Carbon-14 Distribution and Elimination in Chickens Given Methoprene-^{14}C

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Distribution and elimination of carbon-14 given to chickens as methoprene-^{14}C (isopropyl (2*E*,4*E*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate-5-^{14}C) were investigated. When about 4 mg of methoprene was given in a single oral dose to colostomized chickens, elimination of carbon-14 was greatest in exhaled air; however, when 105 or 107 mg of methoprene was given, elimination of carbon-14 was greatest in urine. Up to 19% of the carbon-14 from a single dose of methoprene was eliminated over a 14-day period in the eggs of laying hens, and carbon-14 was detected in all tissues and organs examined.

Methoprene (isopropyl (2*E*,4*E*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate) is a synthetic insect growth regulator that mimics the physiological action of insect juvenile hormone (Henrick et al., 1973). There is interest in using methoprene as a feed-through larvicide to prevent house flies from developing in poultry droppings. This report is the first of a two-part cooperative study to determine methoprene metabolism by chickens. For the second part of the study, see Quistad et al. (1976).

MATERIALS AND METHODS

Colostomized (Paulson, 1969) or intact adult White Leghorn hens weighing 1.22 to 2.20 kg were used. Each hen was placed in a 41-cm^3 Lucite box equipped for exchange of air and collection of expired carbon dioxide. The methoprene was given orally in gelatin capsules. Food and water were supplied ad libitum.

Dosing Materials. Methoprene-5-^{14}C (Schooley et al., 1975), specific activity 5 mCi/mmol, diluted with nonradioactive methoprene was used for chickens 6, 10, 13, 20, 24, 30, and 37. Purity of the methoprene-5-^{14}C (radio-GLC) was 95.9% 2*E*,4*E* isomer, 4.1% 2*Z*,4*E* isomer and with no detectable radioactive impurities, and purity of nonradioactive methoprene was 95.6% 2*E*,4*E* isomer and 2.4% 2*Z*,4*E* isomer. Methoprene-5-^{14}C, specific activity 25.5 mCi/mmol, purity 99.3% 2*E*,4*E* isomer, 0.6% 2*Z*,4*E* isomer, was used for chickens 3, 4, 5, and 9. Preparation and purification of methoprene-5-^{14}C has been described (Schooley et al., 1975), and nonlabeled methoprene was a select production sample supplied by Zoecon Corporation.

Collection of Expired Carbon Dioxide. All air from the Lucite box containing the chickens was aspirated through solutions of ethanolamine (Jeffay and Alvarez, 1961) or 5% KOH (chicken 24 only) to trap the carbon dioxide. With either solution, two 2-l. Erlenmeyer flasks equipped with fritted glass aerators and containing 600 to 800 ml of trapping solution were connected to the box and in series, and finally in the series was a trap containing 2 l. of water to prevent caustic materials from entering the vacuum pump. Air was aspirated through the box at about 25 l./min.

Radioactivity trapped in KOH was verified as $^{14}CO_2$ (Quistad et al., 1975b). Purging of acidified KOH solution with carbon dioxide (dry ice) quantitatively removed the carbon-14. Precipitation of the carbon dioxide as barium carbonate removed 96% of the radioactivity from the KOH solution.

Radioassays. Radioactivity was measured on a Packard Model 3375 liquid scintillation spectrometer equipped with an automatic external standard. Samples of urine and the KOH solution were assayed in Insta-Gel (Packard Instrument Co., Inc.), and samples of the ethanolamine solution were assayed in a toluene cocktail (Hayes, 1963). Feces, eggs, and tissues were lyophilized, combusted in a Packard Model 306 oxidizer, and assayed for carbon-14 by liquid scintillation techniques. Assays of the water collected in the lyophilizer indicated that the losses of carbon-14 during lyophilization were negligible.

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RESULTS AND DISCUSSION

Dosages varied from 1 mg of methoprene per chicken to 138 mg (Tables I–III). In colostomized chickens given about 4 mg of methoprene (Table I), elimination of carbon-14 was greatest in respired air and lowest in feces. In contrast, when 105 or 107 mg of methoprene was given, elimination of carbon-14 was greatest in urine and was lowest in feces.

