Metabolism of Aldicarb Pesticide in Laying Hens

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Aldicarb metabolism in laying hens was investigated and the nature and levels of residues in the eggs and tissues were determined. Single oral doses of aldicarb and/or aldicarb sulfone at 0.7 mg/kg were excreted rapidly, with 75% of the doses in the feces by 24 hr. A large portion of the feces metabolites (50–60%) was as water-soluble materials, 10% as unextractables, and the remainder primarily as known hydrolytic products of aldicarb. Only minute quantities were as toxic carbamate compounds. Aldicarb equivalents in eggs reached a maximum of 0.18 ppm on the day after treatment but had declined to 0.01 ppm by 10 days. In muscle tissues, residues of 0.2 to 0.3 ppm 6 hr after treatment declined to 0.01 ppm or less by 10 days. Residue levels in the liver and kidney were about twice those in the muscle tissue. The nature of the aldicarb metabolites in the eggs and tissues was similar to that in the feces. Aldicarb in the diet of hens for 21 days did not appear to alter the fate of the carbamate in the birds when compared to that when single oral doses were administered.

Aldicarb [2 - methyl - 2 - (methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime] is a systemic pesticide whose metabolism has been studied extensively in insects, animals, and plants. Recent reviews of this subject (Dorough, 1970; Kuhr, 1970) pointed out that sulfur oxidation and hydrolysis of the carbamate ester were of major importance in the metabolism of this carbamate. Subsequent work supported this view and has extended the study of aldicarb metabolism to include the synthesis and identification of highly polar products not considered previously (Bartley *et al.*, 1970; Durden *et al.*, 1970). The metabolic pathway for aldicarb presented by Bartley *et al.* (1970) is the most complete of any reported thus far. It is likely that this pathway is representative of the type of metabolism which aldicarb undergoes in the other biological systems tested.

It was the intent of the current study to determine if laying hens metabolized aldicarb in the same manner as reported for other organisms. In addition, these investigations were designed to provide evidence for the levels and chemical nature in residues in eggs and tissues of hens which consume aldicarb residues in the diet. The sulfone analog was included since it is a common metabolite in plants which may serve as poultry feed (Bartley *et al.*, 1970).

EXPERIMENTAL

Insecticides. Aldicarb-*S*-methyl-^{14}C and aldicarb sulfone-*S*-methyl-^{14}C, each with a specific activity of 5 mCi/mmol, and aldicarb-S^{35}, 45.7 mCi/mmol, were supplied by the Union Carbide Corp., as were a series of metabolite standards (Table I). The radioactive materials contained less than 1% radioactive impurities as determined by thin-layer chromatography (tlc) and radioautography.

Treatment and Sampling. In the first experiment, ten White Leghorn laying hens were treated with a single oral dose of aldicarb-S^{35} at a rate of 0.7 mg/kg. The birds, each weighing approximately 1.5 kg, were administered the radioactive aldicarb *via* a 5 grain gelatin capsule which contained a small amount of laying mash. Two hens were sacrificed at 6 hr, and at 1, 3, 5, and 10 days after treatment. Eggs and feces were collected at 6-hr intervals during the first 12 hr and then at 24-hr intervals thereafter. In this and all other tests described herein, the birds were maintained in air-conditioned housing under continuous lighting. Water and laying mash were provided *ad libitum*.

In another test, six laying hens received a 1:1 molar ratio, single oral dose of aldicarb-^{14}C and aldicarb sulfone-^{14}C. Doses of the insecticides were prepared in gelatin capsules so that each hen received 0.7 mg/kg of aldicarb equivalents. The birds were sacrificed 6 hr, 1 day, and 3 days after the carbamates were administered. Eggs and feces were collected as described above.

To study the fate of aldicarb residues when consumed by hens for an extended period, aldicarb-^{14}C and aldicarb sulfone-^{14}C, 1:1 molar ratio doses, were administered to hens every 12 hr for 21 days. Based on an average feed consumption of 80 g per bird per day, the treatment levels corresponded to aldicarb levels in the diet of 0.1, 1.0, and 20.0 ppm. Six hens were used for each treatment level, and another six birds served as control animals. Nonradioactive insecticides were given to the birds for 7 days prior to initiating the radioactive feeding. Each capsule contained half the total amount of aldicarb equivalents required in 1 day's ration to obtain the desired ppm level in the diet. Eggs and feces were collected twice daily just prior to administering the radioactive aldicarb and aldicarb sulfone. Three hens from each feeding level were sacrificed 12 hr after the last treatment and the remaining three hens were killed on the seventh day following the last treatment. Tissue samples were collected and frozen until analyzed.

