waxy tissue of the apple fruit.

Experiments were conducted to evaluate the possible effect of chlorothalonil on ethephon metabolism in view of the suggestion that chlorothalonil can increase the uptake of ethephon by apple foliage in the first 3 to 4 days after chlorothalonil treatment (Holm and Edgerton, 1976). Consequently, the increased level of foliar [^{14}C]ethephon found on the first day in the presence of chlorothalonil (Figure 4) may represent chlorothalonil-enhanced ethephon uptake. Edgerton and Hatch (1972) found that leaf uptake of [^{14}C]ethephon was important to subsequent levels of [^{14}C]ethephon in the fruit and to production of ethylene in fruit. Thus, chlorothalonil may enhance the fruit ripening response of ethephon by increasing foliar ethephon uptake. The radioactive residue remaining in either the fruit or leaf total extracts in the 4- to 12-day harvest interval was not significantly affected by the presence of chlorothalonil (Figure 4). Examination of autoradiographs of TLC plates developed from the apple or leaf total extracts indicated only one spot that had the same corresponding R_f value as that of authentic [^{14}C]-ethephon. These observations indicate that chlorothalonil apparently affects neither the chemical breakdown nor the rate of disappearance of [^{14}C]ethephon from either McIntosh apple or leaf tissues.

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LITERATURE CITED

Ballee, D. L., Diamond Shamrock Corporation, Painesville, Ohio, personal communication, 1976.

Edgerton, L. J., Hatch, A. J., *J. Am. Soc. Hortic. Sci.* **97**, 112 (1972).

Gilbert, M. D., Monselise, S. P., Edgerton, L. J., Maylin, G. A., Hicks, L. J., Lisk, D. J., *J. Agric. Food Chem.* **23**, 290 (1975).

Holm, R. E., Edgerton, L. J., *J. Am. Soc. Hortic. Sci.*, in press (1976).

Schlenk, H., Gellerman, J. L., *Anal. Chem.* **32**, 1412 (1960).

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Metabolism of the Herbicide Methazole in Lactating Cows and Laying Hens

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[*phenyl*-^{14}C]Methazole [2-(3,4-dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-3,5-dione] was fed to hens at 0.3, 1.0, and 3.0 ppm in the diet for 14 days, and administered daily to cows via gelatin capsule at dosages equivalent to 0.5, 2.5, and 10 ppm in the diet for the same period. Maximum residues in the body after 14 days were located in the liver and were 0.026, 0.101, and 0.201 ppm of [^{14}C]methazole equivalents for hens at the three feeding levels; for cows, the residues in liver were 0.016, 0.065, and 0.406 ppm. Eggs contained ^{14}C-labeled residues at 3% the dietary parts per million levels and milk at about 0.3% that in the diet. Radiocarbon in tissues, eggs, milk, and excreta was characterized by extraction and partitioning behavior, and by TLC analysis of metabolites extractable into organic solvent. Major components of the latter were 3-(3,4-dichlorophenyl)-1-methylurea, 3,4-dichlorophenylurea, and *N*-(2-hydroxy-3,4-dichlorophenyl)urea and its 4,5-dichlorophenyl analogue.

The herbicide methazole [2-(3,4-dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-3,5-dione] has shown promise for control of broadleaf and grassy weeds in several crops and has recently been registered for use on cotton (Whitacre and Whitehead, 1976). The metabolic fate of methazole has been studied in several crop plants (Butts and Foy, 1974; Dorough et al., 1973; Dorough, 1974; Jones and Foy, 1972). These studies show methazole to be transformed largely to 3-(3,4-dichlorophenyl)-1-methylurea (DCPMU) and 3,4-dichlorophenylurea (DCPU) which may be present in the free and/or conjugated form.

A study of the metabolism of methazole in rats (Dorough et al., 1974) demonstrated that *N*-(2-hydroxy-4,5-dichlorophenyl)urea was the major urinary metabolite and was present almost entirely in the glucuronide form. The glucuronide of *N*-(2-hydroxy-3,4-dichlorophenyl)urea was also detected in the urine. DCPU was the major metabolite in the feces and existed almost completely in the free form. The highest levels of methazole equivalents were in the kidney, liver, and fat.

