeled malathion were obtained from American Cyanamid. The radiolabeled material was labeled at the 2- and 3-positions of the butane-

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dioate acid moiety. The radiochemical purity was greater than 96% when analyzed using reverse phase HPLC with on-line radiochemical detection.

Reference Standards. Desmethylmalathion (DMM), isomalathion, the potassium salt of monoethyl maleate, and diethyl 2-mercaptosuccinate were provided by Cheminova Agro A/S (Lemvig, Denmark). The unlabeled mono- and dicarboxylic acids of malathion (MCA and DCA, respectively) were provided by American Cyanamid (Princeton, NJ). The label stated a mixture of 85% α and 15% β isomers. Radiolabeled DMM, MCA, and DCA, obtained from test goat urine, were used as retention time/fortification standards after identification by retention time/migration coincidence to the unlabeled standards by reverse phase HPLC and TLC. Maloxon was obtained from Riëdel de-Hahn (Germany). [2,3-^{14}C]Fumaric acid, [UL-^{14}C]glycerol, L-[UL-^{14}C]glutamic acid, D-lactose monohydrate, D-(+)-glucose, [1-^{14}C]stearic acid, casein, L-tyrosine, and protease type XXV (Pronase E, 4.1 units/mg) were obtained from Sigma (St. Louis, MO). D-Galactose, succinic acid, and glycerol were obtained from Fisher Scientific (West Haven, CT). Citric and L-glutamic acids were purchased from Merck (Darmstadt, Germany), and L-tryptophan and malic and lactic acids were bought from Aldrich (Milwaukee, WI). The sodium salt of [^{14}C]pyruvic acid was purchased from NEN (Wilmington, DE). The sodium salt of [U-^{14}C]-L-lactic acid was purchased from ICN Biochemicals (Cambridge, MA). Lactose monohydrate (D-glucose 1-^{14}C-labeled) and [1-^{14}C]oleic acid were purchased from American Radiochemical Company Inc. (St. Louis, MO).

Goat-Feeding Study. Three female Alpine dairy goats (ranging in age from 2 to 3 years) were received from a local supplier. The goats were weighed upon receipt, at study initiation, and at termination. During quarantine (9 days) and treatment periods (5 days), feed consumption and the production of milk, urine, and feces were determined daily; observations were made twice daily for mortality, morbidity, and toxic signs. The goats were individually housed in 2 ft × 4 ft metabolism stalls maintained on a concrete floor. Purina Goat Chow (0.5 kg) and clover-prairie hay (1 kg) were given twice daily (a.m. and p.m.). Natural well water was provided *ad libitum*. Two goats were orally dosed with encapsulated malathion [21 756 disintegrations per minute (dpm)/μg] diluted with cornstarch (size 12, Eli Lilly) at the morning milking once daily for 5 consecutive days. One control animal received an equivalent amount of encapsulated cornstarch. The dose levels were 0 mg/day (control) and 172.2 mg/day (treated), which was equivalent to approximately 86 ppm in feed when calculated using an average daily intake of 2.1 kg of feed/day. The goats were sacrificed approximately 24 h after the last dose, and analytical and adjunctive samples were collected. Analytical samples collected during and at sacrifice included heart, kidney, liver, milk, longissimus dorsi muscle, semimembraneous muscle, omnental fat, perirenal fat, and back fat. Adjunctive samples, collected as a source of potential malathion metabolites, included urine, feces, and the rumen and contents. Complete gross necropsy examinations were conducted for all animals.

Poultry-Feeding Study. White leghorn chickens were obtained from local suppliers and had been previously vaccinated against Marek's Disease, bronchitis, and Newcastle's Disease. Chickens were individually housed during quarantine (8 days) and the test period (4 days) in a 61.0 × 53.3 × 38.1 cm wire pen maintained over steel pans. Purina Layena Poultry Feed (Purina Mills, Inc., St. Louis, MO) was offered twice daily. The chickens were orally dosed via gelatin capsule (size 00, Eli Lilly and Co.) once daily for 4 consecutive days. The dose levels were 0 mg (four control animals) and 3.8 mg (four treated animals), which was equivalent to approximately 28 ppm in feed calculated using an average daily intake of 113.5 g per chicken per day. The specific activity of [^{14}C]-malathion was 20 868 dpm/μg. The chickens were sacrificed approximately 24 h after administration of the last dose, and analytical and adjunctive samples were collected. Analytical samples collected during and at sacrifice included heart, kidney, liver, skin (plus fat), dark meat, white meat, egg yolk and white, and fat. Feces were collected as an adjunctive sample for use as a source of malathion metabolites. Complete gross necropsy examinations were conducted on all of the chickens.

All egg yolks and whites obtained from the group (treated or control) on each collection day were pooled. Eggs were wiped clean of debris and separated into whites and yolks. All of the egg, excreta, organ, and tissue samples were frozen immediately after collection and stored at less than −10 °C.

Sample Processing and Characterization. *Radioactive Residues.* The radioactive residues in the analytical samples (egg whites and yolks, whole milk, and edible tissues) were characterized by radiometric, solubility, and chromatographic procedures. Tissues, with the exception of fat (all types), were homogenized in 4 volumes of 10% ethanol, and aliquots equal to approximately 500 mg of tissue were combusted. The tissue homogenates were combusted using a Packard Tri-Carb Tissue Oxidizer (model C306, C307, or D306) with Permafluor in combination with Carbosorb (Packard, Downers Grove, IL) as the scintillation fluid. Aliquots of egg whites and yolks, fat (other), and skin (plus fat) were solubilized and counted directly in ScintiVerse LC scintillation cocktail (Fisher Scientific, West Haven, CT). All sample types (∼50−500 mg each) were analyzed in replicate. Samples were counted in a liquid scintillation analyzer (Packard models 2000 CA, 2500 TR, and 4530) for a single 5−10 min period. The background value for directly counted samples (egg whites, yolks, and fat) was less than 40 dpm. The background for combusted samples [40 counts per minute (cpm)] was taken from a historical average of blank samples processed under the same conditions. Similar values were obtained during this study by combustion of tissues from control samples. Counts per minute were converted to disintegrations per minute using an internal AES versus efficiency curve. The curve was derived from a set of commercially prepared quenched standards. The total radioactive residue (TRR) was calculated from the radioactivity determined from the quench curve and the quantity of analytical sample taken for analysis.