Radioassay. Radioactive measurements were accomplished on a Packard Tri-Carb Model 3380/544 liquid scintillation counter. The scintillation mixture and the details of counting liquid and solid samples were the same as described in a similar study with carbofuran (Hicks *et al.*, 1970). The blood was radioassayed by oxygen combustion techniques, as were all solids, after 1 g of blood was evaporated to dryness in a bag made from dialysis tubing (Andrawes *et al.*, 1967).

Extraction of Residues. Feces, 20 g, were extracted by blending with 40 ml of water, followed by the addition of 150 ml of acetonitrile with continued blending for about 1 min. The homogenate was filtered and the feces solids were extracted with 60 ml of a 2:1 mixture of acetonitrile and water.

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Table I. Chemical Identity of Aldicarb Metabolites Formed by Laying Hens Treated Orally with Aldicarb[a]

Chemical name	Designations
2-Methyl-2-(methylsulfinyl)propionaldehyde *O*-(methylcarbamoyl)oxime	Aldicarb sulfoxide
2-Methyl-2-(methylsulfonyl)propionaldehyde *O*-(methylcarbamoyl)oxime	Aldicarb sulfone
2-Methyl-2-(methylsulfonyl)propionaldehyde *O*-(hydroxymethylcarbamoyl)-oxime	Aldicarb-NCH_2OH sulfone
2-Methyl-2-(methylthio)propionaldoxime	Oxime
2-Methyl-2-(methylsulfinyl)-propionaldoxime	Oxime sulfoxide
2-Methyl-2-(methylsulfonyl)-propionaldoxime	Oxime sulfone
2-Methyl-2-(methylsulfinyl) propionitrile	Nitrile sulfoxide
2-Methyl-2-(methylsulfonyl)propionitrile	Nitrile sulfone
2-Methyl-2-(methylsulfinyl)propanol	Alcohol sulfoxide
2-Methyl-2-(methylsulfonyl)propanol	Alcohol sulfone

[a] Authentic samples of these metabolite standards were supplied by the Union Carbide Chemicals Corp., S. Charleston, W. Va.

The combined homogenates were washed five times with 80-ml portions of hexane which was discarded because the hexane contained only negligible amounts of radioactivity. The acetonitrile–water phase was extracted thoroughly with chloroform and the aqueous and organic solvent layers were radioassayed. The latter phase was decolorized with a small quantity of activated carbon, filtered, and concentrated for spotting on tlc.

Egg whites, 40 g, were extracted three times; each time the homogenates were filtered and the solids returned to the blender for further homogenization. The first solvent was 60 ml of acetonitrile, the second was 90 ml of a 2:1 mixture of acetonitrile and water, and the third was 60 ml of hexane. All extracts were combined, shaken, and the layers allowed to separate completely. The acetonitrile–water extract was removed and washed twice with 40-ml portions of hexane. Chloroform was added to separate the acetonitrile and water, and the procedure continued as described above for the feces.

Egg yolks (20 g) were homogenized in 40 ml of water, 60 ml of acetonitrile were added, and homogenization continued for 3 min. The remainder of the extraction and cleanup was identical to that used for the egg whites. Equal extraction efficiency, but an acetonitrile extract with less oils, was obtained when the egg yolks were extracted, using the more involved method described below for tissues.

The various tissues were chopped into small pieces and a 40-g subsample, or all available, was analyzed for residues. The subsample was homogenized in 60 ml of a 2% potassium oxalate solution for 5 min. Ethanol (50 ml) was added to the homogenate and the mixture was homogenized for 3 min. The entire homogenate was transferred to a separatory funnel containing 100 ml of ether and 50 ml of pentane, mixed, and the water layer removed. The water was again extracted with the ether and pentane mixture. After separating the layers, 100 ml of acetone were added to the aqueous phase and shaken thoroughly. The solids, formed on shaking with acetone, were removed by filtration and 10 ml of acetone and 100 ml of ether were used to wash the solids. The filtrate was transferred to a separatory funnel, shaken, and the layers were separated. All organic solvent extracts were combined, dried with anhydrous sodium sulfate, and concentrated to an oily residue.

The residue was transferred to a separatory funnel with 30 ml of hexane and 30 ml of acetonitrile. The funnel was shaken, phases were separated, and the hexane was extracted twice more with acetonitrile. The combined acetonitrile extracts were washed with 20 ml of hexane. Following this final wash to remove the oils, the acetonitrile extract was prepared for tlc analysis.

