Determination of Sevin Insecticide and Its Metabolites in Poultry Tissues and Eggs

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Colorimetric methods are described for determining Sevin and its metabolities, 1-naphthol and 1-naphthol conjugates, in poultry tissues and eggs. Free naphthol is reacted with *p*-nitrobenzenediazonium fluoborate to produce a colored product which is measured spectrophotometrically. Sevin and 1-naphthol conjugates are hydrolyzed to 1-naphthol which is then determined in the same manner. Residue studies with poultry dusted with Sevin showed highest concentrations of residues to be in skin tissue where an average of 19.3 p.p.m. Sevin was found 1 day after the final treatment. A 7-day waiting period between treatment and slaughter reduced these residues to 2.15 p.p.m. No significant amount of either 1-naphthol or its conjugates was found in any tissue of birds processed 7 days after treatment, and eggs were found free of residues throughout the study.

SEVIN (1-naphthyl *N*-methylcarbamate), a broad spectrum insecticide developed by Union Carbide Chemicals Co., has proved highly effective for controlling chicken mites, northern fowl mites, lice, and other ectoparasites of poultry (*1*). Control is gained by treating the litter in the cages or by direct application of the insecticide to the birds.

To study the extent of contamination that may result in the edible tissues of birds treated with Sevin, a dusting experiment was conducted jointly between the Research and Development Department of Union Carbide Chemicals Co. and the Zoology-Entomology Department of Auburn University. The experiment was designed to provide residue data in all types of edible tissue for both the parent insecticide and its metabolite, 1-naphthol. Each type of tissue, including skin, dark and white muscle, liver, and gizzard, was analyzed separately to show the distribution of residues in the carcass. In addition, the liver and gizzard were checked for glucuronide and sulfate conjugates of 1-naphthol, the natural detoxification products of that compound.

The experiment was also designed to determine if, by metabolic processes, residues of Sevin or 1-naphthol were translocated into eggs.

Experimental

Dusting Experiment. For the dusting program, 18 white, leghorn, laying hens, averaging 1.58 kg. in weight, were maintained under controlled conditions for 1 week. During this period, all eggs collected were separated into white and yolk and the two portions frozen and reserved as control samples for the analytical program.

At the end of 1 week, six hens, selected at random, were sacrificed and processed in the usual manner for food purposes except that no detergent was used and the skin was removed. Each type of edible tissue, including skin, breast muscle, leg muscle, liver, and gizzard, was packaged separately, quick-frozen, and reserved for control analyses.

The remaining 12 hens were dusted three times at 4-day intervals, using 4 grams of 5% Sevin dust per bird per application. The insecticide was applied directly to the bird, using a polyethylene dust dispenser in such a manner as to assure thorough contact with the skin. Eggs collected during the dusting period were processed in the same manner as the control specimens and reserved for analysis.

Twenty-four hours after the final Sevin treatment, six of the treated hens were sacrificed, and the tissues of each were individually prepared for analysis in the same manner as the control specimens.

The remaining six hens were maintained without further treatment for 7 days. During this post-treatment period, eggs were collected again and processed for analysis. On the seventh day after the final treatment, these birds were sacrificed and individually prepared for analysis. Like all previous samples, the tissues were immediately frozen for precautionary purposes to minimize possible enzymatic decomposition of the residues.

Analytical Methods. Methods used to determine residues after isolation from the poultry products are based on a modification (*3*) of the procedure described by Miskus *et al.* for determining 1-naphthol by reaction with *p*-nitrobenzenediazonium fluoborate to produce an intense color (*2*). Free naphthol is determined directly in acetic acid medium, whereas Sevin and naphthol conjugates are first hydrolyzed to naphthol, followed by determination of the latter. Sevin is hydrolyzed with alcoholic sodium hydroxide while the conjugates are hydrolyzed by refluxing in aqueous hydrochloric acid.

Apparatus and Reagents. Chromatographic tubes, 18 inches long, 20 mm. O.D., with medium-porosity, fritted-glass disk above stopcock.

Three-ball Snyder evaporator columns.