The average percentage elimination of carbon-14 in the 0–48-h period via respiration was 36.9 ± 3.0% (mean and standard deviation) when 4.2 mg or less of methoprene was given (Tables I–III) and was 24.0 ± 3.5% when 46 mg or more of methoprene was given. Apparently, the proportion of methoprene metabolized to carbon dioxide was higher when the lower dosages of methoprene were given than when the higher dosages were given.

Relatively large amounts of radioactivity were eliminated in eggs (Tables II and III). Most of the radioactivity in eggs was in the yolk (Table IV). The amount of carbon-14 in the yolk peaked at 1 to 3% of the dose on the 2nd to 7th days and was less than 0.1% of the dose by the 13th day.

Relatively small amounts of carbon-14 appeared in eggshells. Eggs from a chicken given 46 mg of methoprene (chicken 6) were examined qualitatively to determine whether the radioactivity was in the shell membranes or in the demembranized shell. Membranes were removed by soaking the shell in a dilute solution of EDTA. The demembranized shell was dissolved in 2 N HCl; the HCl solution was then purged with carbon dioxide (dry ice), and the gases were trapped in a solution of ethanolamine and assayed. Radioactivity in membranes of the egg laid on the 1st day after dosing was 7700 dpm/g and on the 2nd day was 220000 dpm/g, whereas radioactivity in the demembranized shell was 107000 dpm/g for the 1st day and 26000 dpm/g for the 2nd day.

The sequence of physiological events associated with egg formation would explain why more carbon-14 was incorporated in the shell than in the shell membranes of the egg laid on the 1st day. In laying hens, ovulation occurs about 0.5 h after oviposition, shell membranes are formed about 4 h after ovulation, and shell formation begins immediately after the membranes are formed. The chickens used in these studies were dosed immediately after an oviposition. Thus, development of a new egg

Table I. Dosages and Recoveries of Carbon-14 Given to Colostomized Chickens as Methoprene-5-^{14}C

Dosages and materials examined	Chicken 20	Chicken 37	Chicken 13	Chicken 24
Dosages				
Methoprene, mg (mg/kg)	4.2 (1.9)	4.1 (3.4)	105 (59)	107 (64)
Methoprene-^{14}C, μCi	67.7	66.8	67.7	67.7
Recoveries, % of dose				
Respired air, 0-24 h	38.7	33.4	22.0	28.3
24-48 h	5.1	8.1	3.0	5.0
Total in respired air	(43.8)	(41.5)	(25.0)	(33.3)
Feces, 0-24 h	8.7	7.6	16.5	13.3
24-48 h	2.3	2.3	2.3	3.9
Total in feces	(11.0)	(9.9)	(18.8)	(17.2)
Urine, 0-24 h	11.0	22.2	33.3	28.6
24-48 h	0.6	2.6	0.8	10.2
Total in urine	(11.6)	(24.8)	(34.1)	(38.8)
Bile	0.04	0.07	0.08	0.20
Feathers	1.19	0.16	0.52	0.90
Gizzard	0.24	0.76	0.13	0.20
Heart	0.07	0.10	0.05	0.06
Intestines	2.35	3.07	1.25	1.90
Kidneys	0.24	0.45	0.35	0.40
Liver	1.12	1.50	0.85	1.36
Lungs	0.07	0.12	0.04	0.82
Carcass remains	7.0	12.0	8.2	8.4
Total recovered, %	78.7	94.4	89.4	103.5

Table II. Dosages and 48-h Recoveries of Carbon-14 Given to Laying Hens as Methoprene-5-^{14}C

Dosages and materials examined	Chicken 3	Chicken 4	Chicken 6	Chicken 30
Dosages				
Methoprene, mg (mg/kg)	1 (0.6)	1 (0.6)	46 (31)	138 (77)
Methoprene-^{14}C, μCi	81.0	81.0	81.0	67.7
Recoveries, % of dose				
Respired air, 0-24 h	21.7	36.1	14.1	NC[a]
24-48 h	1.6	3.0	2.6	NC
Total in respired air	(23.3)	(39.1)	(16.7)	
Excreta, 0-24 h	50.3	27.9	52.3	57.3
24-48 h	0.8	1.1	4.2	1.3
Total in excreta	(51.1)	(29.0)	(56.5)	(58.6)
Eggs[b]	2.42	2.16	2.22	3.04
Feathers	0.12	0.09	0.05	NC
Viscera	9.0	4.8	12.9	1.38
Carcass	4.2	22.1	3.1	17.1
Total recovered, %	90.1	97.2	91.5	80.1

[a] NC = not collected. [b] Total for two eggs in all cases.