Thin-Layer Chromatography. Because tlc was used to separate the metabolites of aldicarb, and cochromatography of the unknown products with authentic standards was the major means of metabolite identification, great care was taken in developing several efficient tlc systems. The supports consisted of silica gel plates coated 0.3 mm thick and Chromar 500 thin-layer sheets (Mallinckrodt, St. Louis, Mo.). A series of two-dimensional solvent systems was utilized in achieving complete separation of metabolites and for establishing cochromatography in multiple solvent systems. The basic system was a 2:1 ether–hexane + 20% acetone for the first direction and a 2:1 methylene chloride–acetonitrile mixture for the second dimension (Dorough and Ivie, 1968). Organic extracts of the various substrates were spotted on the tlc along with a mixture of metabolite standards (Table I). Following development of the tlc, the plates, or sheets, were exposed to iodine vapors or sprayed with a 1% potassium permanganate solution to visualize the standards. Radioautography was used to locate the radioactive areas on the tlc's.

When there was an indication that one of the standards cochromatographed with an unknown metabolite, the metabolite was isolated and additional tests for cochromatography were conducted. For these experiments, various combinations of solvent systems were used to confirm or rule out the two-dimensional cochromatography of the two materials. The solvent systems were as follows: 5:1 ethyl acetate and methanol; 1:1 dioxane and hexane; 9:1 dioxane and methanol; and a 5:1:1 mixture of chloroform, ethyl acetate, and hexane.

RESULTS AND DISCUSSION

Effect of Treatments on Hens. There were no symptoms of carbamate poisoning in any of the hens treated either with the aldicarb, *per se*, or with the combination of aldicarb and its sulfone analog. As shown in Table II, the 21-day treatment of hens with aldicarb and aldicarb sulfone had no deleterious effects on the birds. When compared to the control animals, the treated birds showed no appreciable differences in body weight, food consumption, egg production, or quantity of fecal matter voided from the body.

Quantity and Nature of Aldicarb Equivalents Excreted from the Body. The pattern of excretion of the aldicarb doses was similar when hens were treated with aldicarb-S^{35} alone, or with a mixture of aldicarb-^{14}C and aldicarb sulfone-^{14}C (Table III). In both tests, the hens eliminated approximately 80% of the dose in 2 days. Extending the collection of feces from the aldicarb-S^{35} treated animals to 10 days showed that a total of 90% of the single oral dose had been excreted from the body.

The pattern of elimination of aldicarb from the hens was not altered a great deal when the insecticide was administered over a 21-day period (Table IV). After 8 to 10 days of treatment, there was an equilibrium formed between the amount of material consumed and the amount excreted. At this point, an equivalent of 80 to 85% of each daily dose was detected in feces excreted during the following 24 hr. These values were lower at the 20-ppm feeding level, 60 to 65%, although there was no apparent reason for this discrepancy in the amount of the daily doses eliminated. Removing the

Table II. Treatment Rates and Criteria for Determining the Effect of Feeding Equimolar Doses of Aldicarb-^{14}C and Aldicarb Sulfone-^{14}C to Laying Hens for 21 Days

	Group numbers[a]			
	I	II	III	Control
Treatment rates				
Aldicarb equivalents in feed, ppm[b]	0.1	1.0	20.0	0
Aldicarb equivalents, mg/kg of body wt[c]	0.005	0.05	1.0	0
Body weight, kg				
0 Day, range, and average	1.3–1.5 (1.4)	1.3–1.7 (1.5)	1.4–1.8 (1.5)	1.4–1.7 (1.5)
21 Day, range, and average	1.3–1.6 (1.4)	1.3–1.6 (1.4)	1.3–1.7 (1.5)	1.3–1.7 (1.5)
Feed consumption				
g/hen/day, range, and average	64–112 (74)	77–103 (84)	66–106 (82)	67–101 (79)
Egg production				
Average/day/hen	0.70	0.50	0.58	0.63
Feces eliminated				
g/hen/day	108	109	118	98

[a] Six hens in each group. [b] Based on an average daily food intake of 80 g for each bird. [c] Based on an average body weight of 1.5 mg for each bird.