Table I. [^{14}C]Methazole Administered in the Diet of Lactating Cows and Laying Hens

ppm of methazole in diet		No. of animals		mg/animal per day	
Cows	Hens	Cows	Hens	Cows	Hens
0.0	0.0	1	6	0	0
0.5	0.3	1	6	11	0.03
2.5	1.0	1	12	55	0.10
10.0	3.0	1	6	220	0.30

Feeding studies with methazole in a dairy cow (Gutenmann et al., 1972) at 5 ppm in the diet for 4 days indicated that there were no detectable residues of the parent compound or its metabolites in the milk, urine, or feces. Because these findings were inconsistent with those reported for rats, a more thorough investigation of the metabolic fate of [^{14}C]methazole in dairy cows was warranted.

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Methazole is intended for use on certain crops which may be fed to livestock and, thus, could result in human exposure. The present study was undertaken to evaluate the propensity of methazole to transfer to tissues of dairy cows and poultry, as well as to milk and eggs.

MATERIALS AND METHODS

Chemicals. Analytical samples of methazole and some of its possible metabolite standards were supplied by Velsicol Chemical Corporation (Chicago, Ill.). Structures of the more important methazole derivatives have been presented in a previous report (Dorough et al., 1974).

[*phenyl*-^{14}C]Methazole (sp act. 22.9 mCi/mmol) was prepared by New England Nuclear Corporation, and was 99% radiochemically pure as determined by thin-layer chromatography (TLC). [^{14}C]Methazole was diluted with methazole analytical reference material to achieve the desired dosage levels. Table I shows the dosage levels administered to animals and the number of animals included in each study.

Sampling and Treatment. Holstein lactating cows weighing between 620 and 790 kg each were held in metabolism stalls and given access to hay and water. Crushed grain pellets were provided twice daily during milking. The milking schedule was the same as when the animals were in the herd, 6:00 a.m. and 4:00 p.m. Separate mechanical milkers were used for each cow to avoid cross contamination.

The cows were placed in the stalls for a pretreatment equilibration period of 5 days. On the 6th day, the animals were catheterized using a number 20 urinary catheter with a 75-cm^3 balloon. The balloon was inflated with 50 ml of a mild antiseptic solution to keep the catheters in place. Treatment of the animals with methazole was initiated 3 days after insertion of the catheter.

Feed consumption, milk production, and the quantity of urine and feces voided were recorded twice daily. The average values for these parameters before and during the treatment period did not change appreciably.

For treatment, a gelatin capsule was filled with crushed grain and the appropriate dose of [*phenyl*-^{14}C]methazole in acetone was pipetted directly into the capsule. The acetone was evaporated under a gentle air stream and the capsules were placed in a freezer until administered to the cows. Each cow was given a capsule containing the appropriate dose of methazole just prior to each milking (Table I). After 14 days of treatment, the animals were slaughtered and tissues collected and frozen until analyzed.

Twenty-four White Leghorn laying hens, 1 year old and averaging 1.35 kg in weight each, were individually housed in poultry battery units, each measuring 33 × 61 × 26 cm. A galvanized sliding tray was kept beneath each unit for collecting the excreta. Hens were given free access to water and a standard laying mash diet for a 10-day pretreatment period.

[^{14}C]Methazole-treated laying mash was prepared at the concentrations shown in Table I and was frozen until used. Treated feed was provided ad libitum. Records of food consumption, egg production, and excreta voided were collected daily throughout the experiment. The average values for these parameters before, during, and after treatment did not significantly change except that a 10% drop in food consumption occurred at the highest dose level.

Two untreated and two treated birds from each level were sacrificed after 7 and 14 days on treatment, and 7 days after withdrawal of the treated diet. Additionally, two birds from the 1.0-ppm level were sacrificed after 2 days on treatment, and 2 and 14 days after withdrawal. Blood and tissue samples were collected and frozen until analyzed.

Radioassay. Direct radioassay was made of 2 ml of milk or 1 ml of urine in 15 ml of a commercial blend of scintillation counting fluid (3a70B, Research Products International Corp., Elk Grove Village, Ill.). All samples were radioassayed using a Packard Tri-Carb Model 3380 scintillation counter. Solid samples were combusted in a Beckman Biological Materials Oxidizer and the $^{14}CO_2$ trapped and radioassayed.

