Chang, A. C.; Page, A. L.; Bingham, F. T. *J.—Water Pollut. Control Fed.* **1981**, *53*, 237–245.
Chang, A. C.; Page, A. L.; Foster, K. W.; Jones, T. E. *J. Environ. Qual.* **1982**, 11, 409–412.
Council for Agricultural Science and Technology *Application of Sewage Sludge to Cropland: Appraisal of Potential Hazards of Heavy Metal to Plants and Animals*, Report No. 64 CAST: Ames, IA, Nov 1976.
Council for Agricultural Science and Technology *Effects of Sewage Sludge on the Cadmium and Zinc Content of Crops*, Report No. 84, 1980.
Dahlquist, R. L. *Appl. Spectrosc.* **1978**, *32*, 1–30.
Dowdy, R. H. Larson, W. E.; Titrud, J. M.; Laterrell, J. J. *J. Environ. Qual.* **1978**, *7*, 252–257.
Epstein, E.; Jeffries, R. L. *Annu. Rev. Plant Physiol.* **1964**, *15*, 169–184.
Hinesly, T. D.; Jones, R. L.; Ziegler, E. L.; Tyler, J. J. *Environ. Sci. Technol.* **1977**, *11*, 182–188.
Hinesly, T. D.; Alexander, D. E.; Ziegler, E. W.; Barrett, G. L. *Agron. J.* **1978**, *70*, 425–428.
John, M. K.; Van Laerhoven, C. J. *Environ Pollut.* **1976**, *10*, 163–173.
King, L. D.; Dunlap, W. R. *J. Environ. Qual.* **1982**, *11*, 608–616.
Lee, C. R.; Page, H. R. *Agron. J.* **1967**, *59*, 237–240.
Milliken, C. R. *J. Aust. Inst. Agric. Sci.* **1961**, *26*, 220–233.
National Research Council, National Academy of Sciences *Mineral Tolerance of Domestic Animals*; NRC: Washington, DC, 1980.
Page, A. L.; Bingham, F. T.; Nelson, C. *J. Environ. Qual.* **1972**, *1*, 288–291.
Peterson, O. *Swed. J. Agric. Res.* **1977**, *7*, 21–24.
Sittig, M *Handboook of Toxic and Hazardous Chemicals*; Noyes Publications: Park Ridge, NJ, 1981; pp 119–122.
Soane, B.D.; Saunders, D. H. *Soil Sci.* **1959**, *42*, 203–216.
SPSS^x: *A Complete Guide to the SPSS*^{x} *Language and Operations* SPSS Inc.: Chicago, Il, 1983.
Tucker, M. R. North Carolina Department of Agriculture, Raleigh, NC, personnel communication, 1983.

Received for review February 27, 1985. Revised manuscript received September 13, 1985. Accepted April 7, 1986. The financial support to conduct this work was granted by USDA-CSRS through the office of Agricultural Research, North Carolina Agricultural and Technical State University, and is very much appreciated.

Fate of [^{14}C]Deltamethrin in Lactating Dairy Cows

M. Humayoun Akhtar,* Kenneth E. Hartin, and H. Locksley Trenholm

Fate and residues of radiocarbon- (^{14}C-) labeled (*gem*-dimethyl or benzyl) deltamethrin were determined in lactating dairy cows after oral administration for 3 consecutive days and slaughtering the animals 24 h after the last dose. Orally administered deltamethrin (10 mg/kg of body weight) appeared to be poorly absorbed, but the deltamethrin that was absorbed was extensively metabolized and excreted in the bile and urine with very little accumulation in major edible tissues. Approximately 36–43% of the total administered radiocarbon was eliminated in feces within 24 h after the last dose. The major portion (78–82%) of ^{14}C compound in feces was deltamethrin. Only 4–6% of the administered ^{14}C was eliminated in urine, and 0.42–1.62% was secreted in the milk. Radiocarbon secreted into milk and was higher for the *gem*-dimethyl portion (0.69 μg/g) than for the benzyl moiety (0.36 μg/g). Deltamethrin was the major identifiable product in milk (0.10–0.14 μg/g). Radiocarbon content in various tissues was generally very low (0.1 μg/g) with the exception of liver, kidney, udder, abdominal, and subcutaneous fats which were higher.

Synthetic pyrethroids belong to a new generation of pesticides introduced as agricultural insecticides about 10 years ago that have been gaining acceptability very rapidly. One of the important members of this class of compounds is deltamethrin, (*s*)-α-cyano-3-phenoxybenzyl (*1R,3R*)-*cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate, also known as RU-22974, NRDC-161, OMS-168, decamethrin, Decis, and K-Orthin. It has been registered throughout the world to control insect pests that attack a variety of crops (FAO, 1981). While it has been developed to control pests of livestock and man, very little is known about its metabolism and residues in milk and meat of farm animals.

The metabolic fate of deltamethrin has been reported for rats (Ruzo et al., 1978), mice (Ruzo et al., 1979), mouse liver microsomes (Shono et al., 1979), and cow and chicken liver homogenates (Akhtar, 1984). Recently Akhtar et al. (1985) have carried out a detailed study on the fate of deltamethrin in laying hens and reported that the insecticide was efficiently absorbed and rapidly metabolized. In the present study the objective was to determine the fate of orally dosed ^{14}C-labeled (*gem*-dimethyl or benzyl) deltamethrin in lactating dairy cows with regard to the nature of ^{14}C residues in milk, fat, muscle, urine, and feces.