Residue Release Experiments. Analytical samples containing greater than 0.01 ppm eq were extracted with a sequence of solvents to characterize the solubility characteristics of the radioactive residues within the sample. An aliquot of tissues and eggs were macroscopically homogenized with a scalpel, and subsamples were transferred to centrifuge tubes. Diethyl ether containing 1% trifluoroacetic acid (TFA) was immediately added, and the samples were homogenized using a Polytron and then centrifuged. The supernatant was transferred to a scintillation vial. The extraction was repeated with a second portion of ether/TFA. The extracts were combined, reduced in volume, and counted after adding scintillation cocktail. The remaining residue was then reextracted with two portions of methanol containing 1% TFA. The methanol extracts were combined in a scintillation vial, reduced in volume, and the extracted radioactivity was determined. The residue was washed with dilute ammonium hydroxide, and the wash was counted. The washed residue was hydrolyzed with 3 N sodium hydroxide at approximately 90 °C for 1 h. The pH of the hydrolysate was adjusted to ≤3 and the hydrolysate centrifuged. The supernatant was decanted and counted. Any remaining residue or precipitate formed on addition of acid was combusted. Milk samples also were treated using these procedures following a hexane extraction. Solvent extracts, washes, or digests containing greater than 0.01 ppm eq were further examined by chromatography. To increase sensitivity, larger sample sizes were taken and modifications to the solvent extraction procedures were made. Furthermore, reconstitution and filtration of the preparations was required to provide compatibility with the chromatographic systems utilized to identify and characterize separated components.

Chromatographic Analysis. Chromatographic profiles of sample extracts were obtained to further characterize the radioactivity released during solvent extraction and hydrolysis experiments. Three systems were routinely used for the analytical samples, and two additional systems were utilized for the identification of specific reincorporation products. The first HPLC system utilized for routine analysis was reverse

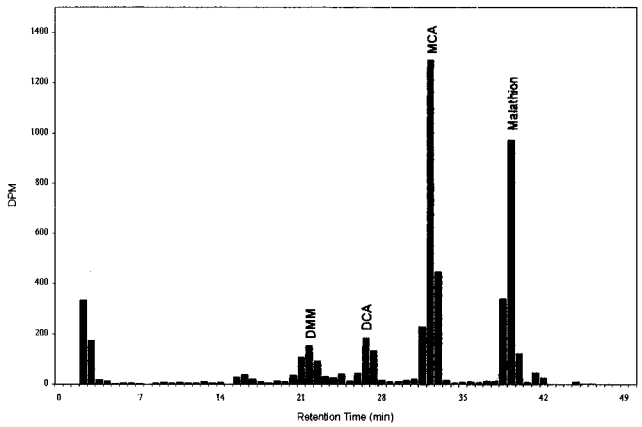

Figure 1. Radiochromatogram of a mixture of a [^{14}C]-malathion standard and goat urinary metabolites: MCA, malathion monocarboxylic acids (α and β); DCA, malathion dicarboxylic acid; and DMM, desmethylmalathion. Separations were obtained using system A (see Materials and Methods).

phase operated in a binary solvent gradient mode designed to separate malathion and immediate metabolites and was used to profile goat urine, chicken feces, and the solvent extracts and hydrolysates from tissues, egg whites and yolks, and milk samples. A chromatogram obtained from a mixture of [^{14}C]-malathion and goat urinary metabolites is illustrated in Figure 1. A second reverse phase system also was used to separate highly nonpolar species (e.g., fatty acids, triglycerides, and lecithins). A third system, based on anion exchange separation, was utilized to separate very polar components extracted with methanol/trifluoroacetic acid or contained in 3 N sodium hydroxide hydrolysates. The two additional systems were based on carbohydrate separation and were used in appropriate experiments.

System A. Reverse phase HPLC of intermediate polarity compounds utilized a Perkin-Elmer model 250 Binary high-performance liquid chromatograph. The analytical column was a Spherisorb ODS-2 analytical column (5 μm, 15 cm $\times$ 4.6 mm inside diameter, stainless steel) which was preceded by a Brownlee NewGuard guard column (RP-18, 7 μm, 1.5 cm $\times$ 3.2 mm inside diameter). Separations were obtained at ambient temperatures. Detection utilized a Spectra-Physics model 100 variable-wavelength detector operated at 230, 254, or 280 nm. Separation of malathion and immediate metabolites was obtained with a program performed with solvent A (water containing 0.2% TFA) which was held for 5 min, followed by a linear program to 80% solvent B (acetonitrile containing 0.1% TFA) in 40 min. A column rinse gradient of 80 to 100% B in 5 min was initiated at the end of the program before returning to initial conditions. The flow rate was 1 mL/min.

System B1. Reverse phase separation of nonpolar radioactive metabolites in chicken tissues and eggs, as observed in diethyl ether extracts and saponified fat samples, was obtained using the same conditions described for system A except a mixture of water/acetonitrile/trifluoroacetic acid (20/80/0.2, v/v/v) was utilized for solvent A and tetrahydrofuran for solvent B. A linear gradient from 100 to 0% A in 30 min was initiated immediately following injection. The final solvent conditions were maintained at 0% A for 20 min before return to initial conditions.

System B2. Studies to characterize and identify residues in goat tissue samples utilized a ternary reverse phase HPLC system with separation comparable to that described for system B1. The operating parameters were exactly as described for system A, except that, after completion of the gradient, tetrahydrofuran was linearly added at a rate of 2% per minute for 50 min.

System C. The anion exchange system was comprised of an Aminex HPX87H column (300 $\times$ 7.8 mm inside diameter, stainless steel) at a column temperature of 60 °C (Fiatron Model 50, Milwaukee, WI) and a mobile phase of 0.01 N H_2SO_4 (isocratic). The mobile phase was delivered at a flow rate of 0.4 mL/min using a Waters 440 pump. A Spectra-Physics variable-wavelength detector was used to monitor the chromatographic eluant.