Thin-Layer Chromatography. Silica gel F_{254} precoated chromatoplates (Merck) were used for resolving methazole and its metabolites in all phases of the study. Unless otherwise specified, the plates were developed in a 5:4:1 mixture of chloroform–petroleum ether–methanol. Two-dimensional chromatography was accomplished using the above-mentioned solvent system and a 95:5 mixture of ethyl ether and methanol. A mixture of 7:2:1 petroleum ether, chloroform, and methanol was also used for developing the plates. The chromatographic behavior of methazole and its metabolites in these solvent systems has been reported (Dorough et al., 1974).

Autoradiography was used to detect radiocarbon on the plates while nonradioactive standards were located by viewing the plates under ultraviolet light. Quantitation of the radioactive areas was accomplished by direct scintillation counting of the gel. When required for further study (e.g., cochromatography), ^{14}C-labeled metabolites were removed from the gel by extraction with methanol.

Sample Analysis. Urine, feces, and tissues containing sufficient radiocarbon were extracted and analyzed according to the procedures described previously (Dorough et al., 1974).

Milk was analyzed by adding 100 ml of 95% ethyl alcohol to 100 ml of whole milk in a 500-ml separatory funnel and shaking for 1 min. Then, 200 ml of diethyl ether was added and shaken for an additional 2 min. After adding 100 ml of pentane and shaking for another minute, the sample was allowed to stand for 10 min for complete separation of phases. The aqueous phase was removed and the organic solvent layer extracted with 50 ml of 2% aqueous NaCl solution. The latter was combined with the original aqueous phase. Anhydrous sodium sulfate was added to the solvent extract and then the extract was filtered into a 500-ml boiling flask and evaporated to an oily residue. Hexane, 100 ml, was used to dissolve the residue which was then extracted 3 times with 50-ml portions of acetonitrile. The phases were separated and both fractions concentrated to near dryness. At this point, the milk was separated into three fractions: that radiocarbon in the water fraction is referred to as water soluble metabolites, that in the acetonitrile as organosoluble metabolites, and that in the hexane as oil soluble metabolites.

The organosoluble metabolites were analyzed directly by TLC. Various methods (solvent partitioning, TLC, and gel permeation chromatography) were utilized in an attempt to remove the radioactive residues from the oils (butterfat) contained in the hexane fraction. However, all attempts were unsuccessful and no information was obtained concerning the chemical nature of the minute ^{14}C-labeled residues associated with the milk fat.

The water fraction of the milk extract was adjusted to 1 N with HCl, heated at 90 °C for 1 h, cooled, and neutralized with NaOH, and then extracted twice with 150 ml of a 2:1 mixture of ether and pentane. The combined extracts were washed with 50 ml of 2% aqueous NaCl, dried with anhydrous sodium sulfate, and then concen-

Table II. Elimination of Radiocarbon from Cows Treated for 14 Days with [^{14}C]Methazole

Days on treatment	Cumulative % of dose[a]/feeding level, ppm											
	0.5				2.5				10			
	Milk	Urine	Feces	Total	Milk	Urine	Feces	Total	Milk	Urine	Feces	Total
1	0.13	78.2	5.6	83.9	0.12	69.7	4.5	74.3	0.11	63.2	4.4	67.7
2	0.17	83.8	7.8	91.8	0.17	79.4	7.6	87.2	0.13	72.7	4.8	77.6
3	0.20	86.9	9.4	96.5	0.19	86.3	13.3	99.8	0.15	74.6	7.2	82.0
7	0.25	86.6	12.3	99.2	0.27	76.3	20.9	97.5	0.20	77.4	10.4	88.0
10	0.25	85.6	13.9	99.8	0.28	79.3	18.9	98.5	0.21	82.0	10.4	92.6
14	0.27	84.0	14.7	99.0	0.28	79.9	17.5	97.7	0.22	82.0	10.8	93.0

[a] (Cumulative [^{14}C]methazole equivalents eliminated)/(cumulative [^{14}C]methazole administered) × 100 = % elimination.

trated to a volume suitable for TLC analysis. Radioactive residues in this fraction are referred to as acid-released metabolites of methazole.