Table III. Nature of Radioactivity in Feces of Hens Treated with a Single Oral Dose of Aldicarb-S^{35} (A) or an Equimolar Dose of Aldicarb-^{14}C and Sulfone-^{14}C (B)[a]

	Percent of total radioactivity in sample after					
	6 hr		1 day		2 days	
Metabolites	A	B	A	B	A	B
Aldicarb sulfoxide	5.1	13.3	0.0	0.6	0.0	0.0
Aldicarb sulfone	3.9	1.1	0	0	0	0
Aldicarb–NCH_2OH sulfone	1.0	1.6	0.9	5.8	2.7	5.5
Oxime sulfoxide	5.2	2.6	1.8	2.5	4.5	4.5
Oxime sulfone	2.8	14.6	4.1	10.6	5.6	9.9
Nitrile sulfoxide	2.3	4.2	2.3	1.3	2.4	2.9
Nitrile sulfone	7.2	4.2	9.2	5.4	16.3	10.7
Alcohol sulfoxide	1.8	1.4	2.0	1.2	5.9	2.8
Alcohol sulfone	1.7	1.6	1.7	3.0	7.7	4.9
Unknown 4	1.3	0.3	1.2	0	3.8	1.6
Unknown 5	5.9	1.9	3.3	2.0	0	0.9
Water-solubles	50.0	44.0	65.1	57.5	44.6	45.1
Unextractables	11.8	9.2	8.4	10.1	6.5	11.2
Cumulative % of dose excreted[b]	50.2	48.0	74.3	76.1	84.5	79.3

[a] Dosage rate = 0.7 mg/kg. [b] Values on day experiments terminated: A = 90% after 10 days, B = 82% after 3 days.

Table IV. Elimination of Radioactivity in the Feces of Hens Fed Equimolar Doses of Aldicarb-^{14}C and Aldicarb Sulfone-^{14}C in the Diet for 21 Days

Days fed insecticides	Percent of consumed doses excreted in the feces[a]		
	0.1 ppm	1.0 ppm	20.0 ppm
1/2	76.0	76.2	64.7
1	75.3	69.8	62.7
3	77.5	71.9	64.9
11	87.9	88.6	69.8
19	83.3	82.7	70.1
21	84.9	82.6	71.0
21-day avg[b]	82.2	80.3	67.5
Days after last treatment			
1	85.3	86.1	72.4
2	85.9	86.4	72.6
4	86.2	86.8	72.8
7	86.2	87.3	73.3

[a] Based on total dose consumed and total radioactivity eliminated by indicated time after first treatment. [b] Average of all daily values over the 21-day feeding period.

Table V. Radioactive Residues in Feces of Hens Fed Equimolar Concentrations of Aldicarb-^{14}C and Aldicarb Sulfone-^{14}C in the Diet for 21 Days

	Percent of total radioactivity in feces at indicated feeding levels[a]		
Metabolites	0.1 ppm	1.0 ppm	20.0 ppm
Aldicarb sulfone	0.0	0.6	0.9
Aldicarb–NCH_2OH sulfone	9.1	8.1	8.7
Oxime sulfoxide	0.0	1.7	0.8
Oxime sulfone	4.3	8.4	4.9
Nitrile sulfoxide	0.0	1.5	1.0
Nitrile sulfone	10.7	12.0	9.6
Alcohol sulfoxide	5.1	2.4	3.8
Alcohol sulfone	8.2	9.6	11.5
Unknown 4	0.0	0.6	1.4
Unknown 5	5.8	3.7	4.9
Water-solubles	48.1	43.8	43.3
Unextractables	8.7	7.6	9.2

[a] Values are averages of analysis of feces collected 1, 2, 4, 12, 17, and 21 days after initiating feeding of the radioactive insecticides.

source of aldicarb resulted in almost 90% of the total doses consumed being excreted from the body within 1 week. Such rapid and thorough elimination of the carbamate would likely prevent the accumulation of large quantities of residues in the tissues.

The chemical fate of aldicarb in laying hens when administered as a single dose or for an extended period of time is shown in Tables III and V. Approximately half of the radioactive residues in the feces consisted of unknown water-soluble metabolites. These materials were formed very rapidly by the hens, as evidenced by the results of the analysis of feces collected 6 hr after a single dose was administered (Table III). No attempts were made to identify these metabolites. However, it is unlikely that the carbamate moiety was intact on these materials, since conjugation and/or degradation of aldicarb to alcohols and acids, processes which yield water-soluble metabolites, would be preceded by hydrolysis of the carbamate ester (Bartley *et al.*, 1970). The unextractable radioactive metabolites, 8 to 10% of the residues, may be similar in chemical nature to the water-soluble metabolites.