Animal Research Centre, Agriculture Canada, Ottawa, Ontario K1A 0C6, Canada.

MATERIALS AND METHODS

Chemicals. Radiocarbon- (^{14}C-) labeled and unlabeled deltamethrin were supplied by Hoechst of Canada through Roussel Uclaf of France. The two forms of [^{14}C]deltamethrin preparations used in the study were [^{14}C]-*gem*-dimethyl (>98% radiochemical purity), and [^{14}C]benzyl (>95% radiochemical purity, *m*-phenoxybenzaldehyde and *m*-phenoxybenzoic acid were the major radioactive impurities). Authentic metabolites and spectral data for positive identification of other metabolites were available from a previous study (Akhtar et al., 1985). The abbreviations used in the text, tables, and figures as structural designations for the products are the same as used in a recent publication (Akhtar et al., 1985).

Preparation of [^{14}C]Deltamethrin for Oral Administration. To a solution of a known amount of unlabeled deltamethrin in acetone was added a calculated quantity of individual preparations of [^{14}C]deltamethrin (*gem*-dimethyl, 55.6 mCi/mmol; benzyl, 59.3 mCi/mmol). The solution was thoroughly mixed, and the solvent was allowed to stand at room temperature in a fumehood to

0021-8561/86/1434-0753$01.50/0 Published 1986 by the American Chemical Society

remove the solvent. The specific activity was analyzed, in triplicate, to give the following (i) [^{14}C]-*gem*-dimethyl, 0.241 μCi/mg; (ii) benzyl, 0.286 μCi/mg. To prepare the daily doses the radiolabeled insecticide was weighed directly into gelatin capsules, size 10 (Torpac Ltd., Willowdale, Ontario, Canada), and securely capped. The empty gelatin capsules disintegrated totally within 1 h when allowed to stand in tap water (pH 6.8) at room temperature.

Animal Treatment. Cows with a daily milk production of 18–22 L (Holstein, 557 kg) and 13–15.8 L (Ayrshire, 504 kg) were used in this study. They were kept in individual stalls and fed to appetite a standard ration (hay, corn silage, grass silage, and a concentrate) for lactating cows for 14 days. Daily feed intake was recorded.

The animals were maintained on the standard ration throughout the entire experiment. They were catheterized for urine collection 3 days prior to commencement of oral dosage of the insecticide. However, as both animals deposited heavy sediment in the catheter, this resulted in no initial urine collection after the first oral dosage. The catheters were removed immediately and rubber tubing used to make artificial bovine vaginas was sutured to the vulvas with the aid of local caudal anesthetic (7 mL, 2% Lidocaine). The free end of the tubing was fitted with a plastic bag for total daily urine collection. Feces was collected in metal trays. Cows were milked individually by a standard portable milking unit (De Laval).

Control feces, urine, and milk samples were collected from each cow just prior to commencement of oral dosages. Both cows were dosed orally for 3 consecutive days after the a.m. milking. A balling gun and capsule facilitated dosing the Holstein with [*gem-dimethyl*-^{14}C]deltamethrin and the Ayrshire with [*benzyl*-^{14}C]deltamethrin.

During the entire experiment, feces and urine were collected on a 24-h basis, while milk was collected on approximately an 8:00 and 16:00 schedule. The urine and feces were weighed, mixed separately (feces was mixed on a Hobart Model 606 mixer), and subsampled. Milk was collected, weighed, and subsampled in the morning and evening and stored in glass containers at 4 °C.

The animals were slaughtered at 24 h after the last dose, and samples of liver, kidney, heart, brain, leg muscle, spleen, udder, lung, and abdominal and subcutaneous fats were removed, frozen, and stored at −20 °C until analyzed.

Measurement of Total Radiocarbon. (*a*) *Feces.* Daily representative samples (50 g), in duplicate, were extracted with methanol (4 × 100 mL); the volume was reduced and adjusted to 250 mL. The radiocarbon content in the extracts was determined by liquid scintillation counting (LSC) of 0.1 mL of the extracts, in duplicate, on a Beckman Model LS-235 using both internal and external standard methods. Extracted residues (~100–120 mg), after air-drying at room temperature for about 1 week, were combusted in a Tri-carb 360D oxidizer (Packard) and counted by LSC. The combined ^{14}C content in the methanol extracts and residues represented the total ^{14}C excreted in the feces.

(*b*) *Urine.* Radiocarbon in the urine was determined by direct LSC of 1 mL of urine, in triplicate, in 15 mL of Biofluor (New England Nuclear) using an internal standard method of adjustment for quenching.

(*c*) *Milk.* Radioactivity in milk was measured by direct LSC of 1 mL of milk, in triplicate, in 15 mL of Biofluor. Again, an internal standard technique was used for quench correction.

(*d*) *Tissues and Blood.* Triplicate samples of tissues, organs, daily a.m. blood (about 250 mg), and fats (approximately 100 mg) were combusted in the Packard oxidizer and counted by LSC using an external standard quench correlation.

