with cold acid permits the determination of free ethylenediamine and enables EBDC to be determined by difference.

The use of $SnCl_2$ as suggested by Keppel (1969) facilitates the decomposition of EBDC to ethylenediamine and, although the mechanism is as yet obscure, its action may be unrelated to the enhanced hydrolysis observed with the addition of EDTA (Pease, 1957).

Two decomposition products of EBDC, ethylenethiourea and ETM (Czeglédi-Janko, 1967; Thorn and Ludwig, 1962), produce small yields of ethylenediamine under the conditions used for hydrolysis and could, if present in large amounts, give rise to erroneously high results. However, ethylenethiourea may be determined by independent methods (Newsome, 1972; Haines and Adler, 1973) while ETM has been determined in foods polarigraphically (Engst and Schnaak, 1970). A gas–liquid chromatographic method for ETM is being developed in this laboratory.

From a study of the stability of EBDC's in kale (Howard and Yip, 1971) it was concluded that significant decomposition occurred during 3 days of refrigeration. The results of the present experiment with lettuce indicate that if maneb reacts upon storage, the reaction products yield the theoretical amount of ethylenediamine after hydrolysis.

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Total ^{14}C Residues and Dieldrin Residues in Milk and Tissues of Cows Fed Dieldrin-^{14}C

John C. Potter,* Ronald L. Marxmiller, George F. Barber, Robert Young, Josef E. Loeffler, William B. Burton, and Lloyd D. Dixon

Two dieldrin-^{14}C cow feeding trials have been carried out. In the first trial two guernsey cows were fed dieldrin-^{14}C at a level equivalent to 0.11 ppm in their total ration for 3 weeks. The pooled mean residues of ^{14}C and dieldrin determined by a specific glpc method in milk collected at 15, 17, 19, and 21 days were 0.019 ppm equivalents and 0.017 ppm, respectively. In the second trial three guernsey cows were fed dieldrin-^{14}C at a level equivalent to 0.21–0.36 ppm in their ration for 42 days. The pooled mean residues of ^{14}C and dieldrin determined by a specific glpc method in milk collected at 28, 34, 39, and 41 days were 0.036 ppm equivalents and 0.036 ppm, respectively. The mean fat-feed ratios in the mesenteric fat, subcutaneous fat, and butter fat are 1.8, 1.3, and 3.4, respectively. None of the known or hypothetic dieldrin metabolites were detected in the milk or tissues of the cows.

The presence of dieldrin (Technical dieldrin contains 85% 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8α-octahydro-1,4-*endo,exo*-5,8-dimethanonaphthalene (HEOD) and 15% related compounds; following Chemical Abstracts usage we have used the name dieldrin for 100% HEOD) in the milk and fat of dairy cattle and other farm animals fed aldrin and dieldrin has been reported by Bann *et al.* (1956). Subsequently, metabolic fate studies of dieldrin in nonruminant animals have led to the identification of three metabolites which are (see Table I for chemical name) aldrin-*trans*-diol (Korte and Arent, 1965), the pentachloro ketone (Richardson *et al.*, 1968; Klein *et al.*, 1968), and 9-hydroxydieldrin (Richardson *et al.*, 1968; Baldwin *et al.*, 1970). Hedde *et al.* (1970) and Feil *et al.* (1970) have found aldrin-*trans*-diol and 9-hydroxydieldrin in the urine of sheep fed dieldrin. Because the sheep were not lactating the concentration of dieldrin and its metabolites in their milk was not determined.

Biological Sciences Research Center, Shell Development Co., Modesto, California 95352.

In order to determine whether these or other metabolites are excreted in milk or stored in the tissues of cows, two feeding experiments with lactating dairy cattle have been made. This article describes the experiments and the results.

EXPERIMENTAL SECTION

Dieldrin Synthesis. Dieldrin-^{14}C was synthesized by the Mallinckrodt/Nuclear Corporation (St. Louis, Mo. 63145) from bicycloheptadiene and uniformly labeled hexachlorocyclopentadiene-^{14}C by the method of Burton *et al.* (1957).

The radiochemical purities of the dieldrin-^{14}C preparations were determined by cochromatographing the dieldrin-^{14}C and an authentic sample of dieldrin on thin layer plates of silica gel. Both Eastman thin layer sheets (Eastman Chemicals, Eastman Kodak Co. catalog 6060, 100 μ thick with poly(vinyl alcohol) binder and lead–manganese activated calcium silicate fluorescence indicator) and Brinkman tlc plates (E. Merck, A. G. Dormstold, 250 μ thick with organic binder and manganese activated zinc

Table I. Common and Chemical Names of Dieldrin and Its Metabolites

Common name	Chemical name
Aldrin, HHDN	1,4:5,8-Dimethanonaphthalene, 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-*endo,exo*-
Photoaldrin, UVCP-aldrin	2,4,7-Metheno-1*H*-cyclopenta(a)pentalene, 1,1,2,3,3a,7a-hexachloro-2,3,3a,3b,4,6a,-7,7a-octahydro-
Photodieldrin, UVCP-dieldrin	2,4,7-Metheno-1*H*-cyclopenta(a)pentalene, 1,1,2,3,3a,7a-hexachloro-5,6-epoxy-2,3,3a,3b,4,5,6,6a,7,7a-decahydro-
9-Hydroxydieldrin, rat fecal metabolite	1,4:5,8-Dimethanonaphthalen-9-ol, 1,2,3,4,-10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,-8a-octahydro-, *endo,exo*-
Aldrin-*trans*-diol	1,4:5,8-Dimethanonaphthalene-2,3-diol, 5,6,-7,8,9,9-hexachloro-1,2,3,4,4a,5,8,8a-octahydro-, *trans*-
Pentachloro ketone, rat urine metabolite	2,4,7-Metheno-1*H*-cyclopenta(a)pentalen-3-one, 1,1,2,3a,7a-pentachloro-5,6-epoxy-2,3,3a,3b,4,5,6,6a,7,7a-decahydro-
9-Ketodieldrin	1,4:5,8-Dimethanonaphthalen-9-one, 1,2,3,4,-10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,-8a-octahydro-, *endo,exo*-
Aldrindicarboxylic acid, ADA	4,7-Methanoindene-1,3-dicarboxylic acid, 4,5,6,7,8,8-hexachloro-2,3,3a,4,7,7a-hexahydro-

silicate fluorescence indicator) were employed. The four solvent systems employed and typical results (R_f values) are as follows: *n*-hexane–acetone (3:1, v/v), 0.52 (major), 0.38 (impurity); ethyl ether–cyclohexane (3:97, v/v), 0.41; methanol–benzene (3:97, v/v), 0.72; and chloroform–carbon tetrachloride (1:1, v/v), 0.65. Only the first solvent system gave two spots. The normalized proportions of radioactivity at R_f 0.52 and 0.38 determined by liquid scintillation counting were 99.3 and 0.7%, respectively, for the material used in the first experiment and 99.8 and 0.2%, respectively, for the material used in the second experiment.

The radiochemical purity of the dieldrin-^{14}C employed in the first experiment was also determined by inverse isotopic dilution. A mixture of dieldrin-^{14}C and recrystallized nonradioactive dieldrin was crystallized three times from methanol. The specific activities of the original material and the final material were determined by liquid scintillation counting using the internal standard method. The radiochemical purity calculated from ratios of the specific activities was 98.8%.

The chemical purity determined by infrared spectrometry was 98% or above for both samples. The spectra were measured in an 8% (w/v) carbon disulfide solution with a Beckman IR-12 spectrometer equipped with a 1-mm cell with sodium chloride optics.

In the first and second experiments, the dieldrin-^{14}C was diluted with analytical grade nonradioactive dieldrin to specific activities of 2102 and 15,000 dpm/μg, respectively.