Of the metabolites which were identified, aldicarb sulfoxide was the only product detected following a single dose that also was not found in the feces of birds treated repeatedly. It was apparent from the single-dose study that the sulfoxide was formed and excreted rapidly. Only trace amounts were detected in feces collected after 6 hr. Assuming that the birds on the continuous treatments did excrete aldicarb sulfoxide in

Table VI. ppm Aldicarb Equivalents in Tissues and Eggs of Hens Treated with a Single Oral Dose of Aldicarb-S^{35} (A) or with an Equimolar Dose of Aldicarb-^{14}C and Aldicarb Sulfone-^{14}C (B)[a]

	ppm (wet wt) Aldicarb-^{14}C equivalents after						
	6 hr		1 day		3 days		10 days[b]
Tissue	A	B	A	B	A	B	A
Kidney	0.59	0.73	0.19	0.21	0.09	0.11	0.020
Liver	0.53	0.68	0.26	0.31	0.10	0.14	0 034
Heart	0.28	0.35	0.12	0.09	0.07	0.05	0.010
Gizzard	0.26	0.31	0.11	0.08	0.04	0.06	0.020
Skin	0.22	0.25	0.08	0.08	0.04	0.05	0.004
Breast	0.20	0.32	0.08	0.08	0.03	0.05	0.006
Thigh	0.20	0.29	0.08	0.08	0.04	0.04	0.008
Leg	0.20	0.27	0.08	0.07	0.04	0.04	0.005
Blood	0.18	0.28	0.11	0.07	0.06	0.04	0.014
Brain	0.18	0.27	0.08	0.09	0.04	0.04	0.008
Fat	0.08	0.06	0.04	0.07	0.03	0.05	0.004
Egg yolk	c	0.14	0.01	0.13	0.07	0.18	0.014
Egg white	c	0.16	0.03	0.18	0.06	0.07	0.007

[a] Dosage rate = 0.7 mg/kg. [b] Experiment involving treatment with B was terminated after 3 days. [c] No eggs laid.

the feces shortly after dosing, it must have been transformed into another material before the feces were collected and frozen. The same may have been true for the aldicarb sulfone, since it was present in higher amounts in the 6 hr feces (Table III) than in the feces of hens used in the continuous feeding study (Table V).

The only other metabolite of the intact carbamate identified was the *N*-hydroxymethyl analog of aldicarb sulfone. This product accounted for 8 to 9% of the radioactive residues in the feces of hens fed aldicarb for 21 days. Based on its relatively low content in the hens treated with a single dose of aldicarb or aldicarb sulfone, aldicarb–hydroxymethyl–sulfone was probably formed rather slowly in the hens. It reached a maximum concentration after the hens were exposed to the insecticides for approximately 8 days.

Hydrolytic products accounted for most of the other residues in the feces of the treated hens. The sulfone forms of these metabolites were predominate, with the nitrile sulfone present in highest amounts in feces of hens treated for a total of 21 days. Nitrile sulfone was also the major identified metabolite in feces of hens given a single oral dose of aldicarb-C^{35}. However, the oxime sulfone concentration was greater in the feces of hens given a single dose of aldicarb-^{14}C and aldicarb sulfone-^{14}C. This was the only marked difference noted in the quantity of individual metabolites in feces of hens receiving the two single treatments.

The two unknown metabolites, designated Unknown 4 and 5 based on earlier studies (Dorough and Ivie, 1968), were present in the feces of all tested hens. Unknown 5 was always present in greater quantities than Unknown 4, and in some cases accounted for 6% of the radioactive residues in the feces. Neither Unknown 4 or 5 cochromatographed with any of the available standards or was present in sufficient quantity for more detailed evaluation of their chemical nature.

Eggs. Adding aldicarb sulfone to the aldicarb dose resulted in higher levels of residues in the eggs than when only aldicarb was given as a single oral dose (Table VI). In neither case did the total aldicarb equivalents exceed 0.2 ppm in the egg yolks or whites. However, maximum aldicarb equivalents of 0.18 ppm were observed when the two insecticides were administered together as compared to a maximum of 0.07 ppm when aldicarb was the only component. Whereas the radioactive residues in the whites had declined markedly by the third day, the residues in the yolks were similar or showed a slight increase by the third day. By 10 days, residues in eggs of hens treated with the aldicarb-S^{35} were 0.014 ppm in the yolk and 0.007 ppm in the whites.

Although these preliminary experiments were not designed to yield sufficient eggs for extensive analysis, the nature of the majority of the residues was tentatively projected (Table VII). Eggs containing the aldicarb-S^{35} residues showed the presence of a number of products not detected in the eggs of hens treated with aldicarb-^{14}C and aldicarb sulfone-^{14}C. This was because the sulfur-35 material was of a very high specific activity and the sensitivity for detecting the individual metabolites was much greater than with the carbon-14 insecticides.

