Radioisotope Residues and Residues of Dichlorvos and Its Metabolites in Pregnant Sows and Their Progeny Dosed with Dichlorvos-^{14}C or Dichlorvos-^{36}Cl Formulated as PVC Pellets

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In three separate trials pregnant sows were fed dichlorvos and dichlorvos-^{14}C, dichlorvos-^{14}C, and dichlorvos and dichlorvos-^{36}Cl, respectively, during the last third of the sows' gestation period at a rate of 4 mg of dichlorvos per kg of body weight per day. In all the trials the dichlorvos was formulated in polyvinyl chloride pellets containing 20–21% dichlorvos. After farrowing, the sows and piglets were held for periods as long as 21 days before being sacrificed. During this period the piglets nursed from their own mothers. The daily rates of excretion of ^{14}C from the sows which had been dosed with dichlorvos-^{14}C only were as follows: feces, 5.4%; the pellets recovered from the feces, 53.6%; urine, 4.3%; and expired air, 6.7%. The recovery of ^{36}Cl from the sows dosed with dichlorvos and dichlorvos-^{36}Cl was as follows: feces, 5.5%; the pellets recovered from the feces, 56.5%; urine, 26.1%; and sow carcass, 8.1%. No residues of dichlorvos, dichloroacetaldehyde, demethyl dichlorvos, dichloroacetic acid, or dichloroethanol were found in the tissues of the sows and piglets, although the tissues contained ^{14}C and ^{36}Cl residues ranging from 0.3 to 18.0 ppm equivalents. The ^{14}C and ^{36}Cl residues in the tissues are believed to be due to degradation of the vinyl group in dichlorvos into ^{36}Cl ions and the incorporation of the ^{14}C into normal tissue constituents such as glycine, serine, creatine, glucose, glycogen, fatty acids, cholesterol, choline, lecithin, and ribonucleic acid.

In vivo and *in vitro* studies have shown that dichlorvos is degraded enzymatically by animal tissue and in whole animals is degraded to dichloroacetaldehyde (DCA), dichloroethanol (DCE), dichloroacetic acid (DCAA), demethyl dichlorvos (DMD), dimethyl hydrogen phosphate, and methyl dihydrogen phosphate (Bull and Ridgway, 1969; Casida *et al.*, 1962; Hodgson and Casida, 1962). Rats dosed with dichlorvos labeled with ^{14}C in the 1 position of the vinyl group excreted part of the ^{14}C as carbon dioxide in the expired air. The fate of dichlorvos in young pigs has also been investigated (Potter *et al.*, 1973). No residues of dichlorvos, DMD, DCA, or DCAA were found in the tissues of pigs 2, 7, or 14 days after the pigs received a single dose of 42 mg of dichlorvos/kilogram formulated as polyvinyl chloride (PVC) pellets.

Dichlorvos formulated in PVC pellets when fed to pregnant sows during the last third of their gestation period at a dosage of 800 to 1200 mg/head/day has been found to increase the pounds of pork per litter at weaning time. Accordingly, the fate of dichlorvos in pregnant sows and their progeny has been investigated (Anderson and Wahlstrom, 1970; England and Day, 1971).

MATERIALS AND METHODS

Feeding Experiments. One range-finding experiment with dichlorvos-^{14}C and two larger experiments, one with dichlorvos-^{14}C and one with dichlorvos-^{36}Cl, were carried out with pregnant sows.

General Protocol. Hand-bred, pregnant Yorkshire-cross sows weighing from 175 to 250 kg were purchased from a commercial hog farm. Two to four days before the sows were scheduled for treatment with dichlorvos-^{14}C or dichlorvos-^{36}Cl, they were transferred to farrowing cages in a special facility designed for housing large or small animals dosed with radioactive materials. The sows were catheterized after they were placed in the farrowing cages to allow separate collection of the feces and urine. Unless otherwise noted, the urine and feces were collected daily and weighed, and an aliquot was retained for analysis.

A standard pig ration containing 13.6% protein and the dichlorvos formulated as PVC pellets at a dose rate of approximately 800 mg of dichlorvos/sow/day or 4 mg of dichlorvos/kg/day was fed in the morning as follows. The unconsumed feed from the previous feeding was removed from each sow's feeding trough, part of the day's ration was placed in the feeding trough, and the pellets were placed on top of this ration. After the sow had ingested this portion, the rest of the daily feed was added to the trough. Less than 1% of the pellets was recovered from the trough at the end of each feeding. The administration of the PVC pellets containing dichlorvos was stopped at the time of farrowing. The piglets were allowed to suckle from their own mothers through each experiment.