Florisil, The Floridin Co., Tallahassee, Fla. Adjust the apparent water content to 3.5% in the following manner. To each of two 250-ml., glass-stoppered Erlenmeyer flasks, add 50 ml. of methanol. Add, from a buret, Karl Fischer reagent (water factor approximately 0.2) until the same permanent coloration is obtained in both flasks. Stopper the flasks and reserve one for a blank or reference. To the other, add 1 to 2 grams of the Florisil, weighed to the nearest 0.1 mg. Stopper and shake well for approximately 30 seconds. Titrate the sample with the Fischer reagent to the same color of the blank. The color should remain stable for approximately 30 seconds. Calculate the apparent water content of the Florisil, and add, with a hypodermic syringe or other convenient means, sufficient water to a supply of the adsorbent contained in a rotating cylinder to raise the apparent water content to 3.5%. Mix the adsorbent continuously in a closed container by rotating or

Table I. Recovery of Known Amounts of 1-Naphthol from Poultry Products

1-Naphthol, P.P.M.		Recovery	
Added	Found	Per Cent	Dev. from Av. Per Cent
	SKIN		
0.35	0.26	74.3	−5.9
0.69	0.55	79.8	−0.4
0.10	0.08	80.0	−0.2
	MUSCLE		
0.23	0.19	82.7	+2.5
0.46	0.39	84.7	+4.5
	0.36	78.2	−2.0
	0.37	80.3	+0.1
	0.36	78.2	−2.0
	0.40	86.9	+6.7
	0.38	82.6	+2.4
	0.38	82.6	+2.4
	0.37	80.3	+0.1
	LIVER		
0.43	0.36	83.7	+3.5
0.60	0.42	70.0	−10.2
	0.54	90.0	+9.8
	0.46	76.7	+3.5
	0.48	80.0	−0.2
	GIZZARD		
0.60	0.46	76.7	−3.5
	0.46	76.7	−3.5
	EGG YOLK		
0.57	0.45	79.0	−1.2
	0.45	79.0	−1.2
	EGG WHITE		
0.57	0.46	80.6	+0.4
	0.46	80.6	+0.4
	Av.	80.2	±3.0

Table II. Recovery of Known Amounts of Sevin from Poultry Products

Sevin, P.P.M.		Recovery	
Added	Found	Per Cent	Dev. from Av. Per Cent
	SKIN		
0.10	0.08	80.0	−6.4
0.96	0.83	86.5	+0.1
	0.84	87.6	+1.2
1.92	1.70	88.6	+3.2
	MUSCLE		
0.10	0.09	90.0	+3.6
0.96	0.80	83.3	−3.1
	0.82	85.5	−0.9
	0.85	88.6	+2.2
	0.83	86.5	+0.1
	LIVER		
1.45	1.13	77.8	−8.6
1.46	1.28	87.6	+1.2
	1.28	87.6	+1.2
	GIZZARD		
0.73	0.63	86.3	−0.1
1.45	1.25	86.2	−0.2
	1.29	88.9	+2.5
	EGG YOLK		
0.29	0.23	79.3	−7.1
0.59	0.56	95.0	+8.4
	EGG WHITE		
0.29	0.24	82.8	−3.6
0.59	0.55	93.3	+6.9
	Av.	86.4	±3.2

Table III. Absorbance of Control Samples as Determined by 1-Naphthol Method

Tissue	Sample, Grams	Absorbance vs. Acetic Acid	Deviation from Av. Absorbance
Skin	10	0.020	0.000
	10	0.022	+0.002
	10	0.023	+0.003
	10	0.020	0.000
Breast muscle	20	0.015	−0.005
	20	0.012	−0.008
	20	0.016	−0.004
	20	0.019	−0.001
	20	0.022	+0.002
Leg muscle	20	0.021	+0.001
	20	0.019	−0.001
	20	0.024	+0.004
	20	0.018	−0.002
Liver	10	0.019	−0.001
	10	0.022	+0.002
	10	0.020	0.000
	10	0.019	−0.001
	10	0.025	+0.005
Gizzard	10	0.021	+0.001
	10	0.018	−0.002
	10	0.025	+0.005
	10	0.021	+0.001
Egg yolk	10	0.021	+0.001
	10	0.017	−0.003
Egg white	10	0.018	−0.002
	10	0.023	+0.003
	Av.	0.020	±0.002