The data (Table VII) show that none of the carbamate metabolites of aldicarb were at detectable levels in the egg yolks or whites. The water-soluble metabolites and nitrile sulfone were the predominant products in the eggs. Other hydrolytic metabolites were detected in the egg whites of the aldicarb-S^{35} treated birds. Although the data were not as complete as desired, these tests indicated the types of metabolites which could be expected in the eggs of hens consuming residues of aldicarb in the diet. The tests also suggested that the aldicarb sulfone contributed more to the residue content of the eggs than did aldicarb. In the yolks this increase was expressed as water-soluble metabolites, while in the whites there was a noted increase in the amount of nitrile sulfone.

Continuous exposure of aldicarb residues to hens resulted in some very interesting patterns of residue levels in the egg

Table VII. Residues in Tissues and Eggs of Hens Treated with a Single Oral Dose of Aldicarb-S^{35} (A) or with an Equimolar Dose of Aldicarb-^{14}C and Aldicarb Sulfone-^{14}C (B)[a]

	ppb in indicated tissue[b]											
	Liver		Kidney		Gizzard		Breast		Egg yolk[c]		Egg white[c]	
Metabolites	A	B	A	B	A	B	A	B	A	B	A	B
Aldicarb sulfoxide	0.0	0.0	7.7	48.9	0.0	0.0	0.0	7.4	0.0	0.0	0.0	0.0
Aldicarb sulfone	30.2	0	0	0	0	0	3.8	0	0	0	0	0
Oxime sulfoxide	8.0	14.3	9.4	0	1.3	9.9	7.0	10.8	3.2	8.2	10.6	0
Oxime sulfone	27.0	102.0	63.7	39.4	9.4	54.9	25.2	45.1	2.1	0	3.6	0
Nitrile sulfoxide	7.4	20.4	20.1	53.3	74.6	6.8	4.6	4.2	0	0	3.6	0
Nitrile sulfone	28.1	17.7	50.8	70.0	23.4	14.9	27.4	20.8	30.9	44.3	7.0	126.9
Alcohol sulfoxide	4.8	0	0	7.3	1.3	7.8	0	0	0	0	2.5	0
Alcohol sulfone	5.8	0	0	0	2.1	0	5.4	7.4	0	9.4	3.1	0
Water-solubles	353.0	439.9	373.5	379.6	105.6	182.0	83.4	160.7	28.1	57.6	24.2	29.3
Unextractables	65.7	85.7	64.9	131.5	42.4	33.8	42.8	60.0	5.7	5.7	5.5	23.8

[a] Dosage rate = 0.7 mg/kg. [b] Tissues from hens sacrificed 6 hr after treatment. [c] A, analysis of eggs laid third day after treatment; B, eggs laid first day after treatment.

Table VIII. Residues in Eggs of Hens Fed Equimolar Concentrations of Aldicarb-^{14}C and Aldicarb Sulfone-^{14}C at Rates of 1.0 and 20.0 ppm in the Diet for 21 Days

	ppb at indicated feeding level[a]					
	1.0 ppm			20 ppm		
Metabolites	Yolk	White	Total	Yolk	White	Total
Acetonitrile solubles						
Aldicarb–NCH_3OH sulfone	0.0	0.0	0.0	0.0	4.8	3.0
Oxime sulfoxide	1.3	3.2	2.5	14.1	40.0	29.7
Oxime sulfone	1.8	1.6	1.7	4.3	21.3	13.5
Nitrile sulfoxide	0.3	0.4	0.4	42.7	27.3	33.0
Nitrile sulfone	21.1	28.5	25.7	304.5	417.4	374.5
Alcohol sulfoxide	0.0	0.2	0.1	8.3	12.0	10.1
Alcohol sulfone	0.2	0.5	0.4	11.6	16.5	14.3
Unknown 5	0.3	0.5	0.4	6.0	12.0	10.0
Hexane-solubles	52.8	0.0	17.8	495.4	0.0	189.6
Water-solubles	6.6	14.3	10.9	192.4	223.8	211.9
Unextractables	4.0	4.1	2.8	82.5	89.6	87.0
Total	88.4	53.3	62.7	1161.8	864.7	976.6

[a] Average of eggs laid 5–21 days of feeding radioactive insecticides.