Preparation of Treated Feed. Grain concentrate containing 1.62 ppm (first experiment) or 2.5 ppm (second experiment) was prepared by applying aliquots of an ethanol solution of dieldrin-^{14}C to 0.5-kg portions of grain concentrate while they were agitated in a tilting table tumbler. The treated portions of grain concentrate were then placed in brown paper bags and stored at 2–3°. The treated grain concentrate, prepared just before the feeding experiment started, was fed to the cows as described below.

Storage Stability of Dieldrin on Grain Concentrate. Portions (50 g) of grain concentrate were fortified as described above with nonradioactive dieldrin at a level of 2 ppm. Portions were stored at −15 and 2° for 21 days and then were extracted for 20 hr in a Soxhlet extraction apparatus with a 1:1 *n*-hexane–acetone mixture. The extract was then analyzed for dieldrin by the glpc method. The samples stored at 2 and −15° were found to contain 1.7 and 1.9 ppm of dieldrin, respectively. A recovery of 90% was obtained on an untreated sample fortified with 2 ppm of dieldrin.

Feeding Experiments. The feeding experiments were carried out in a special building designed for metabolic studies with ^{14}C- and ^{32}P-labeled compounds. The building was maintained at 22–25°. Each stanchion was fitted with an individual manger. Guernsey cows were used in both experiments.

The cows were weighed and placed in the stanchions 12 days (first experiment) and 19 days (second experiment) prior to the administration of the treated feed and held in the stanchions until the test was completed. The lights remained on constantly during the test. Prior to each milking the udders and teats of each cow were washed with a detergent solution (Udder-Du, Kendell Company Fiber Products Division, Walpole, Mass.). The cows were milked at 6 a.m. and 6 p.m. with a bucket-type milking machine. The machine was carefully washed after milking each cow. Thoroughly mixed and weighed milk samples from the evening were stored overnight and combined with the samples from milk collected the following morning. All samples were stored at 2–4° until analyzed.

The cows were fed 14–16% protein dairy concentrate and cubed alfalfa hay as follows. Just before each milking the unconsumed alfalfa hay from the previous feeding was removed from the mangers and weighed. A 0.5-kg portion of treated grain concentrate was then placed in the mangers and milking started. After the treated grain concentrate was consumed, another weighed portion of untreated grain concentrate was placed in each manger. On completion of the milking, the unconsumed grain concentrate was removed from the mangers and weighed. A weighed portion of cubed alfalfa hay was then placed in each manger.

On the tenth day of the first experiment, cow 417 developed symptoms of traumatic gastritis. Her milk production decreased from 18 to 5 kg/day. She was treated with antibiotics and supportive fluids, and a permanent magnet was placed in the rumen. During the last six days of the experiment (days 15–20) the mean milk production of this cow was 10 kg/day.

On the fifth day of the second experiment the milk production of cow 4 decreased from 14.3 kg/day (4 day average) to 5–8 kg/day and her feed consumption decreased.

Table II. Glpc Standard Solutions

Std ref	Compounds	Solvent	Concn, μg/ml: For fortifying samples	Glc 1	Glc 2	Glc 3
A	Aldrin	Hexane	0.10	0.0005	0.0010	0.002
	Dieldrin		1.0	0.0010	0.0020	0.0040
	UVCP-aldrin		0.60	0.0030	0.0060	0.0120
B	9-Hydroxydieldrin	Hexane	0.40	0.0025	0.0050	0.010
	Pentachloro ketone		0.80	0.0050	0.010	0.020
	9-Ketodieldrin		1.20	0.0075	0.015	0.030
	UVCP-dieldrin		1.60	0.010	0.020	0.040
C	Aldrindicarboxylic acid (ADA)	Ethyl ether	0.20			
D	Dimethyl ester of ADA	Hexane		0.0020	0.005	0.010
E	Aldrin-*trans*-diol	1:1 ethyl acetate–ether	0.08	0.010	0.020	0.040

Attempts to raise her feed consumption and milk production to normal level by the substitution of green chop and silage for the alfalfa cubes were not successful. Her milk production for the rest of the experiment continued at a level of 5–8 kg/day.

Determination of Carbon-14. The ^{14}C in the whole milk was determined by liquid scintillation counting (Patterson and Greene, 1965) by adding 5 ml of whole milk, 7 ml of toluene liquid scintillator, and 6 ml of Triton X-100 to a counting vial. The toluene liquid scintillator (LS) contained 5.0 g of PPO and 0.1 g of dimethyl POPOP per liter. The samples were prepared in quintuplicate in the first experiment and quadruplicate in the second experiment. Toluene-^{14}C internal standard was added to two of each set of samples. The samples were counted in a Packard Tri-Carb Model 3380. All the samples were cooled for at least 48 hr before counting. The total ^{14}C levels found in the treat samples were corrected for the apparent ^{14}C content of the pretreat samples.

The total ^{14}C in the tissues was determined by burning two to four 0.5-g portions of each sample in oxygen in a quartz tube packed with copper oxide. The $^{14}CO_2$ was collected in 40 ml of a benzylamine–methanol–toluene LS mixture. After the combustion was complete the absorbent solution was diluted to 50 ml with more absorbent solution and two 20-ml aliquots were transferred to counting vials one of which was fortified with toluene-^{14}C internal standard.

Glpc Analysis. *Standards.* The milk and tissues of the cows were analyzed for dieldrin and the compounds listed in Table I. Solutions of aldrin, dieldrin, and UVCP-aldrin in hexane (standard A), 9-hydroxydieldrin, pentachloro ketone, 9-ketodieldrin, and UVCP-dieldrin in *n*-hexane (standard B), ADA in ethyl ether (standard C), dimethyl ester of ADA in ethyl ether (standard D), and aldrin-*trans*-diol in 1:1 ethyl acetate–ethyl ether (standard E) as shown in Table II were prepared for glpc standards and for fortifying samples from untreated cows for recovery analysis.

Solvents and Reagents. The following solvents and reagents were prepared or purified as described below and were checked for interfering compounds by taking appropriate blanks through the procedural steps for each analysis. If interference were found in a batch of reagent or solvent that batch was discarded. Solvents and reagents used were: dimethylformamide (MCB, Pesticide Quality or AR grade), distilled in glass taking a 60% heart cut; magnesium oxide (Fisher, USP (heavy powder)), as received; Florisil (PR grade), activated at 130° for 15–20 hr and cooled and 3% (w/w) water added, agitated, and allowed to equilibrate overnight; diazomethane, prepare according to the method of Schultz *et al.* (1971).

Table III. Glpc Conditions

A	6 ft × 2 mm[a] of 1:1 mixture of 4% OV-210–2.5% butanediol succinate, each on 80–100 mesh Supelcoport; column temperature, 210°; carrier gas flow rate, 50–60 cm³/min (nitrogen)
B	6 ft × 2 mm[a] of 1:1 mixture of 4% OV-210–2.5% butanediol succinate, each on 80–100 mesh Supelcoport; column temperature, 225°; carrier gas flow rate, 50–60 cm³/min (nitrogen)
C	5 ft × 1/8 in.[a] of 4% OV-210 on 80–100 Supelcoport; column temperature, 210°; carrier gas flow rate, 50–60 cm³/min (nitrogen)
D	5 ft × 2.5 mm i.d.[b] 1:1 (w/w) 10% QF 1 on 80–100 mesh Gas Chrom Q and 2% Reoplex 400 on 80–100 Gas Chrom Q

[a] Glass column. [b] Stainless steel.

Apparatus. A gas chromatograph (Varian Aerograph Model 1440) equipped with a tritium electron capture detector was used for the glpc determination. The conditions and column packings employed are tabulated in Table III.

A glass chromatographic column (10.5 mm i.d. × 130 mm) fitted on the top with a 50-ml reservoir and a 2-mm Teflon stopcock and a vacuum side-arm outlet was employed for chromatographic cleanup of extracts as described in the Analytical Method sections.

A centrifuge manufactured by the International Equipment Co. (Needham Hts, Mass. 02194), Model UV with a No. 239 rotor and cups and a No. 373 tube holder, was employed.