Table IV. Absorbance of Control Samples as Determined by Sevin Insecticide Method

Tissue	Sample, Grams	Absorbance vs. Acetic Acid	Deviation from Av. Absorbance
Skin	10	0.018	+0.003
	10	0.015	0.000
	10	0.023	+0.008
	10	0.012	−0.003
Breast muscle	20	0.012	−0.003
	20	0.010	−0.005
	20	0.015	0.000
	20	0.010	−0.005
Leg muscle	20	0.013	−0.002
	20	0.012	−0.003
	20	0.010	−0.005
	20	0.013	−0.002
Liver	10	0.012	−0.003
	10	0.015	0.000
	10	0.015	0.000
	10	0.015	0.000
Gizzard	7	0.018	+0.003
	7	0.011	−0.004
	7	0.013	−0.002
	7	0.021	+0.006
Egg Yolk	13	0.023	+0.008
	14	0.018	+0.003
Egg White	10	0.014	−0.001
	Av.	0.015	±0.003

otherwise for 24 hours. At the end of this period, check the water content as described above. For best results, the apparent water content should be between 3.2 and 3.8%.

p-Nitrobenzenediazonium fluoborate, Eastman Chemical Co. Prepare fresh before use by dissolving 0.025 gram in 5 ml. of methanol, and diluting to 25 ml. with acetic acid.

Extraction of Sevin and Free Naphthol. Transfer 10 to 20 grams of the sample into each of two Waring Blendor jars, and add 200 ml. of methylene chloride and 50 grams of anhydrous sodium sulfate to each. Blend at high speed for 90 seconds, and filter the mixtures through No. 12 fluted filter papers. Transfer the residue and the filter paper back to the Blendors, and extract again with 170 ml. of methylene chloride (redistilled). Filter the slurries and process for 1-naphthol and Sevin separately as follows:

1-NAPHTHOL. Using a 500-ml. separatory funnel, extract the combined methylene chloride extracts from one of the samples twice with 25-ml. portions of 0.5*N* aqueous sodium hydroxide. Combine the caustic extracts in a 125-ml. separatory funnel and add 4 ml. of concentrated hydrochloric acid. Extract twice with 25- and 20-ml. portions of methylene chloride, combining the extracts in a 250-ml. Erlenmeyer flask containing approximately 5 grams of sodium sulfate. Reserve this solution for the chromatographic cleanup described later.

SEVIN. Combine the methylene chloride extracts of the second sample in a 500-ml. Erlenmeyer flask, and attach the flask to a three-bulb Snyder column. Evaporate the methylene chloride in a hot water bath and dissolve the residue in 200 ml. of petroleum ether. Extract the petroleum ether solution twice in a separatory funnel using 25 and 15 ml. of acetonitrile. Combine these extracts in a 250-ml. Erlenmeyer flask and evaporate in a steam bath. Dissolve the residue in 25 ml. of methylene chloride, and continue as described in the chromatographic step.

For egg yolk, after evaporation of the acetonitrile, add 5 ml. of 0.1*N* alcoholic sodium hydroxide and let stand 3 minutes at room temperature. Transfer to a 250-ml. separatory funnel using 25 ml. of 0.5*N* aqueous sodium hydroxide to rinse the flask. Add 100 ml. of methylene chloride and shake well. Transfer the aqueous extract to a 125-ml. separatory funnel containing 4 ml. of concentrated hydrochloric acid. Extract the methylene chloride layer with an additional 20 ml. of aqueous sodium hydroxide, adding the extract to the same 125-ml. separatory funnel. Extract twice with