System D. The gel permeation chromatographic system was composed of a Waters Sugar-Pac I column (stainless steel) operated at a temperature of 90 °C. The mobile phase, water (18 MΩ resistivity), was delivered isocratically at a flow rate of 0.4 mL/min using a Waters 440 pump. A Shodex refractive index detector or a Spectra-Physics variable-wavelength detector was used to monitor the chromatographic eluant.

System E. Carbohydrate analysis was obtained using a Waters carbohydrate analysis column (300 $\times$ 4 mm inside diameter, stainless steel) with a mobile phase containing 80% acetonitrile/20% water (isocratic) operated at a flow rate of 2 mL/min (ambient temperature). A Shodex refractive index detector was used to monitor the chromatographic eluant.

Injection volumes of 50−100 μL were made using an Alcott model 738 autosampler or a Rheodyne model 7125 fixed loop (100 μL) manual injector. Fractions of the chromatographic eluant were collected using Foxy or Retriever IV fraction collectors (ISCO, Lincoln, NE). The eluant collection rate was 0.5−1.4 mL of eluant collected per vial. The number of fractions collected during a chromatographic run varied from approximately 30 to 100 and was dependent upon the analytical system used. An overlay technique was used which permitted increased sensitivity. This technique utilized repeated injections of a single sample with fraction collection at specific intervals. Collected fractions at each specific interval were overlaid with those of previous injections. Collected fractions were counted by LSC after adding scintillation cocktail. Chromatograms were reconstructed and integrated using commercial and custom software packages. Quantitation of components identified during chromatographic analysis was based on the specific activity of malathion in the dose, and the values are reported as parts per million equivalence (ppm eq).

HPLC system recovery was determined for the analytical samples and radiolabeled standards by comparing the radioactivity collected in the fractions to that injected onto the column. The radioactivity contained in the chromatographic eluant was determined from the summation of background-corrected radioactivity contained within the fractions collected. The amount of radioactivity injected was determined directly by counting an aliquot of the same solution that was injected into the HPLC. Typical background activities ranged from approximately 35 to 45 dpm/fraction and were obtained from chromatograms of analytical sample extracts and matrix and solvent blanks.

Goat Urine/Chicken Feces. Goat urine, collected from treated animals during the study, was used as a source of radioactive malathion metabolites. Radiochromatograms of urine samples were obtained using system A. Urine samples were filtered (0.45 μm PTFE, Gelman) and analyzed directly. Chicken feces were extracted with acetonitrile, filtered, and injected. Identification of the major metabolites was performed from HPLC retention time comparison and TLC migration coincidence to authentic standards. TLC utilized a solvent system of methylene chloride/ethyl acetate/formic acid on silica gel 60 F-254 plates as described by Ryan and Fukota (1984, 1985).

Saponification of Fat Samples. The diethyl ether extractable portion of goat and chicken fat was saponified with crushed potassium hydroxide in the presence of glycerol (nonlabeled diluent) at approximately 130 °C for 3 h. After cooling, the mixture was neutralized with sulfuric acid and extracted with 3 portions of hexane. The hexane extracts were combined, evaporated, and reconstituted in tetrahydrofuran. The reconstituted hexane extract was analyzed by reverse phase HPLC (system B). Radiolabeled oleic and stearic acid standards also were chromatographed on the same system. The aqueous phase, radiolabeled glycerol, and a glycerol-fortified sample of the aqueous phase were analyzed using system D, described above.

Milk Fractionation. Aliquots (10 g of whole milk) were centrifuged at 10 000 rpm for 1 h at 4 °C to separate the milk

Table 1. Distribution of TRR in Solvent Extracts and Hydrolysates of Goat Milk[a] during Administration of Five Oral Doses of Malathion (172 mg/Day)

		percent of TRR released[b,c]				
	day:	1	2	3	4	5
treatment	TRR (ppm eq):[b]	1.42	2.05	2.32	2.46	2.39
hexane		5.7	7.7	8.6	5.8	5.6
diethyl ether/1% TFA		15.2	14	13.3	11	13.8
methanol/1% TFA		74.4	75	69.4	67.3	68.5
0.2 N NH_4OH		4.9	5.4	3.7	3.5	4.2
3 N NaOH/90 °C		0.4	1	0.7	0.7	0.6
residue		6.1	8.2	7.3	8.1	8.8

[a] Composited milk sample from a.m. and p.m. collections. [b] Average of duplicate determinations. [c] Summation of columnar values provides cumulative extraction recovery (percent of TRR).

Table 2. Distribution of TRR in Solvent Extracts and Hydrolysates of Goat Tissues following Five Oral Doses of Malathion (172 mg/Day)

		percent of TRR released[a,b]							
	tissue sample:	back fat	omnental fat	perirenal fat	heart	kidney	liver	LD muscle[d]	SM muscle[e]
treatment	TRR (ppm eq):[b]	1.14	0.94	0.99	0.38	1.96	2.26	0.28	0.25
diethyl ether/1% TFA		84.5	83.8	66.5	21.1	16.7	18	58.4	69.3
methanol/1% TFA		4.9	3.7	2.9	29	24.1	40.9	16.3	26.5
0.2 N NH_4OH		ND[f]	2	1	2.7	1.8	4.5	ND	ND
3 N NaOH/90 °C		5.8	3	5.4	50.2	43	30.2	34.8	38.8
residue		*c*	5.7	16.5	19.9	12.8	15.6	8.1	8.2

[a] Average of duplicate determinations. [b] Summation of columnar values provides cumulative extraction recovery (percent of TRR). [c] No residue remained after NaOH hydrolysis. [d] LD, longissimus dorsi. [e] SM, semimembranous. [f] ND, not detectable (MDL <0.01 ppm eq).

into fat, whey, and casein layers. The milk fat, isolated as a thick plug on the surface of the milk, was removed after solidification by cooling to 0 °C. The isolated fat was weighed, and aliquots were counted by LSC. After removal of the fat, the sample was centrifuged. The liquid portion (principally whey) was transferred to a beaker, and the pH was adjusted to 4.5 ± 0.3 with 20% formic acid to precipitate soluble casein remaining in the liquid. The beaker was heated in a water bath (30 °C for 30 min). The contents of the beaker were then added to the solids remaining after the initial centrifugation, and the sample was recentrifuged (10 000 rpm for 30 min at 4 °C). The whey (supernatant) and casein (solids) were separated. Aliquots of whey and casein (dried) were counted by LSC and prepared for HPLC analyses.