Extraction, Isolation and Analyses of ^{14}C Metabolites. Radiocarbon metabolites of [^{14}C]deltamethrin were extracted from various biological samples and analyzed as follows:

(*i*) *Feces.* Feces were extracted with methanol as described above. The methanol extract was concentrated and partitioned between ether and water, and the ether layer was analyzed by TLC followed by GC, after derivatization where necessary (Akhtar et al., 1985).

(*ii*) *Urine.* The acidified (pH 2) urine (100 mL) was extracted with an equal volumes of ether three times. Radiocarbon in the ether and residual aqueous phases was quantitated by LSC after appropriate volume adjustment. The ether extracts, after reduction in volume, were analyzed by TLC followed by GC of the extracts of radiocarbon regions before and after methylation with diazomethane.

(*iii*) *Milk.* Two types of extraction procedures were used:

(a) Milk samples were first extracted with hexane (3× with equal volume of milk) to remove less polar compounds. The butterfat content was determined by evaporation of a portion of hexane layer and weighing the dried residue. A portion of the hexane layer was shaken with equal volume of acetonitrile (3×). The aqueous phase was filtered to remove fatty residues, and the filtrate was adjusted to pH 2 and extracted with ether. The radiocarbon content of each phase was determined.

(b) For analyses of the nature of ^{14}C, metabolites in milk samples (100 mL), in triplicate, were extracted with a mixture of ethanol–ether (1:3) after addition of 5 mL of 5% aqueous potassium oxalate (Gaughan et al., 1978). Radioactivity in both the organic and aqueous phases were determined. The organic extract was evaporated to near dryness in a rotary evaporator, and the residue was dissolved in hexane (~25 mL) and transferred into a 100-mL separatory funnel. The hexane solution was extracted with acentonitrile (3 × 25 mL), and the radioactivity in both the hexane and acetonitrile phases was measured.

Abdominal Fat. Fat samples were extracted with hexane in a blender following the procedure of Gaughan et al. (1978), and the extracts were analyzed by TLC.

Liver. The liver was homogenized in a blender with water and acetone (1:5) (Akhtar, 1982), the homogenate was vacuum filtered, and the aqueous phase was extracted with hexane. The residual aqueous phase was brought to pH 2 by addition of a few drops of concentrated HCl and extracted with ether. Radiocarbon content in residues, hexane, ether, and extracted aqueous phases was quantitated by combustion and LSC. Organic extracts were analyzed by TLC. The extracted aqueous phase was not investigated further.

Kidney. Kidney was homogenized with aqueous methanol (1:5) and the homogenate filtered. The filtrate was concentrated to remove methanol and extracted with ether. Removal of ether gave an oily material that was redissolved in hexane and partitioned with acetonitrile. The aqueous phase was heated with 6 N HCl at 90 °C for 1 h and the reaction mixture cooled and extracted with ether. Radiocarbon was determined at each level. The acetonitrile and ether extracts, after acid hydrolysis, were analyzed by TLC followed by GC.

Chromatography. (*i*) *Thin-Layer Chromatography.* Extracts were applied on precoated silica gel plates (0.25–2.0-mm thickness; Whatman, KGF, PLK5F) and developed in benzene saturated with formic acid–ether (10:3, v/v) in one dimension. The various ^{14}C components

Table I. Elimination of Radiocarbon in Excreta (Urine and Feces of Lactating Cows during and after Three Oral Administrations of [^{14}C]Deltamethrin)

time after init treatment, days	cum % of total ^{14}C admin at that point			
	feces[a]		urine	
	gem-dimethyl	benzyl	*gem*-dimethyl	benzyl
1	15.7[a]	16.5[a]	*a*	*a*
2	31.0	21.7	5.5[b]	4.4[b]
3 (1)[d]	43.3	36.2	4.2[c]	4.9[c]

[a] Urine and feces combined due to problem with catheter. [b] Based on a single dose. [c] Based on two cummulative doses. [d] Value in parentheses is the day after the last dose.

Table II. Deltamethrin Equivalent in the Blood of Lactating Cows during and after a Daily Dose of 10 mg/kg of Body Weight of [*gem*-*dimethyl*-^{14}C]- or [*benzyl*-^{14}C]Deltamethrin on Each of 3 Consecutive Days

time after treatment commenced, h	[^{14}C]deltamethrin equiv, μg/g	
	gem-dimethyl	benzyl
24	0.13 (0.106)[a]	0.17 (0.135)
48	0.13 (0.053)	0.23 (0.093)
72	0.23 (0.059)	0.36 (0.097)

[a] Values in the parentheses (%) refer to percentage of the total administered dose at that point. An average blood volume of 8.11% of body weight was used in calculation (Dukes, 1960).

on a plate were visualized by exposing the plates to X-ray film (Kodak X-Omat AR) and then quantitated by direct LSC of the appropriate gel regions (Ivie and Hunt, 1980; Akhtar et al., 1985). In order to elucidate the structures of ^{14}C components, various gel regions were extracted with ether and/or methanol.

(*ii*) *Gas Chromatography*. Extracts of various gel regions were analyzed on a Perkin-Elmer Sigma 1 gas chromatograph system equipped with both a ^{63}Ni electron capture detector (ECD) and a flame ionization detector (FID). A fused silica capillary column (15 m × 0.256 mm SE-54 (0.25-μm thickness)) was used with ECD, while a glass column (1.52 m × 4 mm) packed with 5% OV-210 was utilized with FID system.