Milk Analysis for Dieldrin (First Experiment). *Extraction.* Well-mixed whole milk (40 g) and 88 ml of acetonitrile were measured into an 8-oz wide mouth polypropylene bottle and shaken for 5 min. This mixture was centrifuged for 5 min at 2000 rpm.

Partition. The supernatant (75 ml, equivalent to 25 g of milk) was transferred to a separatory funnel containing 350 ml of water. The acetonitrile–water extract was then extracted with three 90-ml portions of *n*-hexane distilled from sodium. The hexane extract was then dried with anhydrous sodium sulfate, filtered, and concentrated to approximately 2 ml for column chromatography cleanup.

Cleanup. A glass column (10.5 mm i.d. × 130 mm) was packed with 3 g of Florisil–Celite (5:1, w/w, containing 8%, w/w, water). The hexane extract was then quantitatively transferred to the column and eluted with 30 ml of hexane.

Glpc Determination. Aliquots (5 μl) of the *n*-hexane eluent and standard A were alternately injected into the glpc using condition D.

Milk Analysis for Dieldrin and Compounds Other Than Aldrindicarboxylic Acid and Aldrin-*trans*-diol (Second Experiment). *Extraction.* Well-mixed whole milk (40 g) and 88.0 ml of acetonitrile were measured into an 8-oz wide mouth polypropylene bottle and shaken for 3 min. This mixture was centrifuged for 5 min at 2000 rpm and the supernatant liquid transferred to a 200-ml graduated glass cylinder. Water (25%; 85 ml) in acetonitrile was added to the milk solids and the polypropylene bottle capped and shaken for 3 min. The mixture was then centrifuged as described above and the supernatant liquid added to the first extract in the graduated cylinder. The final volume was adjusted to exactly 200.0 ml with a 25% water–75% acetonitrile mixture. The combined extracts were stored in glass bottles.

Partition. A 10-ml aliquot of the acetonitrile–water extract equivalent to 2.0 g was transferred into a 60-ml separatory funnel and 10 ml of water added. This acetonitrile–water solution was extracted with 25 ml of hexane. The hexane extraction was repeated and the combined hexane extracts were reduced in volume to approximately 2 ml for column chromatography cleanup.

Cleanup. A glass column was packed with 3.0 g of MgO (20% Celite 545). The hexane extract was quantitatively transferred to the column and eluted first with 30 ml of hexane (fraction I) to recover aldrin, dieldrin, and UVCP-aldrin. The receiver was changed and the column was eluted with 10 ml of acetone. This second elution (fraction II) recovered 9-hydroxydieldrin, pentachloro ketone, 9-ketodieldrin, and UVCP-dieldrin. The acetone eluent was exchanged to hexane and the volumes of the two fractions were adjusted to give a sample to solvent ratio of 0.5 g:1 ml for glpc analysis.

Determination by Glpc. Glpc determinations were made by alternately injecting 5-μl volumes of each of the glpc standard A solutions and fraction I of experimental samples using glpc conditions A or 5-μl volumes of each of the glpc standard B and fraction II of experimental samples using glpc condition B (as described under the Reagents, Standards, and Apparatus sections). A standard was injected after injecting two–three samples. Calibration curves were prepared to determine the amounts of compounds present.

Milk Analysis for *trans*-Diol. *Extraction.* The extraction procedure used for dieldrin in the milk from the second experiment was used for the aldrin-*trans*-diol.

Partition. A 25.0-ml aliquot (equivalent to 5 g of milk) of the acetonitrile water extract was transferred into a 250-ml separatory funnel with 100 ml of water and 100 ml of ethyl ether and shaken. The aqueous phase was discarded and the ether was washed with an additional 100 ml of water. The ether solution was dried over 20 g of sodium sulfate in an erlenmeyer flask, and quantitatively transferred into a graduated centrifuge tube and its volume was reduced using a stream of air to near dryness. Hexane (5 ml) and 5 ml of hexane-saturated dimethylformamide (DMF) were added to the sample and the mixture was shaken vigorously.

The upper hexane phase was aspirated using a disposable pipet and discarded. The DMF solution was subjected to a stream of air until the dissolved hexane was evaporated, then transferred into a 125-ml separatory funnel with 50 ml of water and 60 ml of ethyl ether, and shaken vigorously for 3 min. The lower aqueous phase was discarded and the remaining ethyl ether solution was washed with 50 ml of water. The ethyl ether solution was dried over 10 g of sodium sulfate. The ether solution volume was reduced to near dryness in a graduated tube and exchanged to 1 ml of hexane.

Cleanup. A glass column was packed with 25 g of glass powder (disposable pipets pulverized using a Waring Blendor and carefully washed with acetone followed by hexane and oven dried). The packed glass adsorbent was prewashed with 20 ml of hexane and the 1-ml hexane solution of sample was quantitatively added to the column using a disposable pipet. Hexane (2 ml) was used to quantitatively rinse the sample from the tube and disposable pipet. Using the same graduated tube, 30 ml of hexane was measured into the column and allowed to drain. The receiver was changed and 15 ml of ethyl acetate was added to the column using the same graduated tube and rinsing the disposable pipet. The hexane eluent was discarded, the ethyl acetate was concentrated to approximately 1 ml, and the final volume was adjusted to 5.0 ml with a 1:1 mixture of ethyl acetate–ethyl ether to give a final sample to solvent ratio of 1 g:1 ml. The solution was analyzed by glpc.

Determination by Glpc. Analysis of experimental samples for the *trans*-diol was carried out by alternate 5-μl injections of experimental samples and glpc standard E using glpc condition C. A standard was injected after injecting two–three samples. A calibration curve was prepared to determine the concentrations of compound present.

Milk Analysis for Aldrindicarboxylic Acid. *Extraction and Partition.* Well-mixed whole milk (40 g), 88.0 ml of acetonitrile, and three potassium hydroxide pellets were measured into an 8-oz wide mouth bottle and shaken vigorously for 3 min. This mixture was centrifuged at 2000 rpm for 5 min. A 60.0-ml (20.0 g) aliquot of the acetonitrile–water extract was transferred into an erlenmeyer flask and 300 ml of hexane added. (The additive effect of the water content of the milk and the acetonitrile together with the shrinkage factor for this mixture gives a final extract having a sample to solvent ratio of 1 g:3 ml.) The volume of this mixture was reduced on a steam bath until only the aqueous portion remained. The flask was removed from the steam bath and the aqueous solution quantitatively transferred into an 8-oz wide mouth polypropylene centrifuge bottle. Hexane (50 ml) was added and the mixture was shaken vigorously for 2 min and centrifuged at 2000 rpm for 5 min. The hexane layer was removed by aspiration using a disposable pipet being careful not to remove any of the lower aqueous layer. The alkaline aqueous layer was quantitatively transferred into a 250-ml erlenmeyer flask. The aqueous solution was acidified with 6–7 ml of sulfuric acid (5–6 *N*) while swirling the flask in an ice bath. The flask was adjusted to room temperature, 60.0 ml of ethyl ether was added, and the mixture was shaken vigorously for 2 min. A 30-ml aliquot (10 g) of ethyl ether was quickly transferred into a 35-ml graduated centrifuge tube and concentrated to approximately 3 ml. Diazomethane reagent (1–2 ml) was added and the mixture was allowed to stand about 30 min to form the dimethyl ester derivative. The excess diazomethane reagent was removed by evaporation using a stream of air. The ethyl ether was exchanged to hexane by reducing the volume two or three times after addition of 5–10 ml of hexane never allowing the sample to go completely dry. The final volume was adjusted to 4.0 ml for column cleanup.

Cleanup. Deactivated Florisil (3 g) (3% (w/w) water) was weighed into a small beaker and suspended in 2–5 ml of hexane. This mixture was quantitatively transferred into a glass chromatographic column and about 0.5 in. of granular sodium sulfate was added to the top of this column. The column was prewashed with 25 ml of hexane and the elution rate adjusted to 2–3 drops/sec. The sample was quantitatively transferred in 4.0 ml of hexane to the column. The sample was rinsed into the column with an additional 2 ml of hexane. The column was eluted first with 40.0 ml of hexane and then with 32 ml of 40% ethyl ether in hexane. The final 25–27 ml of ether–hexane was retained for analysis. The ethyl ether–

hexane solution was concentrated to give a sample to solvent ratio of 0.2 g:1 ml. This solution was analyzed by glpc.