Eggs were analyzed by extracting 5-g aliquots of homogenized eggs, yolk and white, three times in 20 ml of acetonitrile and 6 ml of distilled water. The combined filtrates were concentrated until no acetonitrile remained, and the water extracted three times with ethyl acetate. The extract was dried with sodium sulfate, filtered, and evaporated until only fat remained. The sample was transferred to a 150-ml separatory funnel with 60 ml of hexane and extracted three times with 30 ml of acetonitrile. Both fractions were concentrated and the acetonitrile fraction (organosolubles) retained for TLC analysis. The hexane fraction contained the oil solubles. The water-soluble and solid fractions were combined, the water adjusted to 1 N HCl, and the mixture heated for 1 h at 90 °C. After cooling, the mixture was neutralized, filtered, and extracted with ethyl acetate. The different phases were radioassayed, and the ethyl acetate extract was analyzed by TLC.

RESULTS AND DISCUSSION

Excretion. Methazole was very efficiently voided from the dairy animals (Table II). At the two lower treatment levels, 0.5 and 2.5 ppm, a near equilibration between intake and excretion was accomplished after only 3 days. The same situation occurred with the cow fed 10 ppm of methazole after about 10 days. By 14 days, there was over 90% elimination of the [^{14}C]methazole consumed by each cow during the treatment period.

The urine provided the major route of elimination with approximately 80% of the doses excreted therein, while 11 to 18% was voided in the feces. Data obtained from the three cows relative to the concentration of [^{14}C]-methazole equivalents in the milk show that cows on a diet contaminated with this herbicide would eliminate 0.2 to 0.3% of that consumed via this route. The expression of these levels on a parts per million basis is demonstrated in Table III.

Birds continuously exposed to dietary [^{14}C]methazole also rapidly eliminated the radiocarbon (Table IV). The mean cumulative excretion for the three feeding levels was 64% after 7 days. This increased to 76% after the birds were returned to a normal diet for 7 days. After 3 days on treatment, the levels of [^{14}C]methazole equivalents in the excreta stabilized at approximately 0.2, 0.7, and 1.8 ppm for the 0.3-, 1.0-, and 3.0-ppm feeding levels, respectively. These concentrations in the excreta were 70% of that in the diet for the two lower feeding levels, and 60% of the highest level in the diet. While reduced excretion rate at the 3.0-ppm feeding level was indicated by these data, it may have been that the feeding level was slightly less than the intended 10 ppm.

Parts per million of [^{14}C]methazole equivalent in the eggs are presented in Table III. These data, when converted to percentage of dose, showed that a maximum of 2.6% of the total administered radiocarbon was transferred to eggs. By 7 days of treatment, the levels of [^{14}C]-methazole equivalents in the eggs had maximized and remained rather constant throughout the remaining 17-day feeding period. The mean levels of total [^{14}C]methazole equivalents in eggs at equilibrium for the 0.3-, 0.1-, and 3.0-ppm feeding levels were 0.01, 0.03, and 0.08 ppm, respectively. After the chickens were returned to a normal diet for 7 days, ^{14}C-labeled residues in the eggs had declined to 0.006 ppm or less (Table III).

Table III. [^{14}C]Methazole Equivalents in Milk and Eggs

Days animal on/off treatment	ppm of [^{14}C]methazole equivalents/feeding levels for cows (A = 0.5 ppm, B = 2.5 ppm, C = 10 ppm) or hens (A = 0.3 ppm, B = 1.0 ppm, C = 3 ppm)		
	A	B	C
Cows, days on treatment		Milk	
1	0.001	0.005	0.020
2	0.001	0.010	0.032
3	0.002	0.011	0.039
7	0.002	0.014	0.039
10	0.002	0.013	0.045
14	0.002	0.014	0.038
Hens, days on treatment		Eggs	
1	0	0	0.001
2	0.002	0.001	0.003
3	0.008	0.003	0.033
7	0.012	0.025	0.050
10	0.013	0.029	0.078
14	0.013	0.028	0.078
Hens, days off treatment			
1	0.011	0.029	0.072
4	0.006	0.021	0.037
7	0.001	0.001	0.006

Table IV. Radiocarbon in the Excreta of Hens Fed a Diet Containing [^{14}C]Methazole

Days	Cumulative % of dose[a]/feeding level, ppm		
	0.3	1.0	3.0
On treatment			
1	37.1	15.4	18.9
2	44.3	28.7	31.3
3	59.3	41.8	41.0
7	73.7	60.3	58.0
10	74.2	62.8	61.7
14	75.3	65.9	65.0
Off treatment			
1	79.5	70.3	68.4
4	82.8	71.8	69.5
7	82.9	73.8	71.5

[a] (Cumulative [^{14}C]methazole equivalents eliminated)/(cumulative [^{14}C]methazole administered) × 100 = % elimination.