Table III. Dosages and 6- to 14-day Recoveries of Carbon-14 Given to Laying Hens as Methoprene-5-^{14}C

Duration, dosages, and materials examined	Chicken 5	Chicken 9	Chicken 10
Duration, days	14	14	6
Dosages			
Methoprene, mg (mg/kg)	1 (0.6)	1 (0.6)	73 (60)
Methoprene-^{14}C, μCi	81.0	81.0	0.85
Recoveries, % of dose			
Respired air, 0-24 h	11.5	35.7	18.5
24-48 h	23.8	2.4	2.6
48-72 h	2.8	1.2	1.1
After 72 h	3.7	2.2	NC[a]
Total in respired air	(41.8)	(41.5)	(22.2)
Excreta, 0-24 h	12.4	29.2	42.8
24-48 h	14.9	1.3	1.2
48-72 h	1.2	0.4	0.6
After 72 h	1.8	1.1	1.6
Total in excreta	(30.3)	(32.0)	(46.2)
Eggs	11.1 (12)[b]	19.0 (12)	12.5 (6)
Carcass	3.8	3.9	8.0
Total recovered, %	86.9	96.4	89.0

[a] NC = not collected. [b] Total for the number of eggs indicated in parentheses.

Table IV. Recovery of ^{14}C in Eggs of Hens Given Methoprene-5-^{14}C

	% of dose					
	Chicken 5		Chicken 9		Chicken 10	
Day	Shell and white	Yolk	Shell and white	Yolk	Shell	White and yolk
1	1.07	0.015	0.78	0.002	0.188	0.71
2	1.43	0.295	0.84	2.60	0.060	1.72
3	0.90	0.84	0.24	3.10	0.028	2.18
4	0.16	1.11			0.015	2.42
5			0.11	3.08	0.008	2.50
6	0.11	1.32	0.06	3.12	0.009	2.35
7	0.07	1.33	0.05	2.61		
8	0.05	0.98	0.03	1.46		
9	0.04	0.58	0.02	0.43		
10	0.03	0.32	0.02	0.14		
11	0.02	0.183	0.01	0.11		
12	0.02	0.146				
13	0.01	0.099	0.01	0.079		
14			0.01	0.064		

began after the chickens were dosed. The carbonate part of the eggshell apparently comes from carbon dioxide (Mueller and Leach, 1974); the maximum rate of elimination of carbon-^{14}C dioxide by metabolism of methoprene-^{14}C usually occurred during the first 24 h, but most likely occurred later than 4 h after dosing (Figure 1).

Some carbon-14 remained in every tissue and organ examined from chickens 2, 6, and 14 days after dosing

Table V. Methoprene Equivalents in Various Tissues of Chickens 48 h Postdosing with Methoprene-^{14}C

Dose and tissues examined	Chicken 4	Chicken 20	Chicken 37	Chicken 13	Chicken 24
Dose, mg (mg/kg)	1 (0.6)	4.2 (1.9)	4.1 (3.4)	105 (59)	107 (64)
Equivalents in tissue, μg/g of dry matter					
Adipose tissue	0.25	0.08	0.48	5.4	7.6
Adrenals	NC[a]	0.80	NC	NC	34.1
Breast	0.09	0.66	NC	7.6	12.0
Feathers	0.01	0.60	0.20	16.6	39.4
Femur, diaphysis	0.26	0.50	NC	11.3	10.8
Gizzard[b]	0.30	0.95	3.19	18.6	20.5
Heart	0.45	1.36	2.44	19.7	34.5
Intestines[b]	0.47	2.11	5.21	36.1	71.6
Kidneys	1.68	3.35	NC	80.8	104.1
Liver	1.64	5.73	10.26	106.1	152.4
Lungs	0.46	1.05	3.90	30.5	39.2
Skin	0.13	0.48	NC	22.7	17.7
Carcass remains[c]	0.46	0.58	1.09	13.0	16.1

[a] NC = not collected. [b] Includes digesta. [c] Carcass less viscera and feathers, whole organs listed, and samples of adipose tissue, breast, femur, and skin.