Determination by Glpc. Analyses of experimental samples for the dicarboxylic acid (ester derivative) were made by alternately injecting 5-μl volumes each of the experimental samples and glpc standard D. A standard was injected after injecting two-three samples. Glpc condition C was used for these analyses. A calibration curve was prepared to determine concentrations of the aldrindicarboxylic acid (ester derivative) present.

Analysis of Milk for Dieldrin Metabolites. Milk collected from cow 4 on the 29th day of the treatment period was extracted in a manner that provided maximal opportunity for the detection of ^{14}C labeled compounds more polar than dieldrin. A flow diagram of the extraction procedures is given in Figure 1. The milk (757 g) was added as a thin stream to 2 l. of vigorously stirred acetonitrile. The supernatant fluid (fraction A1) was decanted and the remaining solids were reextracted in a homogenizer with 1 l. of acetonitrile and the supernatant was decanted (fraction A2). The remaining solids were extracted two times with methanol (fractions B1 and B2) and once with benzene and the solids (fraction D) were separated from the benzene (fraction C) by filtration through a Buchner funnel. Fractions A1 and A2 were combined and concentrated to dryness, the solid residue extracted once with 250 ml of acetonitrile, and the solids separated by filtration from the acetonitrile. The filtrate (fraction A3) was concentrated and the concentrated solution chromatographed on precoated tlc plates (silica gel, 0.250 mm thick, F-254, EM Laboratories, Elmsford, N. Y.) with an equal volume mixture of ether and *n*-hexane (solvent system I) and hexane-acetone-formic acid in the volume ratio 50:49:1 (solvent system II).

The material remaining on the filter was dissolved in methanol and water. After addition of a small amount of microgranular cellulose (CC 31, Whatman Chromedia) the solvents were evaporated and the contents quantitatively transferred to a column of microgranular cellulose which was placed in series with a silica gel column. The eluting solvents were hexane–acetone (4:1), 200 ml; acetone, 400 ml; acetone–water (1:1), 600 ml; and methanol–water (1:1), 600 ml. Radioactive material was found only in the first 25 fractions out of 250 fractions collected. These 25 fractions were combined (fraction A4, 2.40 μg), concentrated, and chromatographed on tlc with solvents I and II. The two methanol extracts (B1 and B2) were combined and concentrated. A white precipitate separated out and was filtered off. The filtrate (fraction B3) was saved. The precipitate was dissolved in water, and the solution was extracted with benzene. The benzene phase (fraction B5) was combined with the benzene fraction C to give fraction E. Further analysis of this fraction by tlc was not possible because of the large amount of lipid material (about 35 g) present. The aqueous phase (fraction B4) remaining after the benzene extraction was combined with fraction B3 and exhaustively extracted with ethyl ether in a liquid–liquid extractor. The aqueous phase remaining after the ether extraction was designated fraction B6. The other extract (fraction B7) was concentrated and chromatographed with both solvent systems. The ^{14}C contents of fractions A3, A4, B6, B7, D, and E were all determined by liquid scintillation counting.

Tissue Analysis for Dieldrin and Compounds Other Than Aldrindicarboxylic Acid and *trans*-Diol. *Extraction.* From a representative sample of ground tissue a 5.0-g sample was weighed into a 250-ml erlenmeyer flask. Sodium sulfate (25 g) was added and the sample was ground to a particulate texture using a thick glass rod. Hexane (80 ml) was added to the flask and a water-cooled Alihn-type condensor was attached. The sample was refluxed for 1 hr on a hot plate in a well-ventilated fume hood. After refluxing, the hexane extract was decanted into a 100-ml volumetric flask. The volume was adjusted to exactly 100.0 ml with hexane rinses of the extracted tissue to give a sample to solvent ratio of 1 g:20 ml.

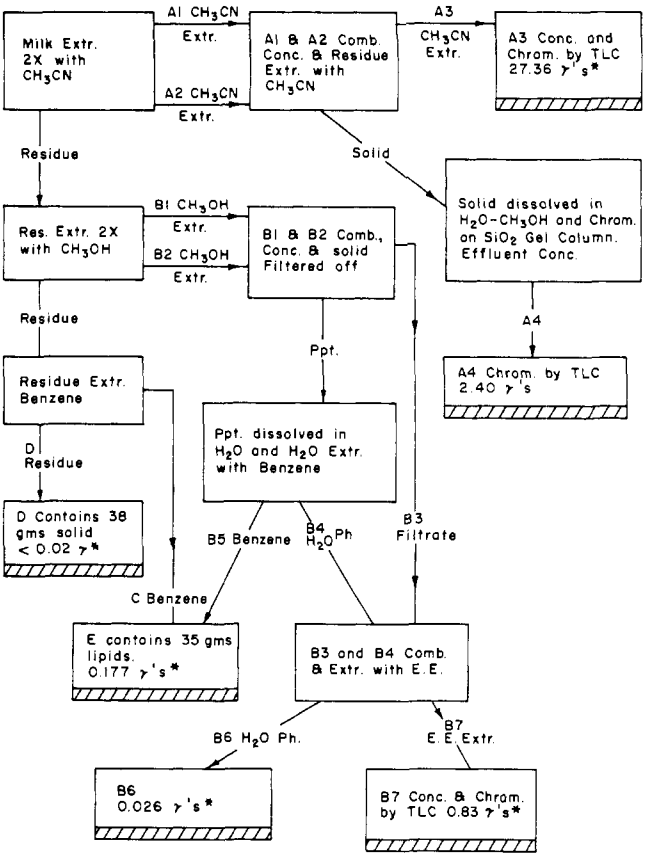

Figure 1. Fractionation of milk. The asterisk indicates micrograms of dieldrin-^{14}C.

Partition. A 20-ml aliquot (1 g) of the hexane extract was transferred into a graduated centrifuge tube and concentrated to 5 ml using a stream of air. Hexane-saturated dimethylformamide (DMF) (5 ml) was added and the mixture was shaken vigorously for 1 min. The hexane layer was removed with a disposable pipet, reextracted with an additional 5 ml of DMF, and discarded. The DMF layers were combined and a stream of air was di-

Table IV. Limits of Detection of Glpc Methods

	Limit of detection[a]			
	Milk		Tissue	
Compound	Response, % FS	Concn, ppm	Response, % FS	Concn, ppm
Aldrin	12	0.001	9	0.001
Photoaldrin	3	0.002	5	0.003
Photodieldrin	3	0.005	3	0.008
9-Hydroxydieldrin	7	0.002	5	0.002
Aldrin-*trans*-diol	5	0.002	5	0.003
Pentachloro ketone	6	0.002	5	0.003
9-Ketodieldrin	4	0.002	4	0.004
Aldrindicarboxylic acid	5	0.004	5	0.003

[a] The limit of detection is the concentration in parts per million corresponding to five times the noise level at the retention time of the compound. The response in per cent of recorder chart full scale and the concentrations in parts per million corresponding to the limit of detection are tabulated.

Table V. Feed Consumption, Dosage, and Milk Analysis of Cows Fed Dieldrin-^{14}C

	Expt no., cow no., and wt of cows, kg						
	1, 3, 503	1, 417, 587	2, 1, 499	2, 2, 485	2, 3A, 496	2, 4, 480	2, 5, 528
Feed consumption, kg/day							
Treated feed	1.0	0.88			1.0	1.0	1.0
Dairy concentrate[a]	7.0	5.2	5.6	6.0	6.0	2.7	6.0
Alfalfa hay	8.9	6.7	5.6	5.9	5.9	4.2[b]	5.9
Milk production, kg/day	20.7	11.4	8.4	11.8	14.9	5.3	20.4
Milk analysis, % (w/w)							
Dry matter	13.2	13.2	14.0	13.7	13.4	12.8	13.9
Butterfat	4.4	4.8	4.9	4.7	4.7	4.7	5.3

[a] Includes weight of treated feed. [b] Cow 4 also consumed 9.0 kg of silage on days 31–33 and 45 kg of green chop on days 38–40.