Table V. Residues in Tissues of Cows after Treatment with [^{14}C]Methazole for 14 Days[a]

Tissues	ppm of [^{14}C]methazole equivalents/feeding level[b]		
	0.5 ppm	2.5 ppm	10.0 ppm
Liver	0.016	0.065	0.406
Kidney	ND	0.046	0.162
Blood	ND	0.017	0.043
Lung	ND	ND	0.035
Brain	ND	ND	0.021
Fat, subcutaneous	ND	ND	0.018
Skin	ND	ND	0.017
Heart	ND	ND	0.013
Muscle, foreleg	ND	ND	0.011
Muscle, neck	ND	ND	0.007
Muscle, hindleg	ND	ND	0.008

[a] Animals slaughtered approximately 14 h after last treatment. [b] The following tissues were assayed but did not contain detectable levels of residues: renal and omental fat, rib and leg bone; ND = none detected.

^{14}C-Labeled Residues in Tissues. As expected from the excretion data, residues of [^{14}C]methazole equivalents in the tissues of cows were very low (Table V). The liver was the only tissue which contained detectable levels of residues at each of the treatment levels. Although the ^{14}C-labeled residues in the liver did not increase in a manner precisely proportionate to the increase in dosage, a good correlation was evident. Based on the average data from the three cows, total [^{14}C]methazole equivalents in the liver were $^{1}/_{30}$th that level consumed in the diet.

Other than the liver, only the kidney and blood of the cow fed 2.5 ppm of methazole contained measurable quantities of radioactive residues. However, at the 10-ppm feeding level, almost all of the tissues assayed contained detectable residues. Even so, the levels of residues were extremely low, with only the liver and kidney containing more than 0.05 ppm of [^{14}C]methazole equivalents. Muscle tissue generally contained 0.01 ppm or less.

Levels of radioactive residues in tissues of hens fed a diet containing [^{14}C]methazole are shown in Table VI. Concentrations of [^{14}C]methazole equivalents were highest in the kidney and liver, one-ninth and one-tenth the parts per million level in the diet after 14 days, and lowest in the brain and muscle tissue. There was rapid dissipation of residues from the tissues upon removal of the herbicide from the diet, with at least a 50% reduction occurring in all samples except the fat after only 2 days. After one week, the residue levels were generally one-tenth or less those found in the tissues after 14 days of treatment.

Nature of ^{14}C-Labeled Residues. *Extraction Characteristics.* The near complete elimination of radiocarbon from cows and hens treated with [^{14}C]methazole resulted in quantities of ^{14}C-labeled residues in most samples which were too low for identification purposes. Nonetheless, the levels were sufficient to determine their general nature as exemplified by the distribution of radiocarbon among the various fractions following extraction and/or partitioning of the residues (Table VII). The data from the analysis of the excreta, which contained large quantities of radioactivity, are included for the sake of comparison.

Excreta. [^{14}C]Methazole equivalents in the cow urine and hen excreta were very similar. Approximately one-half of the residues were extractable directly with organic solvent while another 25–35% could be extracted following acid treatment of the aqueous phase (Table VII). The former were considered as being in the free state and the latter as conjugates and/or salts of certain methazole metabolites as was the case in rat urine (Dorough et al., 1974). TLC analysis of the extractable metabolites of cow urine and hen excreta further demonstrated their similarity. The same metabolites were present in both and each was of about the same magnitude in relation to the total radiocarbon in the sample. Data in Table VIII for the cow urine typify the findings of the TLC analysis of urine and hen excreta extracts in samples collected throughout the 14-day feeding period and at each feeding level. DCPU, R_f 0.33, was the predominant component, 44% of the total residues, and was largely in the free form. The remainder of the extractable radioactivity was composed chiefly of the ring hydroxylated metabolites, R_f 0.15 and 0.24, and the methylurea (DCPMU, R_f 0.53) derivative.