Table V. 1-Naphthol Residues in Poultry Treated with Sevin

Tissue	1-Day Birds: Weight, Grams	1-Day Birds: Net Absorbance	1-Day Birds: 1-Naphthol, p.p.m.	7-Day Birds: Net Absorbance	7-Day Birds: 1-Naphthol, p.p.m.
Skin	10	0.135	0.9	0.000	<0.2
	10	0.108	0.7	0.030	0.3
	10	0.114	0.8	0.028	0.3
	10	0.100	0.7	0.022	0.2
	10	0.210	1.2	−0.002	<0.2
	10	0.098	0.7	0.013	<0.2
Breast muscle	20	−0.008	<0.1	0.002	<0.1
	20	−0.005	<0.1	−0.005	<0.1
	20	−0.007	<0.1	−0.003	<0.1
	20	−0.005	<0.1	0.000	<0.1
	20	0.002	<0.1	−0.006	<0.1
	20	0.003	<0.1	0.002	<0.1
Leg muscle	20	0.003	<0.1	0.004	<0.1
	20	0.002	<0.1	0.006	<0.1
	20	−0.001	<0.1	0.005	<0.1
	20	0.012	<0.1	0.008	<0.1
	20	0.013	<0.1	0.005	<0.1
	20	0.012	<0.1	−0.004	<0.1
Liver	10	0.013	<0.2	0.004	<0.2
	10	0.003	<0.2	0.003	<0.2
	10	0.012	<0.2	0.000	<0.2
	10	−0.006	<0.2	0.002	<0.2
	10	0.009	<0.2	0.003	<0.2
	10	0.008	<0.2	0.006	<0.2
Gizzard	10	0.002	<0.2	0.003	<0.2
	10	0.002	<0.2	−0.002	<0.2
	10	−0.002	<0.2	0.000	<0.2
	10	0.002	<0.2	0.006	<0.2
	10	0.002	<0.2	0.009	<0.2
	10	0.002	<0.2	−0.001	<0.2

Table VI. Sevin Residues in Tissues of Treated Poultry

Tissue	Weight,[a] Grams	1-Day Birds: Net Absorbance	1-Day Birds: Sevin, p.p.m.	7-Day Birds: Net Absorbance	7-Day Birds: Sevin, p.p.m.
Skin	10	2.620	35	0.087	1.2
	10	0.524	6.7	0.137	1.7
	10	0.570	7.3	0.133	1.6
	10	1.110	14	0.188	2.3
	10	2.020	25.5	0.243	3.0
	10	2.026	27.4	0.240	3.0
Breast muscle	20	0.033	0.2	0.000	<0.1
	20	0.018	<0.1	−0.001	<0.1
	20	0.005	<0.1	0.002	<0.1
	20	0.013	<0.1	0.008	<0.1
	20	0.093	0.5	0.007	<0.1
	20	0.195	1.1	−0.001	<0.1
Leg muscle	20	0.158	0.9	0.015	<0.1
	20	0.060	0.3	0.009	<0.1
	20	0.128	0.8	0.006	<0.1
	20	0.053	0.3	0.011	<0.1
	20	0.148	0.9	0.008	<0.1
	20	0.313	2.0	0.016	<0.1
Liver	8	0.005	<0.2	0.001	<0.2
	8	0.006	<0.2	0.003	<0.2
	9, 10	0.002	<0.2	0.000	<0.2
	10, 11	−0.001	<0.2	0.000	<0.2
	7, 10	0.001	<0.2	0.003	<0.2
	7, 10	0.002	<0.2	0.000	<0.2
Gizzard	6, 5	0.002	<0.2	0.002	<0.2
	6, 5	0.002	<0.2	−0.003	<0.2
	7, 6	0.002	<0.2	−0.002	<0.2
	7, 6	0.001	<0.2	0.003	<0.2
	8, 7	−0.001	<0.2	−0.001	<0.2
	7, 6	0.001	<0.2	0.003	<0.2

[a] Duplicate weights represent 1-day and 7-day birds, respectively.

25- and 20-ml. portions of methylene chloride, combining the extracts in a 250-ml. Erlenmeyer flask containing approximately 5 grams of anhydrous sodium sulfate. Continue as described in the chromatographic step using only 40 ml. of methylene chloride rinse solution.