Table VI. Dieldrin and ^{14}C Residues in Milk[a] (First Experiment)

	Cow 3		Cow 417		Pooled means	
Treatment day	^{14}C in ppm[b] equiv	ppm[c] of dieldrin	^{14}C in ppm[b] equiv	ppm[c] of dieldrin	^{14}C in ppm[b] equiv	ppm[c] of dieldrin
1	0.004	0.004	0.003	0.004	0.003	0.004
2	0.010	0.006	0.009	0.006	0.009	0.006
3	0.013	0.009	0.010	0.010	0.011	0.009
6	0.012	0.013	0.011	0.012	0.011	0.013
9	0.016	0.017	0.010	0.013	0.013	0.015
12	0.019	0.023	0.020	0.023	0.019	0.023
15	0.020	0.018[b]	0.020	0.015[b]	0.020	0.017
17	0.018	0.016[b]	0.013	0.015[b]	0.015	0.015
19	0.025	0.021[b]	0.022	0.019[b]	0.023	0.020
21	0.018	0.015[b]	0.019	0.017[b]	0.019	0.016
Pooled mean (days 15, 17, 19, and 21)	0.020	0.017	0.019	0.017	0.019	0.017

[a] Cows fed 0.11 ppm of dieldrin-^{14}C. [b] Mean of triplicate analysis. [c] Results corrected for mean recovery of samples fortified with dieldrin.

rected into the sample tube for 15 min to remove the remaining hexane. The DMF solution was transferred into a 125-ml separatory funnel and diluted with 50 ml of water. Hexane (50 ml) was added and the mixture shaken vigorously for 3 min. The phases were allowed to separate and the aqueous portion drained off. The hexane was washed with an additional 50 ml of water, dried over sodium sulfate, and concentrated to 1.0 ml for column cleanup.

Cleanup. A glass column was packed with 3.0 g of MgO (20% Celite 545). The hexane extract was quantitatively transferred to the column and eluted first with 30 ml of *n*-hexane (fraction I) to recover aldrin, dieldrin, and UVCP-aldrin. The receiver was changed and the column was eluted with 10 ml of acetone. This second elution (fraction II) recovered 9-hydroxydieldrin, pentachloro ketone, 9-ketodieldrin, and UVCP-dieldrin. The acetone eluent was exchanged to hexane and the volumes of the two fractions were adjusted to give a sample to solvent ratio of 0.5 g:1 ml for glpc analysis.

Determination by Glpc. Glpc determinations were made by alternately injecting 5-μl volumes each of glpc standard A and fraction I of experimental samples using glpc condition A or 5-μl volumes each of glpc standard B and fraction II of experimental samples using glpc condition B. A standard was injected after injecting two–three samples. Calibration curves were prepared to determine the amounts of compound present.

Tissue Analysis for Aldrindicarboxylic Acid. *Extraction.* A 10.0-g sample of finely ground tissue was weighed into a 250-ml erlenmeyer flask and 10 ml of 6 *N* sulfuric acid was added while swirling the flask in an ice bath. A 3-ball Snyder column was attached and the sample was digested on a steam bath for 30 min with occasional agitation while heating. The sample was removed from the steam bath and cooled to room temperature. A 100-ml volume of ethyl ether was added to the flask and the mixture shaken on a wrist action shaker for 20 min. After shaking the ethyl ether was quickly decanted into glass bottles to avoid significant evaporation of ether.

Partition. An 80.0-ml aliquot (8.0 g) of the ethyl ether extract was measured into an 8-oz wide mouth polypropylene bottle with 80 ml of 0.05 *N* potassium hydroxide. The mixture was shaken vigorously for 2 min and centrifuged at high speed for 5 min. The ether layer was removed by aspiration using a disposable pipet being careful not to remove any of the lower aqueous layer. A 100-ml volume of hexane was added to the remaining aqueous solution. This mixture was shaken vigorously for 2 min and centrifuged at high speed for 5 min. The hexane was removed and discarded. The alkaline aqueous solution was transferred into a 125-ml separatory funnel and allowed to stand for 10 min. A 60.0-ml aliquot (6.0 g) of the lower aqueous phase was drained into a 250-ml erlenmeyer flask. Concentrated sulfuric acid (11 ml) was added to this solution while swirling in an ice bath. A 60.0-ml volume of ethyl ether was added to the acidified extract and the mixture shaken vigorously for 1 min. The phases were allowed to separate completely and 40.0 ml (4 g) of the ethyl ether solution were decanted into a 50-ml graduated centrifuge tube and the volume reduced to 2–3 ml using a stream of air. Diazomethane reagent (1–3 ml) was added to the ether solution (until the evolution of gas ceased and/or the solution remained yellow). The samples were allowed to react for about 30 min to form the dimethyl ester derivative. Excess diazomethane was removed by vaporization using a stream of air. The evaporation was continued until the sample volume was about 0.5 ml. The sample volume was reduced several times by alternate ad-

Table VII. Dieldrin and ^{14}C Residues in Milk (Second Experiment)[a]

Treatment day	^{14}C residues in ppm equiv and dieldrin residues in ppm in whole milk[b]							
	Cow 3-A		Cow 4		Cow 5		Pooled mean	
	^{14}C	Dieldrin	^{14}C	Dieldrin	^{14}C	Dieldrin	^{14}C	Dieldrin
1–2	0.007	0.001	0.005	0.001	0.005	0.001	0.006	0.001
2–3	0.014		0.014		0.013		0.014	
4–5	0.019		0.011		0.012		0.014	
7–8	0.019	0.028	0.022	0.021	0.013	0.014	0.017	0.020
10–11	0.023		0.021		0.021		0.022	
14–15	0.026	0.024	0.024	0.025	0.022	0.025	0.023	0.025
17–18	0.027		0.033		0.024		0.027	
21–22	0.025		0.036		0.029		0.028	
22–23	0.029	0.029	0.043	0.037	0.030	0.026	0.031	0.029
23–24	0.027		0.038		0.028		0.029	
24–25	0.024		0.037		0.027		0.027	
25–26	0.029		0.038		0.032		0.032	
26–27	0.025		0.042		0.032		0.030	
27–28	0.033	0.032	0.038	0.037	0.031	0.031	0.033	0.032
28–29	0.026		0.042		0.031		0.030	
29–30	0.020		0.050		0.033		0.030	
30–31	0.028		0.061		0.039		0.036	
31–32	0.042		0.066		0.035		0.042	
32–33	0.017		0.062		0.034		0.030	
33–34	0.031	0.030	0.064	0.053	0.036	0.035	0.030	0.035
34–35	0.030		0.060		0.037		0.035	
35–36	0.035		0.056		0.033		0.036	
36–37	0.034		0.058		0.042		0.041	
37–38	0.030		0.064		0.041		0.039	
38–39	0.033	0.038	0.059	0.053	0.045	0.042	0.042	0.040
39–40	0.037		0.053		0.041		0.041	
40–41	0.032	0.041	0.059	0.051	0.041	0.042	0.039	0.038

[a] Cows fed 0.21–0.36 ppm. [b] Means of duplicate analysis.