RESULTS

Absorption and Excretion of Radiocarbon. Orally dosed [*gem*-*dimethyl*-^{14}C]- or [*benzyl*-^{14}C]deltamethrin given at 10 mg/kg of body weight for 3 consecutive days was poorly absorbed and eliminated slowly (Table I). In the first 24 h after the initial administration of the ^{14}C compounds only 15–17% of the dose was eliminated in the excreta (feces and urine were collected together due to problems with catheter). However, more radiocarbon was eliminated in feces on repeated dosing. Approximately 43 and 36% of the total administered radiocarbon from [*gem*-*dimethyl*-^{14}C]- and [*benzyl*-^{14}C]deltamethrin, respectively, was eliminated in the feces within 24 h after the last dose. Only about 4–6% of the total administered ^{14}C from each preparation was excreted in the urine. Moreover, deltamethrin equivalent in blood was also very low (range 0.053–0.136% of the total administered dosage) (Table II). It was also interesting to note that residue levels in blood taken 24 h after the initial dose were higher than those recorded for blood taken 24 h after the subsequent daily dosage. Note: Radioactivity counts of control blood, urine, feces, and milk samples were at, or close to, the background.

Secretion of Radiocarbon in Milk. Radiocarbon was secreted into the milk of cows treated with [*gem*-*dimethyl*-^{14}C]- or [*benzyl*-^{14}C]deltamethrin. As expected, [^{14}C]deltamethrin equivalents were consistently higher

Table III. Deltamethrin Equivalent in the Milk of Lactating Cows during and after a Daily Dose of 10 mg/kg of Body Weight of [*gem*-*dimethyl*-^{14}C]- or [*benzyl*-^{14}C]Deltamethrin on Each of 3 Consecutive Days

time after treatment commenced, h	[^{14}C]deltamethrin equiv,[a] %	
	gem-dimethyl	benzyl
1st dose[b]	5.50 g[c]	5.05 g[c]
p.m. (8 h)	0.34 (0.27)[d]	0.05 (<0.01)
a.m. (24 h)	1.23 (0.38)	0.2 (0.11)
2nd dose[b]	11.0 g[c]	10.10 g[c]
p.m. (8 h)	0.97 (0.58)	0.19 (0.21)
a.m. (24 h)	1.45 (0.37)	0.34 (0.23)
3rd dose[b]	16.50 g[c]	15.15 g[c]
p.m. (8 h)	1.21 (0.69)	0.30 (0.36)
a.m. (24 h)	1.62 (0.54)	0.42 (0.32)

[a] Cumulative data expressed as percentage of the total administered dose(s) at that point. [b] Orally dosed after each a.m. milking. [c] Cummulative dose at that point. [d] Values in parentheses refer to (μg/g) equivalent value.

Table IV. Amount of Deltamethrin Equivalent (μg/g) in Tissues of Lactating Cows Given Orally a Daily Dose of [*gem*-*dimethyl*-^{14}C]- or [*benzyl*-^{14}C]Deltamethrin at 10 mg/kg of Body Weight on Each of 3 Consecutive Days

tissues[a]	[^{14}C]deltamethrin equiv, μg/g	
	gem-dimethyl	benzyl
liver	2.23	3.19
kidney	1.28	2.24
brain	nd	0.09
heart	0.12	0.13
breast muscle	nd[b]	0.06
leg muscle	nd[b]	0.09
spleen	nd	0.08
udder	0.42	0.62
lung	nd	0.21
abdominal fat	0.28	0.56
subcutaneous fat	0.40	0.54
tongue	nd	0.16
bile	6.4	21.4

[a] Cows were killed at 24 h after the last dose. [b] Nondetected, detectable limit 0.01 μg/g.

with [^{14}C]-*gem*-dimethyl than the [^{14}C]benzyl preparation (Table III). Radiocarbon was detected early, in the evening milk samples, obtained about 8 h after the first dose but constituted <0.01 μg/g for the benzyl and 0.27 μg/g for the *gem*-dimethyl moieties (Table III). The amount of radiocarbon secreted in milk continued to increase on repeated dosages. Even at this high dosage level and total administered amounts (16.5 g for *gem*-dimethyl- and 15.15 g for benzyl-labeled deltamethrin over 3 days), the highest level of deltamethrin equivalents secreted into milk was still very low, e.g. 0.69 μg/g for the *gem*-dimethyl and 0.36 μg/g for the benzyl moieties on the p.m. milk after the last dose represented only 1.62% and 0.42% of the total administered doses for [*gem*-*dimethyl*-^{14}C]- and [*benzyl*-^{14}C]deltamethrin, respectively. Almost all [^{14}C] residues (78–96%) of the ^{14}C in the milk were found in the cream.

Radiocarbon in Tissues. The amounts of deltamethrin equivalents in various tissues and organs of cows treated with [*gem*-*dimethyl*-^{14}C]- and [*benzyl*-^{14}C]-deltamethrin and killed 24 h after their last dose are shown in Table IV. Although trace levels of radiocarbon were found in all tissues and organs assayed, levels were considerably higher in liver, kidney, abdominal and subcutaneous fat, and bile. In contrast ^{14}C levels in brain, heart, spleen, muscle, lung, and fat were very low or nondetectable.