Statistical Analysis. Calculation of mean, standard deviation (SD), and relative standard deviation (RSD) followed generally accepted methods. Statistical evaluations of the egg production and egg quality data were conducted using Contingency Analysis.

RESULTS

Clinical Signs and Pathology. Comparison of body and organ weights, feed consumption, egg quality and production, and urine and feces production during quarantine and the treatment period with malathion indicated no statistical differences. No gross pathological signs were observed.

Goat-Feeding Studies. *Magnitude and Distribution of Residues in Edible Tissues and Milk.* Following repeated oral dosing of radiolabeled malathion to goats for 5 days in an amount equivalent to 86 ppm in feed, radioactivity was found in all of the analytical samples. Characterization of the residues solubilized by extraction and hydrolysis indicated a wide diversity of chemical entities shown by differing polarities in goat milk (Table 1) and tissues (Table 2).

Poultry-Feeding Studies. *Magnitude and Distribution of Residues in Edible Tissues and Eggs.* The radioactivity following repeated oral dosing of radiolabeled malathion to chickens for 4 days in an amount equivalent to 28 ppm in feed was widely distributed among egg whites and yolks (Table 3) and edible tissues

Table 3. Distribution of TRR in Solvent Extracts and Hydrolysates in Egg Whites and Yolks following Four Oral Doses of Malathion (3.8 mg/Day)

Egg Whites

		percent of TRR released[a,b]			
	day:	1	2	3	4
treatment	TRR (ppm eq):[a]	0.32	0.18	0.21	0.33
diethyl ether/1% TFA		62.5	22.2	19.0	6.1
methanol/1% TFA		9.4	11.1	23.8	21.2
0.2 N NH_4OH		3.1	5.6	4.8	3.0
3 N NaOH/90 °C		3.1	38.9	81.0	48.5
residue		ND[c]	33.3	57.1	21.2

Egg Yolks

		percent of TRR released[a,b]			
	day:	1	2	3	4
treatment	TRR (ppm eq):[a]	<0.01	0.03	0.35	0.96
diethyl ether/1% TFA		ND	33.3	62.9	77.1
methanol/1% TFA		ND	ND	5.7	7.3
0.2 N NH_4OH		ND	ND	ND	ND
3 N NaOH/90 °C		ND	66.7	28.6	26
residue		ND	ND	8.6	8.3

[a] Average of duplicate determinations from pooled sample. [b] Summation of columnar values provides cumulative extraction recovery (percent of TRR). [c] ND, not detectable (MDL < 0.01 ppm eq).

(Table 4) and within the solvent extracts and hydrolysates used to characterize residue release.

Identification and Characterization of Residues in Goat and Poultry Samples. *Malathion and Immediate Metabolites.* Chromatographic analysis of goat and chicken excreta (system A) confirmed the absence of malathion but revealed the presence of MCA, DCA, and DMM. These metabolites are consistent with those previously reported for rat (Ryan and Fukota, 1984, 1985), poultry (March et al., 1956a,b), and humans (Dary et al., 1994) as well as with our own investigations in rat (unpublished results). Analysis of acidic diethyl ether extracts, using the chromatographic conditions described for system A, indicated the absence of malathion at or above the minimum detection limit

Table 4. Distribution of TRR in Solvent Extracts and Hydrolysates in Chicken Tissues following Four Oral Doses of Malathion (3.8 mg/Day)

		percent of TRR[a,b]						
treatment	tissue: TRR (ppm eq):[a]	heart 0.28	kidney 1.31	liver 0.74	light meat 0.11	dark meat 0.11	fat 0.18	skin 0.16
diethyl ether/1% TFA		14.3	1.1	29.7	27.3	27.3	100.0	62.5
methanol/1% TFA		17.9	17.6	21.6	18.2	18.2	5.6	6.3
0.2 N NH_4OH		ND[c]	2.3	1.4	ND	ND	ND	ND
3 N NaOH/90 °C		35.7	35.9	23.0	63.6	63.6	5.6	43.8
residue		14.3	19.8	13.5	18.2	18.2	ND	ND

[a] Average of duplicate determinations. [b] Summation of columnar values provides cumulative extraction recovery (percent of TRR). [c] ND, not detectable (MDL < 0.01 ppm eq).

(MDL, 0.01 ppm eq). Pooled egg whites collected on day 1, however, contained a broadly eluting peak, which appeared to contain multiple components near the retention time of malathion and MCA. Fortification experiments indicated the retention time of the radioactivity was inconsistent with malathion and inconclusive for MCA. The observation was concluded to be the result of a crack in the eggshell which permitted passage of radioactivity because no similar profiles were obtained for egg white samples collected on subsequent days as well as the higher concentration of radioactivity on this day as compared to those of subsequent days (Table 3). Further characterization of these components was not pursued. MCA and DCA, the only other significant malathion metabolite observed in the tissues, were extracted with ether from a goat kidney sample. The chromatographic profile (system A) indicated DCA was 0.06 ppm eq for duplicate preparation and analyses; MCA was observed at 0.02 ppm eq. With these exceptions, the absence of malathion and metabolites in these tissues is consistent with that previously reported by others. The presence of these metabolites was attributed to unexcreted urine residues remaining in the kidney tissue. Significant quantities of these metabolites were identified in goat urine.