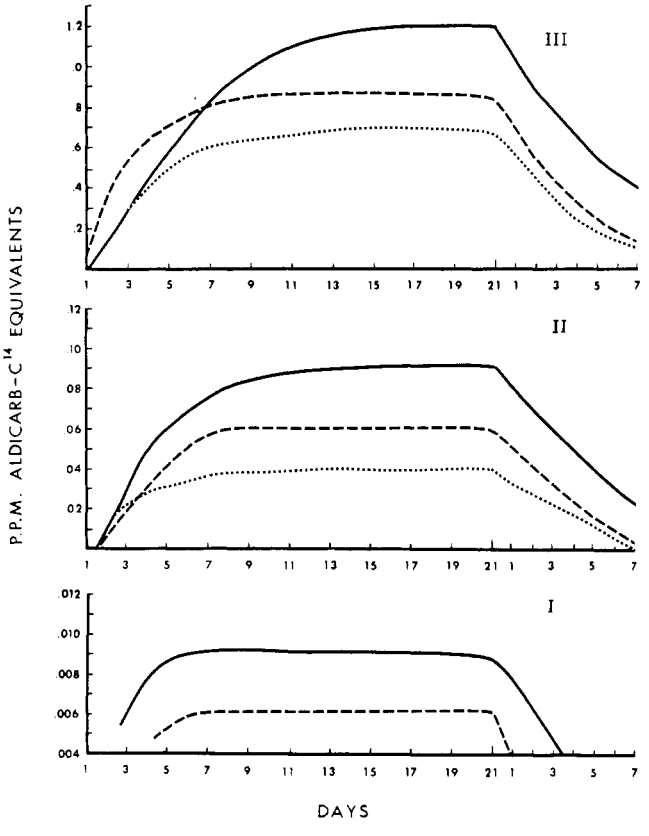

Figure 1. Residues in eggs of hens fed aldicarb and aldicarb sulfone in the diet for 21 days and when returned to normal feed for 7 days. Aldicarb equivalent in diet; I = 0.2 ppm, II = 1.7 ppm, III = 20.0 ppm (- - - - white, ——— total in yolk, - - - - - - - total in yolk less hexane solubles)

(Figure 1). At feeding levels of 1.0 and 20.0 ppm aldicarb equivalents in the diet, it was observed that the total ^{14}C residues in the yolk did not reach a plateau until after 12 to 15 days of feeding. This occurrence was not noted at the 0.1 ppm feeding level because the amount of residues in the eggs was only slightly above the level of sensitivity, which was 0.005 ppm total aldicarb-^{14}C equivalents when analyzing 1 g of sample.

Table IX. ppm Aldicarb-^{14}C Equivalents in Tissues of Hens Killed 12 Hr (A) and 7 Days (B) After the Birds were Fed Equimolar Concentrations of Aldicarb-^{14}C and Aldicarb Sulfone-^{14}C in the Diet for 21 Days

	ppm Aldicarb-^{14}C equivalents in tissues of hens fed[a]			
	1.0 ppm		20.0 ppm	
Tissues	A	B	A	B
Liver	0.14	0.02	1.40	0.36
Kidney	0.12	0.03	1.38	0.39
Heart	0.07	0.02	0.92	0.35
Brain	0.07	0.02	0.90	0.40
Gizzard	0.07	0.02	0.81	0.33
Blood	0.07	0.03	0.76	0.34
Leg	0.06	0.03	0.71	0.30
Skin	0.06	0.02	0.70	0.36
Thigh	0.06	0.02	0.70	0.31
Breast	0.06	0.02	0.68	0.28
Fat	0.05	0.02	0.52	0.22

[a] Residues in tissues of hens fed 0.1 ppm were below the level of sensitivity, 0.005 ppm, except in the following tissues taken 12 hr after the last treatment: blood, 0.015 ppm; kidney, 0.012 ppm; and liver, 0.011 ppm.

Residues in the egg whites stabilized on the sixth or seventh day of treatment. In this case, the total aldicarb-^{14}C equivalents reached a plateau of approximately 0.006, 0.06, and 0.7 ppm for the three feeding levels. Although there were day-to-day variations in the quantities of residues in the egg whites, there were no significant increases or declines in the residue levels until the insecticide source was removed. Once the treatments stopped, the aldicarb-^{14}C equivalents in the egg whites dropped rapidly and by 7 days were almost nondetectable.

Unlike the residues in the egg yolks of hens treated with single doses of aldicarb, there was a portion of the radioactive materials in the egg yolks of these hens which partitioned into the hexane fraction (Table VIII). This was an unexpected occurrence because the use of the hexane in the cleanup procedure was a means of removing the fats and oils from the acetonitrile extracts as had been done with the feces.