Nature of Radiocarbon in Feces, Urine, Milk, and Tissues. *Feces*. Extraction of fecal samples of cows

Table V. Metabolites in the Methanol Extracts of Feces of Lactating Cows Given Orally [*gem*-*dimethyl*-^{14}C]- or [*benzyl*-^{14}C]Deltamethrin

compd	% of ^{14}C	
	gem-dimethyl	benzyl
deltamethrin	80	73
Br_2CA	8	
PBald		12
PBacid		3
unidentified	12	12

treated with [*gem-dimethyl*-^{14}C]- or [*benzyl*-^{14}C]-deltamethrin with methanol recovered 94–97% of the radiocarbon present in feces. TLC analyses of the methanol extracts showed that the major portion of the extractable radiocarbon was associated with the unchanged deltamethrin. From the *gem*-dimethyl moiety, 80% of the ^{14}C in the methanol extract was due to deltamethrin and about 8% to Br_2CA, and the remaining 12% consisted of unidentified materials. Similarly, about 73% of the extracted radiocarbon from the benzyl moiety was in the form of deltamethrin, 12% as PBald, and 3% as PBacid, and remaining radiocarbon (12%) was not authenticated (Table V). There did not appear to be major differences in the relative amounts of unchanged deltamethrin eliminated in feces of day 1 to day 3. Presence of a high percentage of unchanged insecticide suggested that the insecticide was poorly metabolized and degraded by rumen microflora and enzymes in the gastrointestinal tract. Similar observations were made when deltamethrin was incubated with rumen fluid at 37 °C for up to 72 h. Only 20% degradation of the insecticide occurred. Moreover, about 92–96% of the applied radioactivity was recovered from the incubation mixture, suggesting that deltamethrin was not degraded to $^{14}CO_2$ to any appreciable extent Length of incubation (24–72 h) did not show any appreciable difference in the amount of deltamethrin metabolized.

Urine. Small amounts (4–6%) of radiocarbon were excreted in the urine. Approximately 78 and 85% of radiocarbon present in urine samples of the cows treated with [*gem-dimethyl*-^{14}C]- and [*benzyl*-^{14}C]deltamethrin, respectively, were extracted with ether from acidified (pH 2) urine samples. TLC analyses of extracts for two preparations revealed the presence of several ^{14}C regions. Extracts from the [^{14}C]-*gem*-dimethyl moiety exhibited regions due to c-Br_2CA, c-CH_2OH-c-Br_2CA-lactone, c-COOH-c-Br_2CA, c-CH_2OH-c-Br_2CA, t-CH_2OH-c-CH_2OH-c-Br_2CA-lactone. Identities of these compounds were established by comparing TLC and GC retention times, after derivatization where necessary, with metabolites that were recently identified in laying hens (Akhtar et al., 1985). Similarly, extracts of urine treated with [*benzyl*-^{14}C]-deltamethrin exhibited radioactive regions due to PBald, PBacid, and 4′-HO-PBacid along with other unidentified and water-soluble materials. The amounts and nature of metabolites identified, from the two preparations, in urine samples of day 3 (24 h after the last treatment) are shown in Table VI. Aqueous fractions, after extraction with ether at pH 2, were not investigated further.

Milk. Cream was separated by centrifugation of the whole milk (10 mL), in triplicate, at 14000 rpm for 30 min. The radioactivity in the cream phase for the [^{14}C]-*gem*-dimethyl preparation was 86% and 96% for the [^{14}C]-benzyl moiety in day 3, a.m. milk. The butterfat content of milk was 3.79% for Holstein and 3.27% for Ayrshire. Organic solvent (ethanol–ether, 1:3) extracted 43 and 80% of total ^{14}C from the milk of [^{14}C]-*gem*-dimethyl- and [^{14}C]benzyl-treated cows, respectively. Trace amounts of radioactivity were detected in the solid residues in milk

Table VI. Metabolites in Urine of Lactating Cows Given Orally [*gem*-*dimethyl*-^{14}C]- or [*benzyl*-^{14}C]Deltamethrin

compd	% of ^{14}C	
	gem-dimethyl	benzyl
deltamethrin	tr	tr
Br_2CA[a,b]	15	
c-CH_2OH-c-Br_2CA-lactone[b]	34	
c-COOH-c-Br_2CA[a,b]	8	
c-CH_2OH-c-Br_2CA[a,b]	5	
t-COOH-c-CH_2OH-c-Br_2CA-lactone[a,b]	12	
water-sol plus unident	26	
PBald		8
PBacid[a]		35
4′-HO-PBacid[a]		9
water-sol plus unident		48

[a] Both free and conjugates. [b] See Figure 1 for structures and abbreviations.

Table VII. Location of ^{14}C in Milk of Cows Treated Orally for 3 Consecutive Days with [^{14}C]-*gem*-Dimethyl- or [^{14}C]Benzyl-Labeled Deltamethrin

	% of ^{14}C in whole milk		
	acetonitrile sol	hexane sol	water sol
gem-dimethyl[a]	16	30	54
benzyl[b]	62	4	34

[a] p.m. milk (0.69 μg/g) after the third dose, method b (see section on extraction). Trace amounts of radiocarbon residues were detected in the extracted milk residues. [b] p.m. milk (0.21 μg/g) after the second dose, method a (see section on extraction).