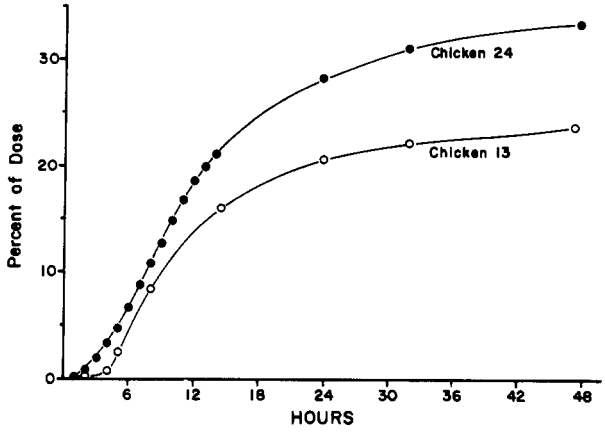

Figure 1. Cumulative expiration of carbon-14 by chickens given methoprene-5-^{14}C.

(Tables I–III and V), and the amount of methoprene or its equivalents which remained in the various tissues generally increased with increased size of the dose (Table V). Concentrations of carbon-14 were highest in liver and kidneys and lowest in adipose tissue.

Total recovery of radioactivity ranged from 78.7 to 103.5%. Recovery was low (80%) for a chicken given 138 mg of methoprene (chicken 30) because respired air was not collected. The low recovery (78.7%) for a chicken given 4.2 mg of methoprene (chicken 20) cannot be explained.

When methoprene-5-^{14}C was given to cattle, demethylated and deisopropylated metabolites of methoprene were identified in feces and urine (Chamberlain et al., 1975), but radioactivity was associated with milk fat, lactose, lactalbumin, and casein (Quistad et al., 1975c). Methoprene can be partly metabolized to acetic acid by cattle (Quistad et al., 1974), and some of this acetic acid may be used for synthesis of cholesterol (Quistad et al., 1975a). Thus, it has been demonstrated that methoprene can be metabolized by cattle so that some of the carbon from methoprene can appear in normal body constituents. However, additional rigorous investigation is necessary to determine whether methoprene was metabolized sufficiently to become a part of the normal carbon skeleton of all natural products in which radioactivity was found.

Ideally, all of the carbon-14 residues in the tissues and eggs from hens fed methoprene-^{14}C should be identified so that their biological significance can be determined. Some metabolites of methoprene have been identified in tissues and eggs of some of the chickens listed in this report (Quistad et al., 1976).

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LITERATURE CITED

Chamberlain, W. F., Hunt, L. M., Hopkins, D. E., Gingrich, A. R., Miller, J. A., Gilbert, B. W., *J. Agric. Food Chem.* **23**, 736 (1975).

Hayes, F. N., *Packard Tech. Bull.* **No. 1** (1963).

Henrick, C. A., Staal, G. B., Siddall, J. B., *J. Agric. Food Chem.* **21**, 354 (1973).

Jeffay, H., Alvarez, J., *Anal. Chem.* **33**, 612 (1961).

Mueller, W. J., Leach, R. M., Jr., *Annu. Rev. Pharmacol.* **14**, 289 (1974).

Paulson, G. D., *Poultry Sci.* **48**, 1331 (1969).

Quistad, G. B., Staiger, L. E., Bergot, B. J., Schooley, D. A., *J. Agric. Food Chem.* **23**, 743 (1975a).

Quistad, G. B., Staiger, L. E., Schooley, D. A., *Life Sci.* **15**, 1797 (1974).

Quistad, G. B., Staiger, L. E., Schooley, D. A., *J. Agric. Food Chem.* **23**, 299 (1975b).

Quistad, G. B., Staiger, L. E., Schooley, D. A., *J. Agric. Food Chem.* **23**, 750 (1975c).

Quistad, G. B., Staiger, L. E., Schooley, D. A., following paper in this issue (1976).

Schooley, D. A., Bergot, B. J., Dunham, L. L., Siddall, J. B., *J. Agric. Food Chem.* **23**, 293 (1975).

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