The identity of the hexane-soluble radioactivity from the yolks was not determined. Attempts to separate these prod-

Table X. Residues in Tissues of Hens after a 21-Day Period of Feeding Equimolar Concentrations of Aldicarb-^{14}C and Aldicarb Sulfone-^{14}C at a Rate of 20.0 ppm in the Diet

	ppb at indicated time after last treatment						
	12 hr				7 days		
Metabolites	Breast	Gizzard	Liver	Kidney	Breast	Gizzard	Liver
Aldicarb sulfoxide	0.0	0.0	3.0	0.0	0.0	0.0	0.0
Oxime sulfoxide	9.3	6.4	2.6	4.1	0	0	0
Oxime sulfone	19.7	23.1	14.4	22.0	0	0	0
Nitrile sulfoxide	8.1	5.7	10.1	8.2	0	0	0
Nitrile sulfone	412.6	410.4	418.0	401.6	144.1	74.2	184.5
Alcohol sulfoxide	2.5	2.4	0	0.7	0	0	0
Alcohol sulfone	3.0	3.9	10.6	10.6	0	0	0
Unknown 5	6.4	6.1	7.2	7.1	0	0	0
Hexane-solubles	5.3	13.3	95.4	96.5	4.0	12.0	19.8
Water-solubles	80.0	98.5	160.3	321.6	22.7	15.1	29.0
Unextractables	138.7	242.8	681.9	503.3	112.4	231.5	129.3

ucts from the oils by tlc and extractions of various kinds were unsuccessful, and it may be that they were naturally occurring materials synthesized from *S*-methyl-^{14}C fragments from the carbamate materials. They were certainly not the typical types of aldicarb metabolites usually encountered (Table I).

Substracting the hexane-soluble radioactivity from the total aldicarb-^{14}C equivalents in the egg yolks revealed that the pattern of accumulation of the remaining residues in the yolk was very similar to that in the egg whites (Figure 1). These residues reached a plateau on the sixth or seventh day of treatment and did not vary greatly until the carbamate treatments were terminated. Once the treatments were stopped, the nonhexane-soluble residues in the yolks declined as did those in egg whites.

With the exception of the increasing hexane-soluble radioactive materials, the relative concentrations of aldicarb metabolites remained fairly constant. For this reason, the nature of the residues in the eggs is presented in Table VIII as averages of the data gathered on eggs laid the fifth through the 21st days of treatment. The *N*-hydroxymethyl analog of aldicarb sulfone was the only carbamate material identified in either the yolks or the whites and then only in eggs of hens at the 20-ppm feeding level. As was the case with the feces and eggs from the single-treatments studies, hydrolytic and unknown water-soluble metabolites accounted for most of the residues. The very low quantities of aldicarb-^{14}C equivalents in the eggs and their chemical nature suggested that small levels of aldicarb residues in the diet of laying hens would not result in toxicologically significant levels of residues in the eggs.

Tissues. The situation in regards to the levels and nature of residues in the tissues of the hens was very much like that described for the eggs. Feeding a combination of aldicarb and aldicarb sulfone appeared to cause slightly higher residues in the various tissues than did the treatment with just aldicarb (Table VI). Residues in the tissues declined sharply as the carbamates were eliminated from the body, and by 3 days the levels had fallen from a high of about 0.6 ppm aldicarb equivalents to levels generally below 0.1 ppm. By 10 days the radioactive residues were less than 0.03 in all the tissue analyzed.

Small quantities of the residues in the kidney and breast of hens killed 6 hr after treatment with a single dose of the insecticide were identified as aldicarb sulfoxide. Similarly low levels of aldicarb sulfone were detected in the liver and breast. All other detectable metabolites were hydrolytic products of known identity or unknown metabolites in the water fraction or were unextractable from the tissues.

Generally, the magnitudes and nature of residues in the tissues of hens treated 21 days with aldicarb and aldicarb sulfone were not too different than what had been observed with the single treatments (Tables IX and X). The liver and kidney contained the highest levels of aldicarb-^{14}C equivalents and the majority of these were hydrolytic products, mainly nitrile sulfone, and water-soluble unknowns. The only carbamate identified was a trace amount of aldicarb sulfoxide in the liver of birds sacrificed 12 hr after the last treatment.

There were considerable quantities of hexane-soluble products in the gizzard, liver, and kidney. As with the eggs, these materials were not present in hens other than those on the continuous treatments. Tissues of hens killed on the seventh day after receiving their last aldicarb treatment contained only nitrile sulfone in the acetonitrile fraction of the tissue extracts. The remainder of the residues was distributed among the hexane, water, and in the tissue solids as unextractable metabolites. As pointed out for the eggs, the data gathered by this investigation do not indicate that low levels of aldicarb residues in the diet of poultry would result in harmful levels of residues in the meat.

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