Table VIII. Proportion of Daily Dose Recovered in Milk

Interval, days	Proportion of daily dose recovered in milk, %									
	First experiment				Second experiment					
	Cow 3		Cow 417		Cow 3A		Cow 4		Cow 5	
	^{14}C	Dieldrin	^{14}C	Dieldrin	^{14}C	Dieldrin	^{14}C	Dieldrin	^{14}C	Dieldrin
1–2	13	8	11	8						
2–3	17	13	14	13						
5–6	15	17	13	14						
7–8					11	16	6	6	12	13
8–9	20	22	6	8						
11–12	26	31	7	8						
14–15	25	23	16	12	16	15	6	6	21	24
16–17	23	20	9	10						
18–19	34	27	15	13						
20–21	22	18	14	12						
22–23					18	18	9	8	22	19
27–28					21	20	6	6	25	25
33–34					20	19	10	8	26	25
38–39					19	20	9	8	32	30
40–41					18	18	10	8	26	27

dition of 10–20 ml of hexane and concentration to remove all traces of ethyl ether never allowing the sample to go dry. The final volume of the sample was adjusted with hexane to 4.0 ml for column cleanup.

Cleanup. Deactivated Florisil (3 g) (3% (w/w) water) was weighed into a small beaker and suspended in 2–5 ml of hexane. This mixture was quantitatively transferred into a glass chromatographic column. The sides of the column were rinsed with hexane and the solvent level allowed to drain to just above the top of the adsorbent. About 0.5 in. of granular sodium sulfate was added to the top of the column. The column was prewashed with 25 ml of hexane and the elution rate adjusted to 2–3 drops/sec.

The sample (in 4.0 ml of hexane) was quantitatively transferred to the column and allowed to reach the surface of the sodium sulfate. The sample was washed onto the column with an additional 2 ml of hexane. The sample was first eluted with 40.0 ml of hexane and this hexane was discarded. The receiver was changed and the ester derivative was eluted from the column using 32 ml of 40% ethyl ether-hexane, discarding the first 5–7 ml and collecting the remaining 25–27 ml. A precise elution profile was periodically determined by chromatographing ≥ 1 μg of the dimethyl ester in the presence of tissue extractives and examining fractional portions of the eluent. The ethyl ether–hexane eluent was concentrated to give a sample to solvent ratio of 0.2 g:1 ml and analyzed by glpc.

Determination by Glpc. Analyses of experimental samples for the dicarboxylic acid (ester derivative) were made by injecting 5-μl volumes each of the test samples and

Table IX. Dieldrin Residues in Fat

Interval, days	Residues in butterfat, ppm of dieldrin[a]				
	First experiment		Second experiment		
	Cow 3	Cow 417	Cow 3A	Cow 4	Cow 5
15	0.47	0.36	0.5	0.6	0.5
17	0.36	0.28			
19	0.44	0.33			
21	0.36	0.38			
23			0.65	1.01	0.58
28			0.62	0.79	0.66
34			0.63	1.43	0.68
39			0.77	1.75	0.96
41			0.64	1.53	0.83
Mean	0.41	0.34	0.66	1.30	0.74

[a] Parts per million of dieldrin in butterfat = [(ppm of dieldrin in whole milk) × (100)]/(% butterfat in milk).

Table X. Recovery of Potential Dieldrin Metabolites from Whole Milk

Compound	No. of analyses	ppm added	Recovery, %	SEM
9-Hydroxydieldrin	20	0.01	102	2.2
	13	0.02	102	2.2
Pentachloro ketone	20	0.02	100	1.8
	13	0.04	95	2.1
9-Ketodieldrin	20	0.03	98	1.7
	13	0.06	95	2.3
Aldrindicarboxylic acid	7	0.01	101	1.5
	8	0.02	103	2.4
UVCP-aldrin	20	0.015	95	1.1
	13	0.03	95	1.9
UVCP-dieldrin	20	0.04	87	2.3
	13	0.08	85	2.4
6,7-*trans*-Diol	9	0.02	77	5.5
	5	0.1	75	9.6
Aldrin	19	0.0025	88	1.9
	13	0.005	85	2.3

glpc standard D using glpc condition C. A standard was injected after injecting two–three samples. A calibration curve was prepared to determine concentrations of the aldrindicarboxylic acid (ester derivative) present.

Tissue Analysis for Aldrin-*trans*-diol. *Extraction.* A 5.0-g portion of ground tissue was weighed into a 250-ml erlenmeyer flask. Anhydrous Na_2SO_4 (25 g) (large crystals) was added and they were ground together using a large glass rod. After grinding, 80 ml of 1:1 ethyl acetate–ethyl ether was added and a water cooled reflux condensor fitted to the top of the flask. This mixture was allowed to reflux for 30 min on a steam table. After cooling, the extract was poured through fast flow filter paper into a 100-ml volumetric flask. Small aliquots of the same solvent (1:1 ethyl acetate–ethyl ether) were used to rinse the solid residue and bring the extract volume to a 100-ml mark (for a 1 g/20 ml sample to solvent ratio).

Partition. The extract (20 ml) was transferred to a 35-ml graduated centrifuge tube, concentrated, and exchanged to approximately 10 ml of hexane. Hexane-saturated dimethylformamide (DMF) (5 ml) was added and the mixture shaken vigorously. The upper hexane phase was aspirated off and discarded. The partition was repeated with another 10 ml of hexane. The DMF phase was drained into a 35-ml centrifuge tube and the dissolved hexane evaporated by gentle air blow-down. The DMF was transferred to a 125-ml separatory funnel using 50 ml of deionized water to rinse the centrifuge tube. Ethyl ether (60 ml) was added to the separatory funnel and the

Table XI. Recovery of Dieldrin and Its Metabolites (Mean Recovery (%) + SEM[a,b])

Tissue	Dieldrin	Aldrin-*trans*-diol	9-Keto-dieldrin	Pentachloro ketone	9-Hydroxy-dieldrin	ADA	UVCP-aldrin	UVCP-dieldrin	Aldrin
Brain	97 ± 6 (3)		98 ± 6 (3)	97 ± 10 (3)	94 ± 13 (3)	122 (1)	97 ± 15 (3)	81 ± 10 (3)	74 ± 23 (3)
	0.05–0.01		0.06–0.12	0.04–0.08	0.02–0.08	0.01	0.03–0.06	0.08–0.16	0.005–0.01
Subcutaneous fat tissue	92 ± 1 (2)	97 ± 2 (3)	86 ± 2 (2)	90 ± 2 (2)	94 ± 2 (2)	111 ± 9 (2)	84 ± 9 (2)	79 ± 4 (2)	
	0.25–0.50	0.01–0.10	0.30–0.60	0.2–0.4	0.10–0.20	0.01–0.02	0.15–0.30	0.40–0.80	
Mesenteric fat tissue	99 ± 0 (2)	95 ± 16 (2)	92 ± 7 (2)	94 ± 8 (2)	101 ± 12 (2)	97 ± 14 (2)	92 ± 4 (2)	82 ± 11 (2)	
	0.25–0.50	0.01–0.10	0.30–0.60	0.20–0.40	0.10–0.20	0.01–0.02	0.15–0.30	0.40–0.80	
Kidney	92 ± 6 (3)	86 ± 6 (2)	86 ± 7 (3)	84 ± 7 (3)	95 ± 3 (3)	103 ± 6 (2)	56 ± 20 (3)	47 ± 13 (3)	60 ± 0 (3)
	0.05–0.10	0.02–0.10	0.06–0.12	0.04–0.06	0.02–0.04	0.01–0.02	0.03–0.06	0.08–0.16	0.005–0.01
Liver	75 ± 1 (3)	87 ± 6 (2)	75 ± 15 (3)	75 ± 16 (3)	85 ± 13 (3)	98 ± 14 (3)	47 ± 10 (3)	57 ± 23 (3)	83 ± 11 (3)
	0.10–0.20	0.04–0.10	0.12–0.24	0.08–0.16	0.04–0.08	0.01–0.02	0.06–0.12	0.16–0.32	0.01–0.02
Lungs	102 ± 2 (2)		106 ± 4 (2)	98 ± 5 (3)	96 ± 8 (2)	106 ± 27 (2)	90 ± 12 (2)	75 ± 21 (2)	
	0.05–0.10		0.06–0.12	0.04–0.08	0.02–0.04	0.01–0.02	0.03–0.06	0.08–0.16	
Gastrocnemius muscle	98 ± 6 (3)	84 ± 11 (3)	85 ± 7 (3)	94 ± 7 (3)	97 ± 10 (3)	84 ± 8 (3)	95 ± 8 (3)	77 ± 8 (3)	71 ± 5 (3)
	0.05–0.10	0.01–0.10	0.06–0.12	0.04–0.08	0.02–0.04	0.01–0.02	0.03–0.06	0.08–0.16	0.01–0.05
Quadriceps muscle	92 ± 16 (3)	84 ± 8 (3)	75 ± 6 (3)	78 ± 5 (3)	80 ± 4 (3)	104 ± 5 (3)	90 ± 14 (3)	65 ± 9 (3)	
	0.05–0.10	0.01–0.10	0.06–0.12	0.04–0.08	0.02–0.04	0.01–0.02	0.03–0.06	0.08–0.16	

[a] Samples of tissue from untreated cows were fortified with dieldrin and its metabolites and the tissues then analyzed by the methods given in the text. The number in parentheses is the number of replicates. [b] The range of concentrations in parts per million used in the recovery determinations is tabulated below the recovery.