The sows and piglets were killed by electrocution. All the samples were stored at −10 to −15° prior to analysis.

Range-Finding Experiment. To conserve the supply of dichlorvos-^{14}C, the sows were first fed PVC pellets containing 10% nonradioactive dichlorvos and then PVC pellets containing 20.9% dichlorvos-^{14}C, specific activity 210 dpm/μg of dichlorvos-^{14}C. The sows received the nonradioactive dichlorvos and the dichlorvos-^{14}C, respectively, for the following lengths of time: Sow 026, 8 days (nonradioactive) and 4 days (dichlorvos-^{14}C); Sow 318, 22 days and 4 days; Sow 348, 16 days and 4 days; Sow 349, 12 days and 4 days; Sow 350, 12 days and 3 days; and Sow 351, 8 days and 4 days. The dosage rates of the nonradioactive dichlorvos and the dichlorvos-^{14}C were 800 and 836 mg/sow/day, respectively. The sows were placed in the farrowing cages the day before they were first dosed with dichlorvos-^{14}C. Single sows were killed 2, 6, 12, and 16 days after farrowing. The two remaining sows were killed 21 days after farrowing.

Blood samples taken from the anterior vena cava were collected in ice-cold heparinized tubes and brought immediately to the laboratory for the determination of plasma and red cell cholinesterase.

Multidose Administration of Dichlorvos-^{14}C. PVC pellets containing 21.3% dichlorvos-^{14}C with a specific activity of 204 dpm/μg of dichlorvos-^{14}C were administered at a dose rate of 852 mg of dichlorvos/sow/day to three hand-bred sows until approximately 24 hr before farrowing. The sows received the pellets for the following lengths of time: Sow 11, 29 days; Sow 28, 26 days; and Sow 46, 25 days. The sows were held for 21 days after farrowing and then sacrificed. Piglets were sacrificed at 0, 9, and 21 days.

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Urine and fecal samples were collected daily during each 24-hr period for the first 7 days of the treatment period and during the first 7 days of the posttreatment period. In the remainder of the experiment the urine and feces were collected and weighed every third day. The carbon dioxide in the expired air of the sows was collected during the treatment period only, as described in the following sections. Samples of brain, kidney, liver, quadriceps muscle, and mesenteric fat from the sows to be analyzed for dichlorvos and its metabolites were quick frozen with liquid nitrogen immediately after collection. Samples of muscle and liver from the piglets were analyzed immediately after collection. All the other tissue samples from the piglets and sows and the quick-frozen samples were stored in a freezer at −10 to −15°. After the tissue samples were taken, the remainder of the piglet samples were ground two times in a Toledo food chopper (Model 5120-009-01). The sow carcasses were cooled to 4–6°, cut longitudinally in half with a chain saw, and the right or left half of each sow carcass was ground for determination of ^{14}C.

Multidose Administration of Dichlorvos-^{36}Cl. Except for the items noted below, the protocol was the same in this experiment as the multidose administration of dichlorvos-^{14}C. Not enough dichlorvos-^{36}Cl was available to treat three sows for 25 to 30 days. Accordingly, three pregnant Yorkshire-cross sows (No. 550, 551, and 552) were fed PVC pellets containing 20% dichlorvos (nonradioactive) for 21 days and then PVC pellets containing 20.7% dichlorvos-^{36}Cl were fed for 6 days. The dosage was 800 mg of dichlorvos/sow/day. The specific activity of the dichlorvos-^{36}Cl was 28.7 dpm/μg. All the sows farrowed within 24 hr of receiving the last dose. Sow 552 died 19 days after farrowing due to ascending pyelonephritis with complicating hydronephrosis caused by a blocked catheter.

Glpc Analysis. The glpc methods used for the determination of dichlorvos and its metabolites have been published (Schultz *et al.*, 1971). The accuracy of the glpc analysis was checked by analyzing fortified samples. The levels at which the samples were fortified, the compound added, the mean recovery, and the standard deviation are as follows: dichlorvos, 0.2–0.5 ppm, 92 ± 11%; DMD, 0.2–0.5 ppm, 94 ± 10%; DCAA, 0.25–0.50 ppm, 95 ± 17%; DCA, 0.1–0.5 ppm, 85 ± 20%; DCE, 0.20–0.25 ppm, 105 ± 12%.