The same metabolites detected in the urine were present in the extractable fractions of the cow feces. More of the residues, however, were of an organosoluble nature in the feces than in the urine (Table VII). TLC analysis of the organosoluble fraction yielded erratic quantitative data although the general trend was similar to that distribution noted in the urine. This was attributed to the instability of methazole metabolites in the feces subsequent to ex-

Table VI. Residues in Tissues of Hens Fed [^{14}C]Methazole in the Diet

Feeding level and days	ppm of [^{14}C]methazole equivalents					
	Liver	Kidney	Blood	Fat	Brain	Muscle
0.3 ppm						
On treatment						
7	0.024	0.022	0.011	ND	0.003	0.002
14	0.026	0.030	0.021	0.012	0.002	0.002
Off treatment						
7	0.003	0.004	0.003	ND	ND	ND
1.0 ppm						
On treatment						
2	0.046	0.062	0.047	ND	0.009	0.004
7	0.043	0.069	0.028	0.011	0.005	0.003
14	0.101	0.123	0.062	0.017	0.010	0.008
Off treatment[a]						
2	0.042	0.032	0.029	0.017	0.002	0.003
7	0.007	0.007	0.002	ND	ND	0.002
3.0 ppm						
On treatment						
7	0.158	0.172	0.071	0.025	0.015	0.009
14	0.201	0.286	0.124	0.038	0.013	0.010
Off treatment						
7	0.014	0.024	0.029	0.010	ND	ND

[a] Tissues collected 14 days off treatment were free of detectable radiocarbon.

Table VII. General Nature of [^{14}C]Methazole Equivalents in Samples from Cows and Hens after 14 Days of Treatment at 10 ppm and 3 ppm of [^{14}C]Methazole in the Diet, Respectively[a]

Animal and sample	% of total ^{14}C in sample/nature of metabolites: Organo-soluble	Acid released[b]	Water soluble[c]	Unextracted
Cow				
Urine	49.2	34.5	16.3	
Feces	61.6	2.5	9.0	26.9
Liver	10.9	25.6	11.4	52.1
Kidney	50.8	10.8	20.4	18.0
Blood	15.3	26.3	16.6	41.8
Heart	39.2	5.0	14.4	41.4
Muscle	14.6	7.0	22.1	56.3
Lung	28.0	20.3	13.6	38.1
Brain	23.7	26.2	18.5	31.6
Milk	39.9	25.4	34.7	
Hen				
Excreta	54.4	25.0	12.4	8.2
Liver	39.1	10.9	14.1	35.9
Kidney	53.8	21.4	13.9	10.9
Eggs	61.6	11.9	15.7	10.8

[a] The general nature of the radiocarbon was not influenced by dose, time of treatment, or in the case of samples from hens, the time after treatment was terminated. Therefore, the data for the other two levels and other sampling times are not included. [b] Radiocarbon converted from water-soluble materials by acid treatment. [c] Radiocarbon remaining in water fraction after acid treatment and extraction with organic solvent.

cretion, a point documented by adding [^{14}C]methazole and metabolites to rat feces and allowing it to stand for 24 h (Dorough et al., 1974). In that study, extensive degradation of methazole and DCPMU occurred, but could be prevented by collecting the fecal material in light-tight containers. Such precautions were not taken in the present study.

Tissues. There was considerable variation in the general nature of [^{14}C]methazole equivalents in tissues from the same animal and in the same tissue from the two test species (Table VII). In the cow, the free or organosoluble metabolites were lowest in the liver, blood, and muscle where they accounted for 10 to 15% of the total residues. Total water-soluble ^{14}C-labeled residues in these tissues comprised 30 to 43% of the radiocarbon in the samples, but about 65% of those in the liver and blood was rendered organosoluble by acid treatment. Only 24% of the ^{14}C-labeled water solubles in the muscle was so rendered by identical treatment. Residues which could not be extracted from the liver, blood, and muscle constituted 52, 42, and 56% of the total ^{14}C content, respectively. With the exception of the kidney, the unextracted residues in the other tissues constituted 30 to 40% of the total residues present, while the percentages in the organo extract and the water-soluble fractions showed a corresponding increase. Only 18% of the radiocarbon in the kidney was unextractable and 20% remained in the water-soluble fraction following acid treatment.