Table XII. Total ^{14}C and Dieldrin Residues in Tissues

	Residues in ppm equiv (^{14}C) or ppm (dieldrin)					
	Cow 3A		Cow 4		Cow 5	
	^{14}C	Dieldrin	^{14}C	Dieldrin	^{14}C	Dieldrin
Brain[a]	0.009	0.009	0.017	0.016	0.010	0.010
Kidney[b]	0.020	0.013	0.024	0.030	0.020	0.015
Liver[a]	0.064	0.045	0.10	0.062	0.079	0.050
Lungs[b]	0.018	0.010	0.018	0.09	0.022	0.014
Gastrocnemius muscle[a]	0.011	0.010	0.029	0.032	0.022	0.019
Quadriceps muscle[a]	0.022	0.018	0.023	0.022	0.025	0.020
Mesenteric fat tissue[a]	0.46	0.39	0.58	0.56	0.43	0.40
Subcutaneous fat tissue[a]	0.29	0.26	0.47	0.41	0.41	0.34

[a] Duplicate ^{14}C and glpc analysis. [b] Quadruplicate ^{14}C analysis and duplicate glpc.

mixture shaken vigorously for a few minutes. After complete separation of the phases the lower aqueous portion was drained and discarded. An additional 50 ml of water was used to rinse the ether and this water was also discarded. The ethyl ether was then dried over sodium sulfate, concentrated, and exchanged to 2 ml of ethyl acetate in preparation for column chromatography.

Cleanup. A cleanup column was prepared by packing 2.0 g of 80% MgO–20% Celite 545 into the glass column and capping with approximately 1 g of Na_2SO_4. The column was rinsed with 20 ml of ethyl acetate and the ethyl acetate added to the top of the column. The sample was then eluted through the column with 50 ml of an 80% ethyl acetate–20% acetone mixture, discarding the first 30 ml and keeping the following 20 ml. The 20 ml of eluent was concentrated and exchanged to 1 ml of 1:1 ethyl acetate–ether mixture and analyzed by glpc as in the milk analysis.

Determination by Glpc. The glpc analysis was carried out by the procedures described for the analysis of the milk for the aldrin-*trans*-diol.

Glpc Analysis; Limits of Detection. The limits of detection for the glpc analysis of the dieldrin metabolites were taken as five times the electronic noise at the retention time of each compound. The limits of detection so estimated for each compound in units of per cent of recorder chart full scale deflection times the factor 5 are tabulated in Table IV. The limits of detection in parts per million for each compound were then calculated from eq 1.

$$\text{limit of detection (ppm)} = \frac{(\text{electronic noise})(5)}{(\text{slope of calibration curve})(\text{sample weight})} \quad (1)$$

The slopes of the calibration curves were calculated from the calibration data for each compound in units of peak height (per cent of full scale deflection) per microgram. The sample weight is the weight of original tissue equivalent to the volume of the extract injected into the glpc. The limits of detection are tabulated in Table IV.

Milk Analysis. The dry matter and butter fat content of the milk were determined by methods previously published (Horwitz, 1965).

Recovery Analysis. Recovery analysis on pretreat and control samples of milk and control samples of tissue fortified with dieldrin-^{14}C, dieldrin, and all the dieldrin metabolites were made.

Calculations of Pooled Means for Milk. *Mean Levels of Dieldrin and ^{14}C in the Milk.* The pooled means, $\bar{X}_n$, of the dieldrin level were calculated from eq 2, where

$$\bar{X}_n = \frac{\Sigma W_{i,n} X_{i,n}}{\Sigma W_{i,n}} \quad (2)$$

$\bar{X}_n$ = pooled mean level of dieldrin or ^{14}C in milk collected on the nth day in micrograms of dieldrin per gram of milk or dieldrin microgram equivalents per gram of milk, $W_{i,n}$ = weight of milk produced by the ith cow on the nth day (i = 3, 4, or 5), and $X_{i,n}$ = dieldrin level in micrograms per gram of milk from the ith cow on the nth day or ^{14}C level in dieldrin microgram equivalents per gram of milk from the ith cow on the nth day.

RESULTS

Milk Residues. The mean milk production, feed consumption, milk analysis, etc. are summarized in Table V. In the first experiment the daily intake of dieldrin-^{14}C, 1.62 mg for cow 3 and 1.43 mg for cow 417, corresponds to 0.10 and 0.12 ppm of dieldrin-^{14}C based on the total ration for cows 3 and 417, respectively. In the second experiment the daily intake of dieldrin-^{14}C, 2.5 mg, corresponds to 0.21, 0.36, and 0.21 ppm of dieldrin-^{14}C based on the total ration for cows 3A, 4, and 5, respectively.

The apparent total ^{14}C residues in parts per million equivalents found in the milk collected during the pretreatment period were as follows: cow 3, 0.00063 (standard error of the mean (SEM) = 0.00001, three samples); cow 417, 0.00068 (SEM = 0.000008, three samples); cow 3A, 0.00092 (SEM = 0.00003, three samples); cow 4, 0.00086 (SEM = 0.00006, three samples); and cow 5, 0.0017 (SEM = 0.0004, three samples).

The mean apparent ^{14}C residues in parts per million equivalents in the milk from cows 1 and 2, the controls, were 0.00090 (standard deviation (SD) = 0.00009, 31 samples) and 0.00089 (SD = 0.00007, 30 samples), respectively.

Recoveries of ^{14}C on milk fortified with dieldrin at levels of 0.01 and 0.03 ppm were 93% (SEM = 2.6%, three samples) and 96% (SEM = 4.3%, three samples), respectively. Because the mean recovery was not significantly different from 100% the total ^{14}C residues in the milk were not corrected for the mean recovery, 95%.

The total ^{14}C residues in the milk collected during the treatment periods and the pooled means are tabulated in Table VI, first experiment, and Table VII, second experiment. These results have been corrected for the apparent ^{14}C content of the milk collected during the pretreatment periods. The pooled means are plotted in Figure 2. The proportions of the daily dose of ^{14}C recovered in the milk are tabulated in Table VIII.

In the first experiment the mean apparent dieldrin concentration found by glpc in the pretreatment milk samples was 0.002 ppm (SEM = 0.0005, 15 samples). In the

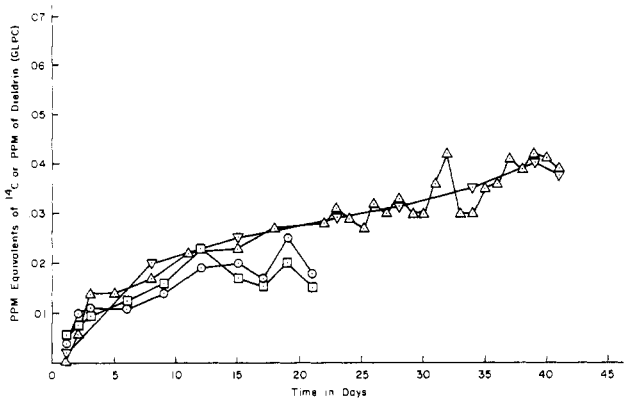

Figure 2. Parts per million of dieldrin and ^{14}C ppm equivalents in milk first and second experiments: first experiment, (⊙) ^{14}C; (⊡) dieldrin; second experiment, (△) ^{14}C; (▽) dieldrin.

second experiment the mean apparent dieldrin content of the pretreatment milk samples for the three treated cows (3A, 4, and 5) was 0.001 pm (SEM = 0.0002, six samples). In neither experiment was there a statistically significant difference between the milk collected from the different cows.