Determination of ^{14}C. A packed tube combustion method and liquid scintillation counting were used to determine the ^{14}C in the tissues. The ^{14}C in the urine was determined by liquid scintillation counting. Details of the procedure have been previously described (Potter *et al.*, 1973). For ease in comparison to the glpc analysis, the ^{14}C residues in both trials have been reported in ppm equivalent of dichlorvos-^{14}C. To convert these residues to dpm of ^{14}C per gram the residues in ppm equivalents should be multiplied by 210 and 204 in the range-finding and multidose experiments, respectively.

Determination of ^{36}Cl. The total ^{36}Cl in fecal material, blood, and liver was determined using the same apparatus as was used for the ^{14}C, except that a roll of platinum screen instead of copper oxide was used as a catalyst. To prevent the retention of ^{36}Cl by sorption on the quartz combustion tube and to volatilize inorganic chloride in the samples, oxygen used for the combustion was bubbled through concentrated hydrochloric acid. Combustion products were absorbed in a 2% sodium hydroxide–0.3% hydrogen peroxide solution. The ^{36}Cl in the absorption solution was determined by liquid scintillation counting.

Total ^{36}Cl in the rest of the tissues was determined by solubilization as follows. The samples were homogenized in a Virtis 45 homogenizer or a Waring blender for 1 or 2 min and 0.5-g portions were placed in a counting vial. Soluene-100 (Packard Instrument Company) was then added, 1 ml of solution per 0.1 g of sample. The vials were capped, heated for 18 hr at 37° and for 2 hr at 60°, cooled, and 0.2 ml of acetic acid/0.1 g of sample and 15 ml of ethanol LSS were added. The ^{36}Cl was then counted in a liquid scintillation counter equipped with an automatic external standard (AES). The AES was calibrated by counting samples fortified with a known amount of chlorobenzene-^{36}Cl standard. The calibration data were fitted to a quadratic equation by the method of least squares. The counting efficiency was taken as the independent variable. For ease in comparison to the glpc analysis the ^{36}Cl residues have been reported in ppm equivalents of dichlorvos-^{36}Cl. To convert these residues to dpm of ^{36}Cl/g, the residues in ppm equivalents should be multiplied by 28.7.

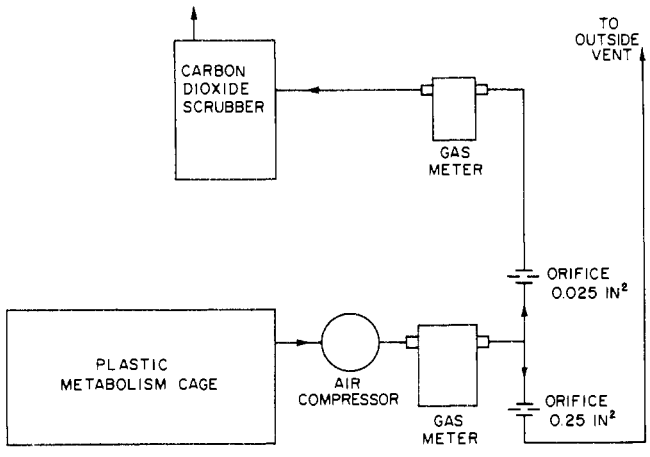

Figure 1. Carbon dioxide collection systems for sows.

Synthesis of Dichlorvos-^{14}C and Dichlorvos-^{36}Cl. The synthesis and methods for determining the purity of dichlorvos-^{14}C and dichlorvos-^{36}Cl have been described by Burton (1971). The highest specific activity ^{36}Cl obtainable was employed.

Formulation of Dichlorvos in Polyvinylchloride. The preparation of polyvinylchloride pellets containing dichlorvos-^{14}C or dichlorvos-^{36}Cl has been described by Potter *et al.* (1973).

Assay of Cholinesterase. The blood samples were analyzed for plasma cholinesterase and erythrocyte cholinesterase as described by Boyer (1967) using a radiometer titrator (The London Company, Westlake, Ohio).