Fatty Acids/Glycerol. The radioactivity present in all of the ether extracts eluted as a component, which was identified as species containing an acyl glycerol moiety. Under system B2 conditions utilized for goat tissue and milk samples, this peak eluted with a retention time of approximately 90 min (Figure 2A). A predominant peak was observed in chicken tissue samples with a retention time of approximately 28 min (system B1, data not shown). This component was preceded by a smaller peak, which eluted at approximately 21 min in egg samples. Following saponification, chromatograms of the lipid portion yielded multiple peaks (Figure 2B) which differed in retention time from peaks in unsaponified samples. However, two peaks in the saponifed sample exhibited retention times which were identical to those of radiolabeled oleic and stearic acids (Figure 2C). Fortification of the lipid portion with ^{14}C-labeled stearic and oleic acid confirmed coelution of these standards with two peaks in the saponifed sample. Analysis of the aqueous phase (system D) indicated a single radioactive component which was identified as glycerol by fortification with ^{14}C-labeled standard (Figure 3). The radioactivity associated with the fat portion of a fractionated milk sample (day 5) represented 17.4% of the TRR.

Pyruvic Acid. Pyruvic acid was extracted from tissues with acidic methanol. The retention time of the radiolabeled standard was 18 min when it was analyzed using system C. Peaks at the same retention time were observed in the extracts of goat (Figure 4B) and chicken liver and kidney and were enhanced on fortification of the extracts with the radiolabeled standard. Radiolabeled pyruvate in liver was 0.62 ppm eq in goat and 0.09 ppm eq in chicken. Kidney pyruvate was 0.14 ppm eq in the goat and 0.02 ppm eq in the chicken. Peaks

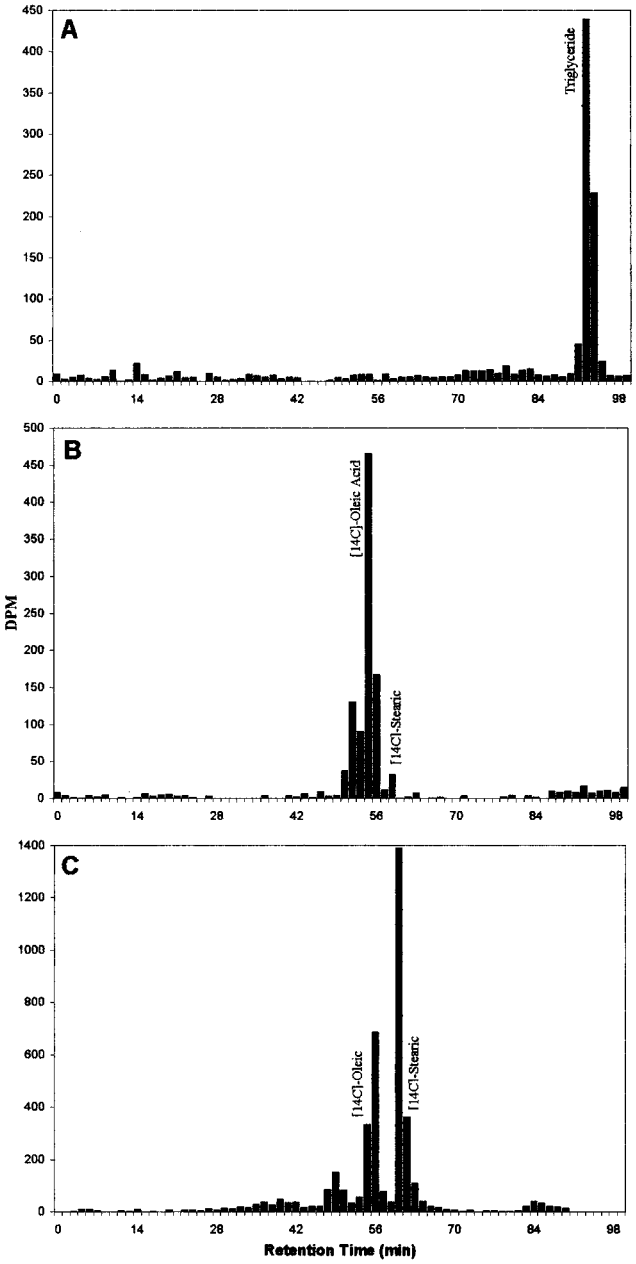

Figure 2. Radiochromatograms demonstrating incorporation of malathion as fatty acids in goat back fat: (A) ether extract of goat back fat, (B) back fat following saponification, and (C) mixed standard containing [^{14}C]oleic and [^{14}C]stearic acids. Separations obtained using system B1 (see Materials and Methods).