In the second experiment the apparent levels of dieldrin in the control cows 1 and 2 did not change significantly during the test. The apparent dieldrin levels were the same at the start and end of the test, 0.001 ppm. The mean of 20 samples was 0.001 ppm (SD = 0.0003 ppm).

In the first experiment the mean recoveries obtained when the milk samples were fortified with dieldrin at levels of 0.01 and 0.02 ppm were 87.3% (SEM = 2.7%, seven samples) and 88.4% (SEM = 1.2%, ten samples), respectively. Because there was not a statistically significant difference between the two means the grand mean of all 17 analyses, 88% (SEM = 1.3%) was used to correct the net dieldrin levels found in the treatment milk samples.

In the second experiment the recoveries obtained on milk samples fortified with 0.01, 0.025, and 0.05 ppm of dieldrin were 94% (SEM = 3.8%, 4 analyses), 94% (SEM = 1.1%, 20 analyses), and 94% (SEM = 1.3%, 13 analyses), respectively. The net dieldrin levels found in the second trial were not corrected for the mean recovery.

The net dieldrin residues determined by glpc in the milk collected during the treatment period are tabulated in Table VI, first experiment, and Table VII, second experiment. The pooled means are plotted in Figure 2. The proportion of the daily dose of dieldrin recovered in the milk is tabulated in Table VIII.

The dieldrin residues in the butter fat are tabulated in Table IX. These residues were calculated from the dieldrin residues obtained by the specific glpc method and the proportion of butter fat found in each milk sample. The recoveries of the compounds listed in Table I except dieldrin from fortified milk samples are given in Table X.

In the second experiment single analyses of the milk from all five cows collected at 0–1, 7–8, 14–15, 22–23, 27–28, 33–34, 38–39, and 40–41 days were performed for all the metabolites and hypothetical metabolites listed in Table I except the aldrin-*trans*-diol. The milk collected at the following intervals, 7–8, 22–23, 33–34, and 40–41 days, was analyzed for the aldrin-*trans*-diol. None of these compounds was found in the milk samples.

The results of the exhaustive extraction and fractionation of the milk collected on day 29 from cow 4 are tabulated in Figure 1. Single radioactive spots were found on the thin layer chromatograms of fractions A3, A4, and B7. The R_f of these spots had the same value as dieldrin and cochromatographed with dieldrin-^{14}C. The limit of the ^{14}C detection on the thin layer chromatograms was equivalent to 0.0001 ppm of dieldrin-^{14}C in the whole milk. No ^{14}C was found in fraction D. Fraction E containing ^{14}C equivalent to 0.0002 ppm of dieldrin could not be analyzed further because large quantities of lipid material were present and fraction B6 could not be analyzed further because only a small quantity of ^{14}C equivalent to 0.00003 ppm of dieldrin in the milk was present. The total recovery of ^{14}C was 96.86%. Therefore, 0.997 μg equivalents or 0.001 ppm equivalents based on the whole milk was lost in the extraction and fractionation of the extract.

Table XIII. Fat–Feed Ratios

Tissue	Fat–feed ratio[a]					
	First expt		Second expt			
	Cow 3	Cow 417	Cow 3A	Cow 4	Cow 5	Mean
Mesenteric fat tissue			1.9	1.6	1.9	1.8
Subcutaneous fat tissue			1.3	1.1	1.6	1.3
Butterfat[b]	4.1	2.8	3.1	3.6	3.5	3.4

[a] Fat–feed ratio = (ppm of dieldrin in fat)/(ppm of dieldrin in feed). [b] Calculated from the mean level in butterfat given in Table IX.

Table XIV. Comparison of ^{14}C and Dieldrin Residues in Milk

Interval, days	Pooled mean residues in ppm equiv (^{14}C) or ppm (dieldrin)		
	^{14}C	Dieldrin	Difference
	First Experiment[a]		
15	0.019	0.017	0.002
17	0.017	0.016	0.001
19	0.024	0.020	0.004
21	0.018	0.015	0.003
Mean	0.019	0.017	0.002
	Second Experiment[b]		
8	0.017	0.020	−0.003
15	0.023	0.025	−0.002
23	0.031	0.029	0.002
28	0.033	0.032	0.001
34	0.030	0.035	−0.005
39	0.042	0.040	0.002
41	0.039	0.038	0.001
Mean[c]	0.036	0.036	

[a] Mean of triplicate analysis. [b] Mean of duplicate analysis. [c] Mean days 28–41.

Table XV. Comparison of ^{14}C and Dieldrin Residues in Tissues

Tissue	Mean residues in ppm equiv (^{14}C) or ppm (dieldrin)[a]	
	^{14}C	Dieldrin
Brain	0.012	0.012
Mesenteric fat tissue	0.50	0.45
Subcutaneous fat tissue	0.39	0.34
Kidney	0.021	0.014
Liver	0.081	0.052
Lungs	0.020	0.011
Gastrocnemius muscle	0.021	0.020
Quadriceps muscle	0.024	0.020

[a] Second experiment. See Table XII for number of replicate analysis.

Tissue Residues. Samples of tissue were fortified with 1 ppm of dieldrin-^{14}C and the fortified samples analyzed for ^{14}C by the packed tube combustion method with the following results: brain, 97%; gastrocnemius muscle, 94%; kidney, 97%, liver, 102%; and lung, 100%. Samples of the tissues were also fortified with dieldrin and the compounds listed in Table I and the fortified samples analyzed by the specific glpc methods. The results are tabulated in Table XI. Single analyses were performed for the metabolites of dieldrin listed in Table I and duplicate or quadruplicate analyses were performed for ^{14}C and dieldrin as shown in Table XI.

The residues of ^{14}C and dieldrin found in the tissues are tabulated in Table XII. None of the metabolites or hypothetical metabolites listed in Table I were found in the gastrocnemius muscle, kidney, and liver. The brain and lungs were not analyzed for the aldrin-*trans*-diol and the subcutaneous fat, mesenteric fat, lung, and quadriceps muscle were not analyzed for aldrin because of interference. None of the other compounds listed in Table I were found in these five tissues.

DISCUSSIONS AND CONCLUSIONS

The dieldrin residues found in the milk (Tables VI and VII) are compatible with those reported by Williams and Mills (1964) and Gannon *et al.* (1959).

The fat–feed ratio in the butter fat, mesenteric fat tissue, and subcutaneous fat tissue in this work is tabulated in Table XIII. The mean feed–fat ratios in the mesenteric fat, subcutaneous fat, and butter fat are 1.8, 1.3, and 3.4, respectively.

A comparison of the ^{14}C and dieldrin residues found in the milk is tabulated in Table XIV. Only the analyses which were replicated in the first experiment are included. In the first experiment all of the ^{14}C residues (days 15–21) were higher than the dieldrin residues. The mean difference is 0.002 ppm. In the second experiment three of the dieldrin residues are higher than the ^{14}C residues and four are lower. The mean difference is <0.001 ppm. The mean difference for both experiments is 0.001 ppm. These results, the absence of any polar material with chromatographic properties different than those of dieldrin, and the absence of any of the known metabolites of dieldrin indicate that no metabolites of dieldrin were present in the milk at concentrations of 0.001 ppm or greater.

A comparison of the mean ^{14}C and dieldrin residues in the tissues is tabulated in Table XV. Considering the sampling error and low recoveries the differences are not considered to be significant.

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