Table VIII. Major Metabolites in Liver, Kidney, and Abdominal Fat of Cows Given Orally [*gem*-*dimethyl*-^{14}C]- or [*benzyl*-^{14}C]Deltamethrin

compd	tissues					
	liver		kidney		fat	
	gem	benzyl	*gem*	benzyl	*gem*	benzyl
deltamethrin	24	23	32	35	60	90
Br_2CA[a]	23		33		16	
PBald		tr		tr		tr
PBacid[a]		32		23		
water sol	16	10	16	18		
unextractable[b]	37	23	19	24		

[a] Also contains other unidentified metabolites in minor quantities. [b] Not extracted with organic solvent.

of the cow treated with [*gem-dimethyl*-^{14}C]deltamethrin.

Organo-extractable ^{14}C from [^{14}C]-*gem*-dimethyl was not fully transferred into the acetonitrile phase. Almost 63% of the total extractable ^{14}C remained in the hexane phase. On the other hand, greater than 75% of the total organo-extractable ^{14}C from [^{14}C]benzyl was transferred into acetonitrile. The only ^{14}C compound identified in the acetonitrile phases from both the [^{14}C]-*gem*-dimethyl and [^{14}C]benzyl was unchanged deltamethrin. Treatment of the aqueous phase from [^{14}C]-*gem*-dimethyl with 3 N HCl at 60 °C for 2 h afforded ether extractable compound(s) (~35% of the total aqueous radioactivity). The major compound identified in the hydrolysate was Br_2CA. The aqueous phase from [^{14}C]benzyl was not subjected to acid hydrolysis. Distribution of ^{14}C in the milk is shown in Table VII.

Liver. About 41–47% of the total ^{14}C in liver for the [^{14}C]-*gem*-dimethyl and 58–69% of total liver ^{14}C from the [^{14}C benzyl] preparations were extracted with organic solvents. A good portion (34–50%) of the extractable ^{14}C was the unmetabolized deltamethrin. The remaining extractable ^{14}C from [^{14}C]-*gem*-dimethyl was mainly associated with Br_2CA while that from [^{14}C benzyl] was due