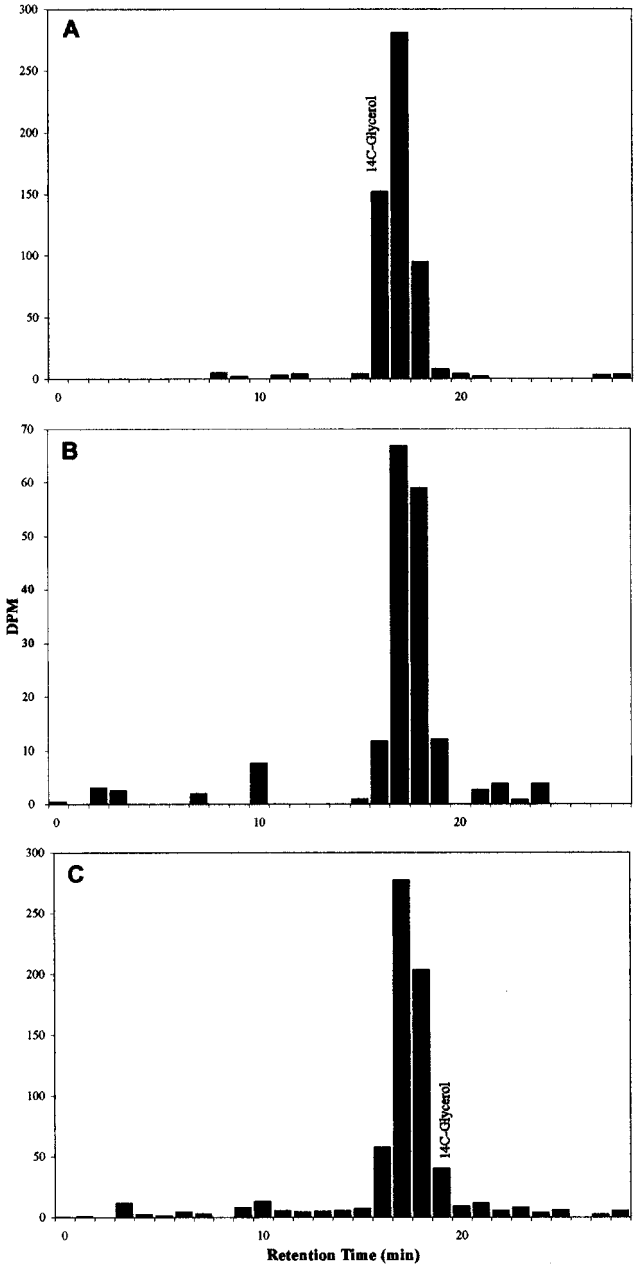

Figure 3. Radiochromatograms demonstrating reincorporation of radioactivity as glycerol: (A) [^{14}C]glycerol standard, (B) aqueous extract of a saponified sample of goat fat, and (C) aqueous extract following fortification with [^{14}C]glycerol. Separations obtained using system D (see Materials and Methods).

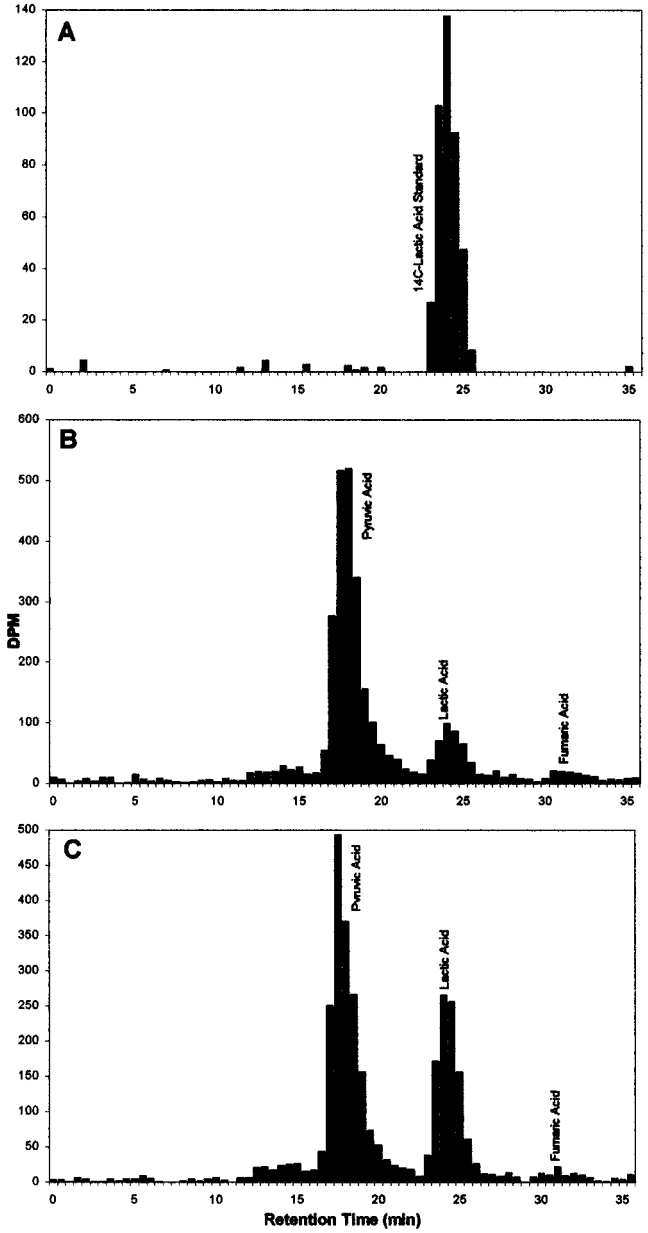

Figure 4. Radiochromatograms demonstrating incorporation of malathion as lactic acid in goat liver tissue: (A) [^{14}C]lactic acid standard, (B) acidic methanol extract of goat liver, and (C) extract following fortification with [^{14}C]lactic acid. Separations obtained using system C (see Materials and Methods).

attributable to pyruvic acid also were evident in other tissue extracts but typically represented less than 0.03 ppm eq.

Lactic Acid. Lactic acid was extracted from tissues with acidic methanol. The retention time of the [^{14}C]-lactic acid standard was 24 min when determined under system C conditions (Figure 4A). A peak at the same retention time was observed in the methanolic extracts of goat (Figure 4B) and chicken liver and kidney samples and was the major component in kidney extracts. The lactic acid concentration was 0.13 and 0.16 ppm eq in goat liver and kidney, respectively. Chicken liver did not contain detectable lactate, but 0.03 ppm was found in kidney extracts. Fortification of the chicken and goat kidney and goat liver extracts with the radiolabeled standard resulted in coelution of the sample and standard peaks (Figure 4C). The concentration of lactic acid in goat and chicken heart tissues was 0.04 and 0.01 ppm eq, respectively, while other tissues, milk, or eggs did not contain detectable quantities of this species.

Fumaric Acid. Fumaric acid was extracted from tissues with acidic methanol. The retention time of [^{14}C]-fumaric acid was 29.5 min when analyzed using system C. This component, identified by fortification experiments, represented 0.01 ppm eq in chicken liver and 0.04 ppm eq in goat liver (Figure 4B) and kidney. Small but significant peaks (less than or equal to 0.01 ppm eq) were observed in the chromatograms for several other tissue extracts. However, the concentration of this component did not exceed 0.01 ppm eq in any other chicken samples and was approximately 0.04 ppm eq in goat samples.