Collection of ^{14}C Carbon Dioxide in Expired Air. The ^{14}C carbon dioxide in the expired air of the sows was collected by enclosing the farrowing cages in a rectangular enclosure made by bolting sheets of plastic 3/8-in. thick to a 1.5-in. angle iron framework. The enclosure had sides and a top but no bottom. It was of sufficient size to fit over the cages, with the bottom edge of the side panels resting on the floor. The air was drawn from the enclosure with a positive displacement air compressor (Gast Manufacturing Company, Model 1022 V1036Z72X) and then forced through a gas meter (American Meter Company, MOD AL-800, 600CFH), as shown in Figure 1. The air was then split in two streams with approximately 90% of the air vented to the outside. The other 10% was passed through a second gas meter (American Meter Company, MOD-AS-11-1, 60CFH) and then into a caustic scrubber (Potter *et al.*, 1973). Daily readings of the gas meters were made and the caustic solution in the scrubbers was replaced daily. The total ^{14}C in the expired air was computed by multiplying the total ^{14}C in the caustic by the ratio of the total volume of air pumped from the cage to the volume passed through the caustic scrubbers.

RESULTS

Range-Finding Experiment. The ^{14}C residues in the tissues of the sows and piglets are tabulated in Tables I and II.

Table I. Total ^{14}C Residues in Tissues of Sows Dosed with Dichlorvos-^{14}C[a]

Tissues	^{14}C Residues,[b] interval in days				
	2	6	12	16	21
Bladder	1.2	3.4	2.2	1.9	1.9
Blood		<0.1	<0.1	0.1	<0.1
Brain	0.5	1.6	1.7	0.7	1.6
Duodenum	3.1	2.9	1.3	1.3	1.1
Gall bladder	6.8	4.9	2.3	0.1	1.3
Heart	2.5	4.2	2.8	2.0	1.9
Kidney	5.8	4.9	2.7	2.0	1.6
Liver	13.0	12.6	7.1	4.3	2.7
Lungs	2.7	6.9	3.0	1.7	3.9
Mammary gland	1.7	2.3	3.4		1.9
Pancreas	7.9	3.5	3.4	0.3	1.5
Quadriceps muscle	1.3	2.2	1.4	0.9	1.7
Renal fat	1.1	1.2	1.5	1.7	1.7
Spiral colon	4.2	1.6	1.8	1.0	1.9
Spleen	4.6	4.8	1.0		1.2
Stomach	2.2	2.2	1.6	0.4	1.5
Subcutaneous fat	1.0	1.4	0.9	0.6	0.7
Thyroid	1.8	3.0	1.5	0.9	1.9
Uterus	3.6	4.2	3.8	0.4	2.5

[a] Multidose range-finding experiment. [b] Although no dichlorvos was found in the tissues, the ^{14}C residues were calculated for simplicity as ppm equivalents of dichlorvos-^{14}C.

Table II. Mean Total ^{14}C Content Expressed as ppm Equivalent of Dichlorvos in Tissues of Piglets at Different Ages[a]

Tissues	^{14}C Residues,[b] interval in days						
	0	1	2	4 and 5	8 and 9	14–16	21
Adrenals	3.4	3.3	3.1	3.4	4.6	2.9	5.2
Bladder	0.9	1.2	2.0	2.2	1.9	1.0	1.2
Blood	0.7	0.8	2.0	1.8	1.6	1.5	0.8
Brain	0.9	0.7	1.3	1.7	1.8	1.2	1.2
Duodenum	1.8	2.8	3.1	3.7	2.6	1.9	1.5
Gall bladder	1.6	4.0	3.0	3.3	2.3	1.9	1.2
Heart	1.1	1.0	1.8	2.6	2.5	1.8	1.5
Kidney	1.6	1.7	2.3	2.3	2.6	2.0	1.4
Liver	3.4	2.7	3.9	4.3	3.6	2.5	1.5
Lungs	1.0	1.2	2.0	2.7	2.7	1.9	1.8
Pancreas	2.2	3.2	3.4	5.7	3.2	1.9	1.5
Quadriceps muscle	1.0	0.9	1.7	2.8	3.2	3.0	2.2
Renal fat				3.3	4.5	3.9	3.2
Spiral colon	1.3	2.0	3.0	2.0	2.1	1.3	1.3
Spleen	1.3	1.4	2.3	3.3	2.9	2.0	1.4
Stomach	0.8	3.9	4.1	5.6	3.1	1.2	0.8
Subcutaneous fat	2.0	2.8	6.5	3.7	6.6	2.5	2.5
Thyroid	0.8	0.7	1.8	3.0	2.9	2.1	4.0
Grand mean	1.5	2.0	2.8	3.2	3.0	2.0	1.9

[a] Multidose range-finding experiment. [b] Although no dichlorvos was found in the tissues, the ^{14}C residues were calculated for simplicity as ppm equivalents of dichlorvos-^{14}C.