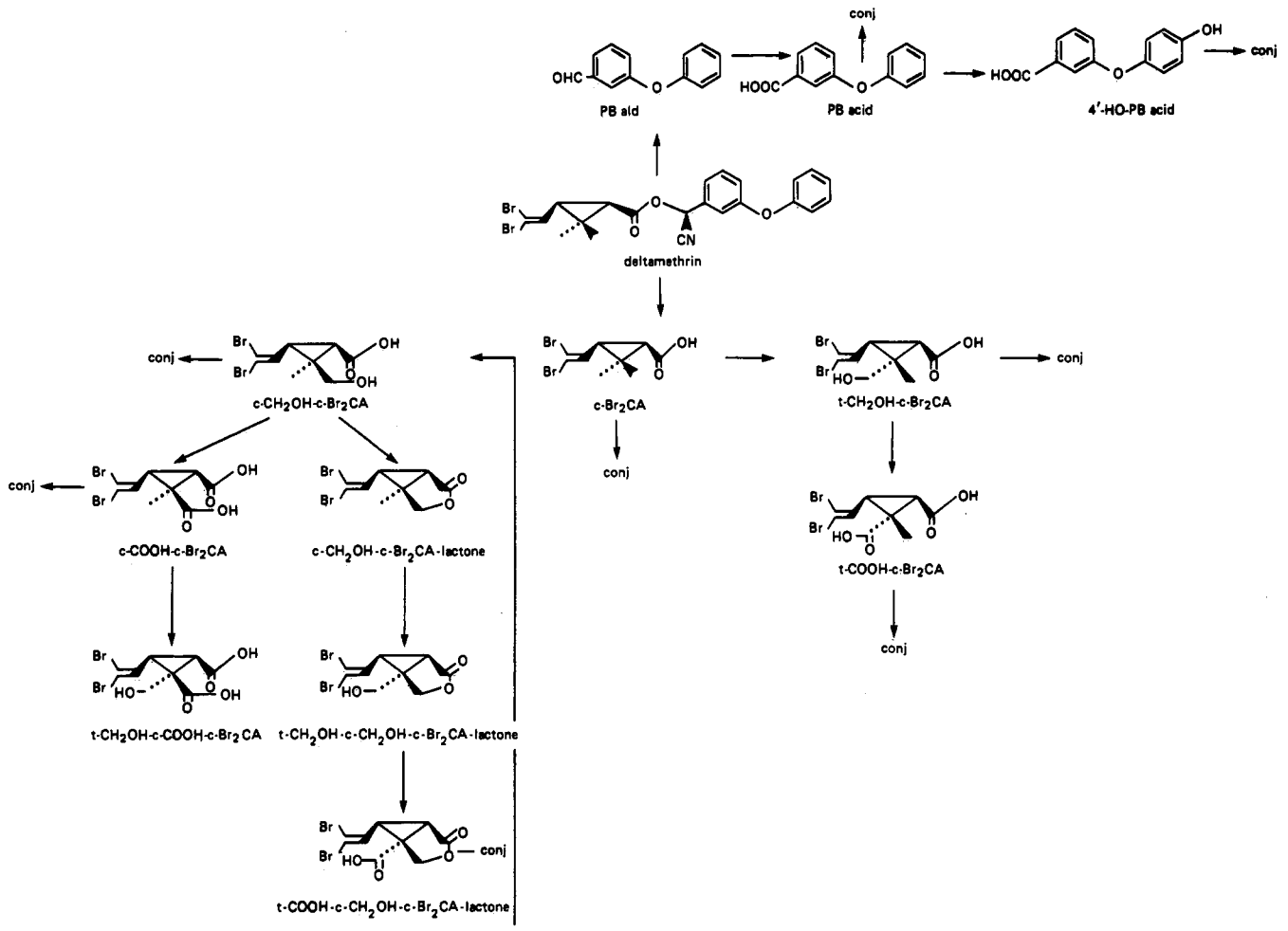

Figure 1. Proposed metabolic pathways of deltamethrin in lactating dairy cows.

to PBacid (major) and some unidentified metabolites. The results of the nature of ^{14}C in the liver are given in Table VIII. The residual ^{14}C in extracted residues and aqueous phase was not investigated further.

Kidney. Unmetabolized deltamethrin constituted 25–35% of the total ^{14}C in kidney. Other major ^{14}C components identified after acid hydrolysis of the aqueous phases were Br_2CA from [^{14}C]-*gem* dimethyl and PBacid from [^{14}C]benzyl preparations (Table VIII). The identities of deltamethrin, Br_2CA, and PBacid were authenticated by GC.

Fat. The abdominal fat from a cow treated with [*gem*-dimethyl-^{14}C]deltamethrin contained 0.284 μg/g of deltamethrin equivalents whereas the value was 0.56 μg/g for a cow treated with [*benzyl*-^{14}C]deltamethrin. About 88–92% of the total radioactivity in fat was extracted with organic solvents. About 90% of the extractable ^{14}C from the [^{14}C]benzyl preparation was in the form of unmetabolized deltamethrin. Extracts from the [^{14}C]-*gem*-dimethyl preparation also contained deltamethrin as the major component along with Br_2CA (Table VIII).

DISCUSSION

The data presented here show that orally dosed deltamethrin was not rapidly absorbed by the gastrointestinal tract within 24 h after the last of the three consecutive daily oral dosages at 10 mg/kg of body weight. The insecticide was slowly eliminated in feces mainly as unchanged deltamethrin. Only a small portion (4–6%) of the total administered dose was excreted in the urine. Furthermore, residues in blood at the time of sacrifice accounted for <0.1% of the total administered dose. In contrast, studies of structurally related pyrethroids with lactating cows showed that radioactivity from [^{14}C]cypermethrin was eliminated in equal proportions in urine and feces (Croucher et al., 1985). Elimination of [^{14}C]-*cis*-permethrin resulted in 22–28% excretion in urine and 60–76% in the feces (Gaughan et al., 1978).

Radioactivity was detected in the milk of cows treated with [^{14}C]deltamethrin within 8 h after the first dose and accounted for 0.27 μg/mL of deltamethrin equivalents for the [^{14}C]-*gem*-dimethyl and <0.01 μg/mL for the [^{14}C]-benzyl preparations. Radiocarbon residues in milk continued to increase after each successive dosage of [^{14}C]-deltamethrin and reached a peak of 0.69 μg/mL for [^{14}C]-*gem*-dimethyl and 0.36 μg/mL for [^{14}C]benzyl in the evening milk after the third and last dose. Most (86–96%) of the total ^{14}C in milk was located in the cream. The amount of ^{14}C (as a percentage of total ^{14}C in milk) in cream was as high as those observed for cypermethrin (95%; Croucher et al., 1985), permethrin (70–90%; Gaughan et al., 1978), and deltamethrin (89%; FAO, 1981). Although ^{14}C was found mainly in the lipophilic compartment of milk (cream), a significant amount of ^{14}C could not be extracted with the organic solvents employed. The unextractable property was much more pronounced in the milk of [^{14}C]-*gem*-dimethyl-treated cow. The only compound identified in the acetonitrile phases was unchanged deltamethrin. On the basis of the ^{14}C content in the acetonitrile phases, the amount of unchanged deltamethrin was calculated to range between 0.10 and 0.14 μg/g. This value appears to be very low when one considers the high level of deltamethrin administered. The aqueous phase also contained Br_2CA (free and conjugated) and explains,

in part, higher values of ^{14}C in the milk of [^{14}C]-*gem*-dimethyl-treated cows.

Radiocarbon reesidues in various tissues were generally very low except for liver, kidney, and abdominal and subcutaneous fats. These data are similar to those reported for cows given cypermethrin (Croucher et al., 1985), permethrin (Gaughan et al., 1978), and deltamethrin (FAO 1981) and with goats (Ivie and Hunt, 1980). About 30–50% of the total ^{14}C in liver and kidney was unmetabolized deltamethrin. In abdominal fat about 90% of ^{14}C from the [^{14}C]benzyl preparation was the parent insecticide. Kidney and liver also contained Br_2CA and PBacid as both free and as conjugate(s).

About 78–82% of the total extractable ^{14}C from feces was in the unchanged deltamethrin. Croucher et al. (1985) have also identified cypermethrin as the only radioactive compound in the feces of cows treated with [^{14}C]cypermethrin. Urine of [*gem-dimethyl*-^{14}C]deltamethrin-treated cows contained ^{14}C metabolites that could be produced by a number of pathways as shown in Figure 1. The pathway appears to resemble closely that observed in laying hens (Akhtar et al., 1985).