Extraction characteristics of the [^{14}C]methazole equivalents in the kidney of hens were similar to those in the cow kidney. However, the residues in the liver of the two animals showed a different pattern of distribution (Table VII). The major difference was that the organosolubles in the cow liver accounted for 11% of the total residues whereas in the hen these residues accounted for 39%. None of the other tissues from the hens were similarly analyzed because of low residue levels and/or small sample size.

Individual components of the organoextractables, free and acid released, from the liver and kidney are shown in Table VIII. Cow kidney extracts gave results somewhat different than the general pattern demonstrated by the other tissues. For example, the two ring hydroxylated metabolites, R_f 0.15 and 0.24, comprised 29% of the total residues in the cow kidney but only 8% in the hen kidney, and even less in the liver of either animal. Moreover, DCPU, R_f 0.33, was only 9% of the total in the cow kidney while it was the major component, 24 to 56%, of the other tissues analyzed. Two unknown metabolites, R_f 0.10 and 0.28, also were of a much greater percentage, 6 and 10%, of the total cow kidney residues than observed in the livers or hen kidney. However, it did not contain detectable quantities of the unknowns having R_f values of 0.38 and 0.42 as did some of the other tissue samples. DCPMU, R_f 0.53, constituted from 2 to 6% of the total residues in all samples and the parent compound was not detected in any of the tissues.

Milk. Centrifugal separation of the whole milk into butterfat and skim milk showed that the residues were almost equally distributed among these two fractions. The same results were obtained when the butterfat was extracted from the milk with organic solvent.

The low levels of radioactive residues in the milk of cows fed 0.5 and 2.5 ppm of [^{14}C]methazole precluded a detailed comparison of the nature of the residues in these samples. However, a comparison was made of the extraction characteristic of the radioactive components in milk from each of the three cows, and the data revealed that the ^{14}C-labeled residues were of a similar nature in all milk samples.

Table VIII. ^{14}C-Labeled Metabolites in the Organosoluble and Acid-Released Fractions of Samples from Cows and Hens after 14 Days of Treatment at 10 and 3 ppm of [^{14}C]Methazole in the Diet, Respectively[a]

R_f[b]	Metabolites	% of total ^{14}C in sample[c]: Cows: Urine	Milk[d]	Liver	Kidney	Hens: Liver	Kidney
0.00	Unknown	3.4	0.7 (100)	1.0 (100)	4.7 (23)	1.4 (71)	2.2 (64)
0.10	Unknown	0	5.8 (100)	1.6	5.9	0.8	0.6
0.15	*N*-(2-Hydroxy-4,5-dichlorophenyl)urea	15.1	10.0 (88)	4.2 (45)	14.1 (8)	1.6 (44)	6.3 (35)
0.24	*N*-(2-Hydroxy-3,4-dichlorophenyl)urea	7.0	4.5 (100)	0.9	14.5 (11)	1.4 (43)	1.5
0.28	Unknown	0	0	0	10.0	0.5	0.3
0.33	3,4-Dichlorophenylurea	43.8 (11)	9.7 (36)	24.3 (80)	8.7 (52)	39.4 (19)	56.4 (22)
0.38	Unknown	0.9	0.5 (60)	2.4	0	1.2	0.9
0.42	Unknown	0	0	0	0	1.0	1.0
0.53	3-(3,4-Dichlorophenyl)-1-methylurea	13.5 (31)	2.0 (95)	2.1 (33)	3.7 (62)	2.5 (36)	6.0 (93)

[a] See Table VII for explanation of fractions. Only those samples shown here contained sufficient radiocarbon for TLC analysis. [b] Solvent system, 5:4:1 chloroform-petroleum ether-methanol; R_f of methazole was 0.98. [c] Value in parentheses is percent of metabolite released by acid treatment of the water-soluble fraction. [d] Average of all samples analyzed during 14-day feeding period; relative distribution of metabolites was similar at each sampling time.