The mean cholinesterase activity in 0.5-ml samples of plasma from untreated sows was 9.5×10^{-5} mmol of acetylcholine per minute (SEM = 1×10^{-5}; eight sows). The activity in the plasma from sows treated for 2–18 days was less than 10% of the activity found in the plasma from the control sows. The mean cholinesterase activity in 0.05 ml of lysed red cells from eight untreated sows was 8.1×10^{-5} mmol of acetylcholine per minute (eight determinations, eight sows). The mean cholinesterase activity in the red cells from the treated sows was 3.1×10^{-5} mmol of acetylcholine per minute (five sows, 14 determinations).

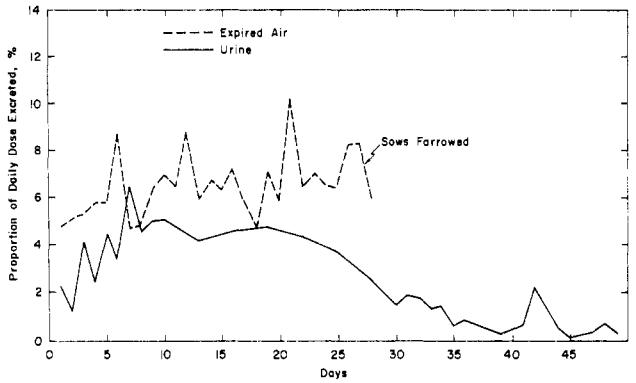

Figure 2. Excretion rates of radioactivity from sows fed dichlorvos-^{14}C—expired air and urine.

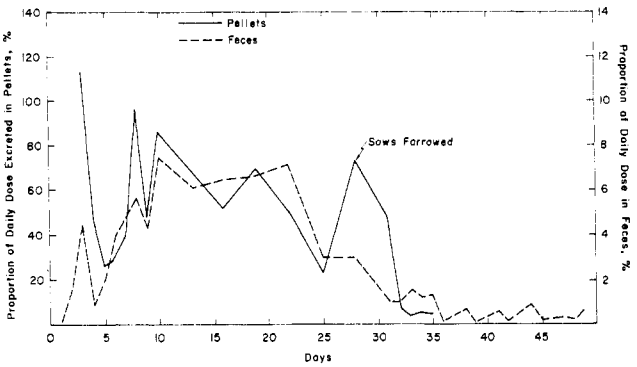

Figure 3. Excretion rates of radioactivity from sows fed dichlorvos-^{14}C—pellets and feces.

The t value for the difference between the means for the treated and control sows is 8.3, which is statistically significant at the 1% confidence level.

Multidose Experiment with Dichlorvos-^{14}C. The total found in the expired air, urine, pellets recovered from the feces, and the feces are plotted in Figures 2 and 3. From the fifth day to the time of farrowing, the average rates of excretion of ^{14}C per day, calculated as a percentage of the daily dose, are as follows: feces, 5.44%; the pellets recovered from the feces, 53.60%; urine, 4.28%; and expired air, 6.65%. Therefore, only 46.40% of each daily dose was lost from the pellets while they were in the gastrointestinal tract. The excretion rates of ^{14}C, based on the amount of ^{14}C lost from the pellets, are as follows: feces, 11.72%; urine, 9.22%; and expired air, 14.33%.

The mean levels of the ^{14}C in the tissues of the sows and piglets are tabulated in Tables III and IV.

Multidose Experiment with Dichlorvos-^{36}Cl. The total ^{36}Cl found in the urine, feces, and pellets isolated from the feces are plotted in Figure 4. The amount of ^{36}Cl recovered in the excreta are as follows: feces, 5.50%; urine, 26.13%; and the pellets recovered from the feces, 56.55%. Of the total dose, 8.07% was recovered in the tissues and carcass. The total recovery of ^{36}Cl was therefore 96.25%.

The total ^{36}Cl residues in the sow and piglet tissues are tabulated in Tables III and V. Because of the death of Sow 552 on the 19th day after farrowing, only the data for Sows 550 and 551 on day 21 are included.

Residues of Dichlorvos and its Metabolites by Glpc. The minimum detectable concentrations of dichlorvos and its suspected metabolites in ppm were as follows: dichlorvos in tissues, 0.03; dichlorvos in blood, 0.10; DMD, 0.07; DCA, 0.05; DCAA, 0.11; and DCE, 0.15.