The data suggest that even when deltamethrin is ingested by dairy cows at very high levels, the pesticide is poorly absorbed. Metabolism by rumen microflora and/or enzymes present in the gastrointestinal tract occurs to only a minor extent. Most of the ingested deltamethrin is excreted as unchanged insecticide in the feces. Consequently, residues are secreted in milk at trace levels and do not accumulate in edible tissues to any extent. Therefore, the data to date suggest that residues at levels that are of toxic concern may not be present in the milk and other dairy food products when deltamethrin is used around dairy cattle at much lower recommended levels. In addition to this short-term feeding trial, a longer term study is required at levels reflecting likely residues in feeds, to confirm our observations reported in this paper.

ACKNOWLEDGMENT

We thank Claude Danis for his technical assistance. We are also grateful to Floyd Fisher, Lorraine Robinson, and John Shackelton in caring for the cows on test.

LITERATURE CITED

Akhtar, M. H. *J. Chromatogr.* **1982**, *246*, 81.
Akhtar, M. H. *J. Agric Food Chem.* **1984**, *32*, 258.
Akhtar, M. H.; Hamilton, R. M. G.; Trenholm, H. L. *J. Agric. Food Chem.* **1985**, *33*, 610.
Croucher, A.; Hutson, D. H.; Stoydin, G. *Pestic. Sci.* **1985**, *16*, 287.
Dukes, H. H. *The Physiology of Domestic Animals*, 7th ed.; Comsotck Publishing Associates: Ithaca, NY, 1960; p 60.
FAO (Food and Agricultural Organization) *FAO Plant Prod. Suppl.* **1981**, *26*, 113.
Gaughan, L. C.; Robinson, R. A.; Casida, J. E. *J. Agric. Food Chem.* **1978**, *16*, 1376.
Ivie, G. W.; Hunt, L. M. *J. Agric. Food Chem.* **1980**, *18*, 1131.
Ruzo, L. O.; Engel, J. L.; Casida, J. E. *J. Agric. Food Chem.* **1979**, *27*, 725.
Ruzo, L. O.; Unai, T.; Casida, J. E. *J. Agric. Food Chem.* **1978**, *26*, 918.
Shono, T.; Ohsawa, K.; Casida, J. E. *J. Agric Food Chem.* **1979**, *27*, 316.

Received for review December 12, 1985. Accepted May 8, 1986. This work was supported in part by Hoechst Canada, Ltd. Contribution No. 1367. This study was part of a coordinated program of research under the sponsorship of the Joint Food and Agricultural Organization and the International Atomic Energy Agency, Vienna, Austria.

High-Performance Liquid Chromatographic Determination of D-*arabino*-Hexos-2-ulose (D-Glucosone) in Irradiated Sugar Solutions: Application of the Method to Irradiated Mango

Laetitia Den Drijver,* Cedric W. Holzapfel, and Hendrik J. van der Linde

A strongly basic anion-exchange HPLC column coupled with an amperometric detector was used for the direct detection of a mutagenic compound, D-*arabino*-hexos-2-ulose, in γ-irradiated sugar solutions. The method was applied to an irradiated model fruit system as well as an irradiated whole fruit. Although D-*arabino*-hexos-2-ulose could be detected in the model system, no indication of its presence was found in the real fruit. This study represents the first direct analysis for radiolysis products in a whole fruit.

The γ irradiation of aqueous sugar solutions has been studied as part of our research on the wholesomeness of irradiated foodstuffs. A number of researchers have shown that γ-irradiated sugar solutions exhibit mutagenic activity (Schubert and Sanders, 1971; Schubert, 1969; Kesavan and Swaminathan, 1971; Kito et al., 1981; Schubert, 1973; Namiki et al., 1973; Aiyar and Subba Rao, 1975, 1977; Niemand et al., 1983), warranting further investigation since sugars are the main components of subtropical fruits. D-*arabino*-Hexos-2-ulose (D-glucosone) is one of the major radiolytic products of glucose and fructose (Dizdaroglu et al., 1975; Dizdaroglu and Von Sonntag, 1973; Von Sonntag, 1980; Kawakishi et al., 1973; Den Drijver, 1979.), the most common sugars in subtropical fruit. In an earlier investigation by Niemand et al. (1983), mutagenicity of glucosone toward *Salmonella* TA 100 was demonstrated. It was, therefore, necessary to develop a reliable and simple method for establishing the presence or absence of this compound in irradiated food.

The methods most commonly used for the determination of alduloses and diuloses in irradiated sugar solutions suffered from several disadvantages. We now report a simple, sensitive, high-performance liquid chromatographic method that allows the direct detection of D-glucosone in γ-irradiated sugar solutions and its application to irradiated fruit. As far as can be ascertained, this study represents the first analysis of an actual irradiated fruit for mutagenic compounds.

Chemistry Department, Atomic Energy Corporation, Private Bag X256, Pretoria 0001, South Africa (L.D.D., H.J.v.d.L.), and Rand Afrikaans University, Box 524, Johannesburg 2000, South Africa (C.W.H.).

0021-8561/86/1434-0758$01.50/0