Lactose. The major peak observed in the radiochromatogram of a whey sample (Figure 5C), isolated from fractionated whole milk, was consistent with that of a

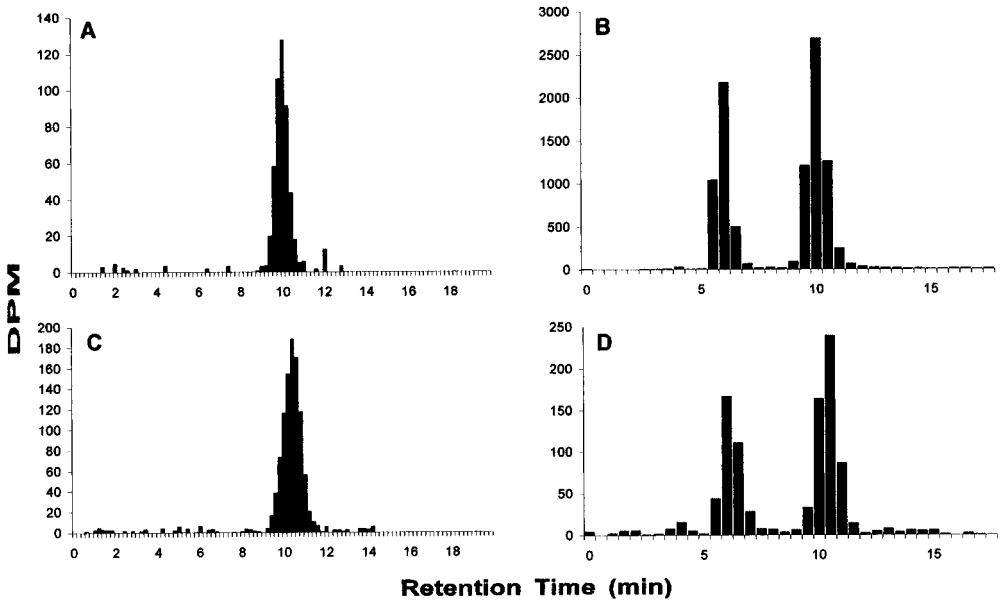

Figure 5. Radiochromatograms demonstrating incorporation of malathion as lactose in goat milk: [^{14}C]lactose standard before (A) and after (B) hydrolysis (90 °C for 220 min with 1 N HCl) and whey isolated from goat milk (day 4) before (C) and after (D) hydrolysis. Separations obtained using system E (see Materials and Methods).

[^{14}C]lactose standard (Figure 5A) when chromatographed using system E and was enhanced after fortification with authentic [^{14}C]lactose. Confirmatory identification was obtained following hydrolysis of the [^{14}C]lactose standard and whey sample. The standard (Figure 5B) and the whey sample (Figure 5D) exhibited similar rates of hydrolysis (90 °C in 1 N HCl for 90 and 220 min). Glucose and galactose, coeluting at 6.5 min, were the expected hydrolysis products of the whey sample and the lactose standard. The radioactivity associated with the whey portion of a fractionated milk sample represented 50.1% of the TRR. Lactose also was extracted by acidic methanol extraction of milk and identified using system C.

Reincorporation as Protein. Reincorporation of malathion into protein was indirectly determined using casein, isolated from the milk of treated goats, as a source of radiolabeled protein. Casein represented approximately 24% of the sample TRR. Identification was obtained by comparing the chromatogram obtained at 230 nm following Pronase E reaction to that of a similarly treated casein standard (system A). Tyrosine, liberated by the action of the enzyme, eluted with a retention time of 15 min in both samples. The experimental conditions utilized for enzymatic hydrolysis indicated an association of radioactivity release with enzymatic action but did not provide sufficient radioactivity for further characterization of the hydrolysis products. Hydrolysis of concentrated solutions of the isolated casein under more rigorous conditions (3 N NaOH at 90 °C) was performed to increase the yield of hydrolyzed products and permit characterization studies.

The radiochromatogram of base-hydrolyzed casein, tissues, milk, and egg samples obtained with system A contained a diffuse and unresolved band of radioactivity eluting over a wide range of retention times (10−30 min) in addition to peaks eluting near (5−10 min) and with the void volume. On the basis of the similarity of the radiochromatogram obtained for milk casein hydrolysate, the majority of the chromatographed radioactivity in tissue and milk samples was interpreted as the release of numerous hydrophilic (void peaks), intermediate polarity (5−10 min), and hydrophobic amino acids and small peptides (broad unresolved band, 10−30 min). The elution of hydrophobic amino acids in the retention time window of 10−30 min was confirmed from the retention time of tyrosine (15 min); the elution of hydrophilic amino acids with the void volume was demonstrated using [^{14}C]glutamic acid. Further investigation of the reincorporation of the radiolabel into individual amino acids was not performed. The estimation of protein content in hydrolyzed tissue, egg, and milk samples was based on the total radioactivity contained in the radiochromatograms (system A). The radioactivity associated with the casein portion of a fractionated milk sample was 24.1% of the TRR.

DISCUSSION

The cumulative results from this study indicated that malathion was rapidly and quantitatively metabolized following repeated oral ingestion by lactating goats and laying chickens. The radiolabel was widely distributed throughout edible tissues and was associated with naturally occurring biochemicals. The products of metabolism were consistent with those arising from catabolic and anabolic reactions of maleic, fumaric, and malic acids as intermediates common to both malathion metabolism and the tricarboxylic acid cycle (TCA cycle). As substrates for the TCA cycle, extensive dilution of the radiolabel occurred with the concurrent production of biochemicals with widely varying physicochemical properties. Typically, more than 80% of these species were identified (Tables 5−7). Investigations to identify additional components using specific radiolabeled standards were not pursued.

The polar radioactive residues following malathion administration (i.e., those extracted with methanol or dilute ammonia or hydrolyzed with base during this study) have not been previously characterized. In proteinaceous tissues, these residues were identified as TCA cycle components and proteins. Only insignificant amounts of polar residues were associated with fat samples.