No dichlorvos was detected in the sows' blood from the range-finding experiment.

No dichlorvos, DMD, DCA, DCAA, or DCE was detected in any of the tissues from the sows and piglets from the

Table III. Total ^{14}C and ^{36}Cl Residues in Sows Dosed with Dichlorvos-^{14}C or Dichlorvos-^{36}Cl

Tissues	ppm Equivalents[a,b] ^{14}C	^{36}Cl
Adrenals	10 ± 1.2	1.0[c]
Bladder	10 ± 1.5	2.1 ± 0.06
Blood	12 ± 0.7	2.1 ± 0.52
Brain	6 ± 0.2	1.1 ± 0.08
Carcass	8 ± 0.9	1.0 ± 0.04
Duodenum	6 ± 1.0	1.7 ± 0.05
Femur	2 ± 0.5	1.4 ± 0.01
Gastrocnemius muscle	9 ± 0.5	1.3 ± 0.01
Heart		1.5 ± 0.28
Kidney	9 ± 0.9	1.7 ± 0.25
Liver	16 ± 0.6	1.1 ± 0.10
Lungs	10 ± 0.7	1.1 ± 0.25
Mammary gland	9 ± 1.9	1.8 ± 0.30
Mesenteric fat	4 ± 1.6	1.0 ± 0.20
Pancreas	8 ± 1.0	1.4 ± 0.30
Quadriceps muscle	10 ± 0.9	0.9 ± 0.10
Salivary gland	8 ± 0.4	1.8 ± 0.04
Skin	6 ± 1.1	1.8 ± 0.06
Spleen	11 ± 1.1	1.3 ± 0.80
Spiral colon	6 ± 0.5	1.6 ± 0.12
Stomach	7 ± 0.8	1.4 ± 0.05
Subcutaneous fat	6 ± 2.1	0.9 ± 0.10
Thyroid	9 ± 1.2	0.9 ± 0.06
Uterus	17 ± 0.7	2.0 ± 0.17

[a] Although no dichlorvos was found in the tissues, the ^{14}C and ^{36}Cl residues were calculated for simplicity as ppm equivalents of dichlorvos-^{14}C or dichlorvos-^{36}Cl. [b] Mean of sows 550 and 551 ± SEM. [c] Sample for sow 550 only.

Table IV. Total ^{14}C Residues in Piglets[a]

Tissues	Total ^{14}C residues[b,c] Age of piglet, 0 days	Age of piglet, 9 days	Age of piglet, 21 days
Brain	5.8 ± 0.32	5.7 ± 0.24	4.6 ± 0.09
Carcass	9.7 ± 0.48	10.3 ± 0.88	7.2 ± 0.36
Femur	10.5 ± 0.57	12.0 ± 0.92	7.9 ± 0.35
Gall bladder	6.8 ± 0.92	5.1 ± 0.42	4.6 ± 0.26
Heart	8.7 ± 0.37	7.9 ± 0.59	5.0 ± 0.14
Kidney	8.2 ± 0.21	7.1 ± 0.68	4.4 ± 0.09
Large intestine	8.6 ± 0.39	9.4 ± 1.39	4.0 ± 0.20
Liver	12.2 ± 0.70	8.1 ± 0.86	4.4 ± 0.12
Lungs	8.1 ± 0.30	8.6 ± 0.73	5.2 ± 0.12
Muscle	7.6 ± 0.31	9.2 ± 0.84	6.1 ± 0.20
Pancreas	12.1 ± 0.44	7.7 ± 0.73	5.1 ± 0.11
Spleen	11.0 ± 0.79	7.2 ± 0.74	4.7 ± 0.12
Stomach	7.2 ± 0.44	6.7 ± 0.52	4.2 ± 0.18

[a] Multidose ^{14}C experiment. [b] Mean ± SEM. [c] Although no dichlorvos was found in the tissues, the ^{14}C residues were calculated for simplicity as ppm equivalent of dichlorvos-^{14}C.

multiple-dose dichlorvos-^{14}C and multiple-dose dichlorvos-^{36}Cl experiments.