Approximately 40% of the radiocarbon was extractable from the milk with organic solvent (Table VII). When these residues were partitioned between acetonitrile and hexane, about 30% of the radioactivity was located in the acetonitrile and 70% in the hexane. Components of the acetonitrile were resolved by TLC but similar attempts to analyze the hexane fraction, or oil solubles, failed. The same was true of attempts at separation by column chromatography, acid and base treatment of the oils, and by slowly heating the oils to the point of charring. In each case, the radiocarbon remained in the oils or in the solids formed from the oils by the various treatments. The quantity or radiocarbon in the oils was too low to obtain reliable distribution data even when the oils were separated into fractions by TLC or column chromatography. With the milk from the 10-ppm level cows, for example, the oils contained less than 20 dpm/mg.

^{14}C-Labeled residues remaining in the aqueous phase after extraction with organic solvent accounted for 60% of the total ^{14}C content of the milk. When treated with acid, about one-half of these water-soluble residues or 25.4% of the total residues was converted to products extractable into ether. These metabolites and those in the acetonitrile fraction, 7.7% of the total, were subjected to TLC analysis. The remainder of the residues, 67% of the total ^{14}C content of the milk, was almost equally distributed between the water layer after acid treatment and the oil-soluble fraction.

Of the ^{14}C-labeled residues extractable from the milk 33% was resolved into seven components by TLC (Table VIII). Almost one-half of these residues was as *N*-(2-hydroxy-4,5-dichlorophenyl)urea and its 3,4-dichlorophenyl isomer. The latter was entirely as a conjugate while 88% of the former was in the conjugated or acid-released form. DCPMU, R_f 0.53, and DCPU, R_f 0.33, together constituted 12% of the [^{14}C]methazole equivalents in the milk; both were present in the organosoluble and acid-released fractions although DCPMU was predominately a component of the acid-released materials. The other radiocarbon in the extractables from milk, 7% of the total in the whole milk, consisted of unknown metabolites. Trace amounts of materials were at the TLC origin and as an unknown product at R_f 0.38. The other unknown, R_f 0.10, was equivalent to 5.8% of the radiocarbon in the milk and was water soluble in nature prior to acid treatment. In the cow and hen tissues, a metabolite with identical TLC position was present but was an organosoluble material. The same characteristics were noted for a methazole metabolite in rat urine, liver, and kidney (Dorough et al., 1974). It may be that the acid-released product from milk is not the same as the free metabolite from other samples having the same R_f. This was impossible to confirm because the total radiocarbon exhibiting an R_f 0.10 was extremely low in all samples.

Eggs. Approximately 90% of the [^{14}C]methazole equivalents in the eggs was extractable with acetonitrile and water (Table VII). Sixty-two percent of the residues was of an organosoluble nature and about 10% of these products was associated with the oils. The water-soluble metabolites constituted 28% of the total ^{14}C content of the eggs but 43% of these, 12% of the total, was converted into organoextractables by acid treatment. Levels of radiocarbon in the organosoluble fraction from the eggs were not high enough for meaningful TLC analysis. However, direct radioassay of the gel after development of the TLC showed that the radioactivity was distributed at R_f values corresponding to DCPMU, DCPU, *N*-(2-hydroxy-3,4-dichlorophenyl)urea, and *N*-(2-hydroxy-4,5-dichlorophenyl)urea. That radiocarbon moving as DCPU constituted about 60% of the residues in the eggs while the others ranged from 5 to 10%.

LITERATURE CITED

Butts, E. R., Foy, C. L., *Pestic. Biochem. Physiol.* **4**, 44 (1974).
Dorough, H. W., *Bull. Environ. Contam. Toxicol.* **12**, 493 (1974).
Dorough, H. W., Cardona, R. A., Lolmaugh, S. C., *Drug Metabol. Disposition* **2**, 129 (1974).
Dorough, H. W., Whitacre, D. M., Cardona, R. A., *J. Agric. Food Chem.* **21**, 797 (1973).
Gutenmann, H. W., Serum, J. W., Lisk, D. J., *J. Agric. Food Chem.* **20**, 991 (1972).
Jones, D. W., Foy, C. L., *Pestic. Biochem. Physiol.* **2**, 8 (1972).
Whitacre, D. M., Whitehead, C. W., *Weeds Today*, in press (1976).

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