Hydrolysis and Extraction of Conjugated Naphthol from Liver and Gizzard Tissue. Blend 10 grams of sample with 40 ml. of water at high speed for 30 seconds. Transfer the slurry to a 250-ml., glass-stoppered Erlenmeyer flask, and add 10 ml. of concentrated hydrochloric acid. Attach the flask to a water-cooled condenser and heat at reflux temperature for 10 minutes. Filter the mixture through a No. 12 fluted filter paper into a 250-ml. separatory funnel marked *A*.

Transfer the residue and filter paper to a Waring Blendor, and blend for 30 seconds at high speed with 150 ml. of methylene chloride. Filter through a No. 12 fluted filter paper into a 500-ml. separatory funnel marked *B*, and repeat the extraction of the residue using 100 ml. of methylene chloride. Filter into the same 500-ml. separatory funnel. To funnel *A*, add 25 ml. of water and extract twice with 100-ml. portions of methylene chloride, adding the extracts to funnel *B*. Extract the contents of funnel *B* twice with 25- and 20-ml. portions of 0.5*N* aqueous sodium hydroxide, and transfer the extracts to a 125-ml. separatory funnel containing 4 ml. of concentrated hydrochloric acid. Extract the aqueous solution twice with 25- and 20-ml. portions of methylene chloride, transferring the extracts to a 250-ml. Erlenmeyer flask containing approximately 5 grams of sodium sulfate. Reserve for the chromatographic cleanup.

Chromatographic Cleanup. Prepare a Florisil column 1 inch deep in a chromatographic tube. Saturate the column with methylene chloride which has been previously saturated with water. Pass the methylene chloride containing the residues through the column, and collect the eluate in a 250-ml., glass-stoppered Erlenmeyer flask. For the free naphthol and conjugate samples, use 40 ml. of water-saturated methylene chloride to rinse the flask and pour the rinse solution onto the column. Use 100 ml. of rinse solution for Sevin samples except as indicated for egg yolk.

Colorimetric Measurement. Add one drop of diethylene glycol to the flasks containing the eluate from the chromatographic columns, and evaporate the methylene chloride at 50 mm. pressure. Add 1 ml. of 0.1*N* alcoholic sodium hydroxide to the flasks containing the free naphthol and the conjugate naphthol, and 2 ml. to the Sevin samples. Rotate the flasks so as to wet the entire inner surface and allow them to stand for two minutes. Add 9 ml. of glacial acetic acid to the naphthol and conjugate samples, and 18 ml. to the Sevin samples. Add 1 ml. of freshly prepared 0.25% *p*-nitrobenzenediazonium fluoborate solution and let stand for 1 minute at 25 ± 2° C. Determine the absorbance of the color at 475 mμ on a suitable spectrophotometer in 1-cm. cells, using acetic acid to zero the instrument. Refer the absorbances to calibration curves to determine the amount of residue in each.

Calibration Curves. NAPHTHOL AND NAPHTHOL CONJUGATES. Prepare a series of standard solutions of 1-naphthol in methylene chloride to contain 2, 4, 6, and 10 μg. per ml., respectively. Pipet 5 ml. of the solutions into a series of 500-ml. separatory funnels containing 300 ml. of methylene chloride. Process these solutions, along with a reagent blank, as described in the extraction section beginning with 1-naphthol and continuing through the chromatographic cleanup and colorimetric measurement sections. Prepare a calibration curve by plotting the net absorbances versus micrograms of 1-naphthol contained in the 5-ml. aliquots of the standards.

SEVIN. Prepare a series of standard

Table VII. 1-Naphthol Conjugates in Liver and Gizzard Tissue from Hens Dusted with Sevin Insecticide

Tissue[a]	Sampling Schedule, Days	Net Absorbance	Conjugates as 1-Naphthol, P.P.M.
Liver	1	0.000	<0.2
	1	0.023	<0.2
	7	0.004	<0.2
	7	0.000	<0.2
Gizzard	1	0.001	<0.2
	1	0.004	<0.2
	7	0.003	<0.2
	7	−0.001	<0.2

[a] 10-gram samples representing composites of six treated birds were used.