DISCUSSION AND CONCLUSIONS

The results obtained in these experiments with the sows are qualitatively similar to the results found in similar experiments with young pigs (Potter *et al.*, 1973). Forty-six percent of the total dose of the dichlorvos was leached from the PVC pellets fed to the sows, while only 38% of the total dose of dichlorvos was leached from the PVC pellets fed to the pigs. The expired air from the sows contained 6% of the ^{14}C fed to the sows as dichlorvos-^{14}C, while the expired air from the pigs contained 14% of the ^{14}C fed to the pigs as dichlorvos-^{14}C. These differences

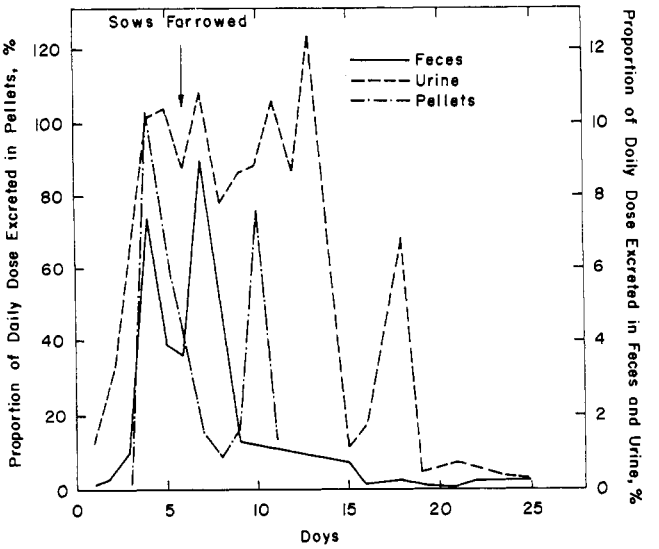

Figure 4. Excretion rates of radioactivity from sows fed dichlorvos-^{36}Cl.

Table V. Total ^{36}Cl Residues in Piglets

Tissues	Total ^{36}Cl residues[a,b] Age of piglet, 0 days	Age of piglet, 9 days	Age of piglet, 21 days
Brain	3.9 ± 0.40	5.3 ± 0.42	3.4 ± 0.09
Carcass	6.0 ± 0.78	6.2 ± 0.51	2.3 ± 0.10
Fat		6.2 ± 0.45	3.2 ± 0.09
Femur	4.4 ± 0.47	5.6 ± 0.34	3.5 ± 0.15
Gall bladder	5.8 ± 0.46	7.8 ± 0.48	4.0 ± 0.30
Heart	4.1 ± 0.41	5.2 ± 0.35	3.0 ± 0.22
Kidney	4.5 ± 0.68	6.3 ± 0.40	3.7 ± 0.12
Large intestine	4.1 ± 0.56	5.7 ± 0.92	1.4 ± 0.08
Liver	3.3 ± 0.36	3.8 ± 0.48	2.0 ± 0.22
Lungs	5.3 ± 0.25	7.2 ± 0.38	3.8 ± 0.16
Muscle	3.8 ± 0.62	4.4 ± 0.44	2.1 ± 0.13
Pancreas	3.9 ± 0.58	5.8 ± 0.49	3.6 ± 0.19
Salivary gland	5.5 ± 0.53	6.3 ± 0.47	3.6 ± 0.13
Spleen	5.0 ± 0.55	6.6 ± 0.61	3.3 ± 0.25
Stomach	5.9 ± 0.82	6.4 ± 0.55	4.1 ± 0.13

[a] Mean from three pigs ± SEM. [b] Although no dichlorvos was found in the tissues, the ^{36}Cl residues were calculated for simplicity as ppm equivalents of dichlorvos-^{36}Cl.

may be caused by the different basis of the measurements. In the trials with pigs a single dose was administered and the ^{14}C in the expired air and excreta was determined for the next 14 days, while in the trials with the sows the sows were dosed daily for approximately 25 days and the ^{14}C was determined daily in the expired air and excreta.

No residues of dichlorvos, demethyl dichlorvos, dichloroacetaldehyde, demethyl dichlorvos, dichloroacetaldehyde, dichloroacidic acid, or dichloroethanol were found in any of the tissues.

The concentrations of ^{14}C and ^{36}Cl in all the tissues of the sows and piglets were 10–600 times higher than the detection limit of dichlorvos. Information presented by Page *et al.* (1971), Hutson *et al.* (1971), and Loeffler *et al.* (1971) showed that the liver and muscle from the sows and from rats treated with dichlorvos-^{14}C contained labeled compounds as glycine, serine, creatine, glucose, glycogen, fatty acids, cholesterol, choline, lecithin, and ribonucleic acid and that the ^{36}Cl was all present as chloride ion. The presence of ^{14}C and ^{36}Cl in the piglets is thus probably due to the degradation of the vinyl group of dichlorvos to normal constituents in the sow and subsequent incorporation of these ^{14}C-labeled compounds and ^{36}Cl

ion by the fetuses prior to farrowing and by the piglets ingesting milk containing ^{36}Cl ion and normal constituents labeled with ^{14}C after farrowing.