The cumulative recovery of radioactivity following oral administration of radiolabel was not determined. Uri-

Table 5. Identity and Concentration (ppm eq) of Residues in Goat Tissues and Milk Samples during Five Daily Oral Exposures to Malathion (172 mg/Day)[a]

tissue sample:[b] TRR (ppm eq):	back fat 1.74	omnental fat 1.5	perirenal fat 1.42	heart 0.39	kidney 1.71	liver 2.23	LD[g] muscle 0.36	SM[h] muscle 0.26	milk samples:[e] TRR (ppm eq):	day 1 1.49	day 3 2.66
malathion	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01		<0.01	<0.01
MCA[i]	<0.01	<0.01	<0.01	<0.01	0.01[c]	<0.01	<0.01	<0.01		<0.01	0.01
DCA[j]	<0.01	<0.01	<0.01	<0.01	0.06[c]	<0.01	0.03	<0.01		<0.01	<0.01
malaoxon	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01		<0.01	<0.01
DMM[k]	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01		<0.01	<0.01
triglyceride	1.25	1.02	0.87	0.05	0.10	0.07	0.14	0.18		0.25	0.52
oleic acid	0.11	0.03	<0.01	<0.01	0.03	0.10	<0.01	0.03		<0.01	<0.01
stearic acid	<0.01	<0.01	0.04	<0.01	<0.01	0.03	<0.01	<0.01		<0.01	<0.01
pyruvic acid	≤0.04	*d*	≤0.03	0.02	0.14	0.62	0.03	<0.01		*d*	*d*
lactic acid	≤0.04	*d*	≤0.03	0.04	0.16	0.13	0.03	0.04		*d*	*d*
fumaric acid	≤0.04	*d*	≤0.03	0.01	0.04	0.04	<0.01	0.01		*d*	*d*
protein	0.04	0.07	0.08	0.18	0.79	0.87	0.10	0.08		0.09	0.11
lactose	*d*	*d*	*d*	*d*	*d*	*d*	*d*	*d*		1.05[f]	1.56[f]

[a] Tissue samples obtained approximately 24 h after receiving last dose. [b] Results from analysis of one tissue sample obtained from one of two treated animals. Residues isolated by solvent extraction and identified by retention time coincidence to radiolabeled standards. [c] Average of duplicate analysis. [d] Matrix not analyzed for this component. [e] Composited milk sample from a.m. and p.m. collections. [f] Identified in whey portion of fractionated milk sample. [g] LD, longissimus dorsi. [h] SM, semimembranous. [i] MCA, monocarboxylic acid of malathion (malathion half-ester). [j] DCA, dicarboxylic acid of malathion. [k] DMM, desmethylmalathion.

Table 6. Identity and Concentration (ppm eq) of Residues in Poultry Tissues following Four Daily Oral Exposures to [^{14}C]Malathion (3.8 mg/Day)[a]

tissue sample: TRR (ppm eq):	fat 0.18	skin 0.16	heart 0.28	kidney 1.31	liver 0.74	light meat 0.11	dark meat 0.11
malathion	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
MCA[b]	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
DCA[c]	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
malaoxon	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
DMM[d]	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
triglyceride	0.12	0.06	<0.01	0.06	0.24	<0.01	<0.01
oleic acid	<0.01	<0.01	<0.01	<0.01	0.14	<0.01	<0.01
stearic acid	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01
pyruvic acid	<0.01	<0.01	<0.01	0.02	0.09	<0.01	<0.01
lactic acid	<0.01	<0.01	0.01	0.03	<0.01	0.02	0.02
fumaric acid	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01
protein	0.04	0.08	0.08	0.34	0.22	0.04	0.04

[a] Results from pooled samples ($n = 4$). [b] MCA, monocarboxylic acid of malathion (malathion half-ester). [c] DCA, dicarboxylic acid of malathion. [d] DMM, desmethylmalathion.

Table 7. Identity and Concentration (ppm eq) of Residues in Poultry Egg Whites and Yolks during Four Daily Oral Exposures to [^{14}C]Malathion (3.8 mg/Day)[a]

egg whites: TRR (ppm eq):	day 1 0.32	day 2 0.18	day 3 0.21	egg yolks: TRR (ppm eq):	day 3 0.35	day 4 0.96
malathion	0.10	<0.01	<0.01		<0.01	<0.01
MCA[c]	0.02	<0.01	0.02		<0.01	<0.01
DCA[d]	<0.01	<0.01	<0.01		<0.01	<0.01
malaoxon	<0.01	<0.01	<0.01		<0.01	<0.01
DMM[e]	<0.01	<0.01	<0.01		<0.01	<0.01
triglyceride	<0.01	<0.01	<0.01		0.23	0.62
oleic acid	<0.01	<0.01	<0.01		<0.01	<0.01
stearic acid	<0.01	<0.01	<0.01		<0.01	<0.01
pyruvic acid	*b*	*b*	*b*		*b*	*b*
lactic acid	*b*	*b*	*b*		*b*	*b*
fumaric acid	*b*	*b*	*b*		*b*	*b*
protein	0.06	0.11	0.14		0.10	0.17
lactose	*b*	*b*	*b*		*b*	*b*

[a] Results from pooled samples ($n = 4$). [b] Matrix not analyzed for this component. [c] MCA, monocarboxylic acid of malathion. [d] DCA, dicarboxylic acid of malathion. [e] DMM, desmethylmalathion.

nary and fecal elimination represented a major excretory pathway. In chickens, fecal elimination represented approximately 29% of the daily administered dose. Daily excretion of urinary radioactivity in goats averaged 50−60%, and feces was 8−14% (data not shown). The loss of radiolabel through expired air was not investigated. However, this pathway may represent significant elimination because of the number of energy-producing substrates identified during this study.

The magnitude of the radioactive residues found during this study was consistent with previous literature reports. The identification and characterization of these residues suggested that malathion metabolites were reincorporated via acetyl-CoA into a variety of biochemical classes. The absence of malathion and potentially toxic metabolites in conjunction with the rapid metabolism and reincorporation of the radiolabel into long-lived endogenous biochemicals strongly suggests that exposure via ingestion of contaminated edible tissues is of no toxicological concern.

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