Table VIII. Sevin Residues in Eggs from Treated Hens

Sampling Period	Weight, Grams	Net Absorbance	Sevin, P.P.M.
	Egg Yolk		
Treatment	16	0.008	<0.1
	13	0.001	<0.1
	14	0.013	<0.1
	16	0.011	<0.1
	13	0.003	<0.1
Post treatment	14	0.007	<0.1
	13	0.001	<0.1
	14	0.008	<0.1
	Egg White		
Treatment	11	−0.002	<0.2
	10	−0.003	<0.2
	12	−0.001	<0.2

Table IX. 1-Naphthol Residues in Eggs from Hens Dusted with Sevin

Sampling Period	Weight, Grams	Net Absorbance	1-Naphthol, P.P.M.
	Egg Yolk		
Treatment	22	0.002	<0.1
	20	0.000	<0.1
	20	0.002	<0.1
Post treatment	10	0.006	<0.2
	9	0.004	<0.2
	12	0.005	<0.2
	11	0.005	<0.2
	Egg White		
Treatment	15	−0.001	<0.1
	16	−0.001	<0.1
	17	0.002	<0.1
Post treatment	15	0.002	<0.1
	16	−0.004	<0.1
	17	0.001	<0.1
	15	−0.005	<0.1

solutions of Sevin in methylene chloride to contain 2, 4, 6, and 10 μg. per ml., respectively. Pipet 5 ml. of the solutions into a series of 500-ml. Erlenmeyer flasks containing 300 ml. of methylene chloride and process, along with a reagent blank, as described in the extraction section beginning with Sevin and continuing through the chromatographic cleanup and colorimetric measurement sections. Prepare a calibration curve by plotting the net absorbances versus micrograms of Sevin contained in the 5-ml. aliquots of the standards.

Discussion

Recovery Experiments. The methods were evaluated by analyzing samples of poultry products containing known amounts of Sevin and 1-naphthol. Along with these samples, portions of the product containing no additives were analyzed to determine if interference is encountered from natural components and to serve as a baseline for calculating recovery data. Tables I and II show average recoveries of 80.2 ± 3.0% for 1-naphthol and 86.4 ± 3.2% for Sevin.

Because suitable naphthol conjugates were not available, free naphthol was used to check the conjugate method. Recoveries averaged 55 ± 5% at 0.5 p.p.m. concentrations. Inasmuch as free naphthol reacts as conjugates in the method, results must be corrected for naphthol to obtain true conjugate values.

Conditions for hydrolyzing naphthol conjugates were established in a previous experiment involving the analysis of cow urine for these substances (*4*).

Calibration curves used for the recovery experiments and for analyzing products from treated birds showed responses of 0.024 and 0.009 absorbance unit per μg., respectively, for 1-naphthol and Sevin.

Analysis of Treated Poultry. Samples from treated birds were analyzed in sets of three with a corresponding sample from control birds processed with each set. The control was included to indicate the sensitivity of the methods and to keep a constant check on the purity of the reagents. All control data obtained throughout the analytical program are tabulated separately for 1-naphthol and Sevin in Tables III and IV. Average control absorbances are 0.020 ± 0.002 for naphthol and 0.015 ± 0.003 for Sevin with no significant difference among the various tissue or egg samples.

Residue data for the treated samples are tabulated in Tables V through IX, according to tissue type and time interval between the completion of the dusting program and the slaughter. The data are based on the difference between absorbance of the treated sample and average absorbance of controls with appropriate factors, as determined from average recovery data, being applied to correct the results to absolute values. No attempt was made to calculate precisely the concentration of residues in samples containing 2 μg. or less of either naphthol or Sevin. Instead, these values are listed as less than 0.1 p.p.m. (<0.1 p.p.m.) or <0.2 p.p.m., depending on the sample weight.

Highest concentrations of residues occurred in the skin where an average of 19.3 p.p.m. of Sevin was found in birds sacrificed 24 hours after treatment. A 7-day waiting period, however, reduced these residues to an average of 2.15 p.p.m. Skin likewise showed the highest level of 1-naphthol with an average of 0.83 p.p.m. in the 1-day birds, but again the 7-day waiting period reduced the residues to less than 0.1 p.p.m. Leg muscle of the 1-day birds, which contained an average of 0.87 p.p.m. Sevin, was the only other product showing significant residues of either Sevin or its metabolites.

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