In summary, the absence of dichlorvos and the known metabolites of dichlorvos, the presence of ^{36}Cl ion and normal constituents labeled with ^{14}C, and the presence of ^{14}C-labeled carbon dioxide suggest that the ^{14}C residues in the tissues of the sows and piglets are due to normal tissue constituents formed by degradation of the dichlorovinyl moiety in dichlorvos.

ACKNOWLEDGMENT

The authors are indebted to D. L. Arnold, D. D. Jordan, W. M. Olson, G. A. Sanders, J. H. Mears, and J. M. Montero, whose help was essential in carrying out this investigation.

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Received for review August 24, 1972. Accepted May 3, 1973.

COMMUNICATIONS

Identification of 2,4,6-Trichloroanisole in Several Essential Oils

The identification of 2,4,6-trichloroanisole (I) as a trace constituent of a number of essential oils (distilled Mexican lime, French petitgrain (Clementine), Bulgarian rose, and Spanish rue) is reported. Likely, although not firmly established, is its presence in French geranium, Italian lemon, and American peppermint (*Mentha piperita*). Circumstantial arguments indicate that I is more of fungal or microbial origin than a pesticide residue.

In the course of analytical work on various essential oils we repeatedly encountered fractions having a strong musty naphthalenic odor. After closer examination by gas and liquid chromatography, it became apparent that the same trace component was involved in concentrations between 0.0001 and 0.0009%. In all cases the characteristic odor was observed at the same retention time while smelling the exit gas eluting from a gas chromatograph operating under standardized conditions.

In the attempts to isolate the musty smelling component, we found that it eluted with the hydrocarbon fraction upon chromatographing the oils on silica gel with *n*-pentane as the eluting solvent (Kirchner and Miller, 1952). Further work showed that the compound eluted toward the end of the chromatography procedure together with aromatic compounds such as alkyl naphthalenes. In order not to introduce impurities contained in the silica gel as purchased, the column was washed thoroughly with (carefully purified) *n*-pentane prior to use.

Since apparently small quantities were involved, we did not succeed in preparing a sufficiently pure sample to obtain an acceptable infrared spectrum. Therefore, appropriate fractions were injected into a gas chromatograph coupled to a double-focusing mass spectrometer. The gas chromatograph was equipped with a stainless steel column packed with 10% SE-30 on 70–80 mesh Varaport 30. The mass spectrum of the compound in question indicated the presence of three chlorine atoms, with M = 210 for the isotope 35, and suggested a trichloroanisole as a possible structure. Comparing gas chromatographic and mass spectral data of the six isomeric trichloroanisoles showed that only 2,4,6-trichloroanisole and the unknown were identical in all respects.

The occurrence of traces of 2,4,6-trichloroanisole has been established in the essential oils of distilled Mexican lime, French petitgrain (Clementine), Bulgarian rose, and Spanish rue. Its presence in oil of French geranium, Italian lemon, and American peppermint (*Mentha piperita*) is presumed, based on mass spectral data, but has not been unequivocally demonstrated. In addition, direct gas chromatographic analysis of the oils of rose and petitgrain, using a Dohrmann cell, showed the presence of a halogen compound having the identical retention time as 2,4,6-trichloroanisole.

DISCUSSION

The origin of the trichloroanisole in these oils is of considerable interest. To our knowledge, no halogen compounds have been reported previously in essential oils and halogen metabolites are rare in higher plants. However, 1,4-dimethoxy-2-nitro-3,5,6-trichlorobenzene, 1,4-dimethoxy-2,3,5,6-tetrachlorobenzene, and 1-hydroxy-4-methoxytetrachlorobenzene have been discovered in fungi of the Fomes species (Butruille and Dominguez, 1972; Kavanagh *et al.*, 1952; Singh and Rangaswami, 1966). One can, therefore, ask whether the 2,4,6-trichloroanisole is produced by fungi that live on the plants or in the soil and are subsequently absorbed in the essential oil-bearing plants.

On the other hand, the use of polychlorophenols in agriculture as pesticides and in packaging materials as preservatives, and consequently the occurrence of metabolites derived from them, should be taken into account. Re-