Results and Discussion

Analytical Method. The method presented is a sensitive procedure for analysis of Kelthane in fats with the advantage that the substrate materials need not be separated from the insecticide prior to analysis. Recoveries from milk and butterfat are somewhat better than those recoveries from body fat. No explanation for low Kelthane recoveries from body fat can be offered since only a limited number and relatively small size of samples were available. Thus, a statistical evaluation of these results was not possible. It is readily understood that the reliability of this procedure is directly proportional to the size of the sample being analyzed.

Freshly prepared tetraethylammonium hydroxide solutions should be used but may also be repurified by evaporating to dryness on a steam bath with a stream of dry air and dissolving the recovered solid in sufficient water to make a 1*N* solution.

The absorbance of 0.005 may be arbitrarily chosen as the limit of readability of the Beckman DU spectrophotometer which was used. This is equivalent to 1.10 μg. of Kelthane or an ultimate sensitivity of 0.06 p.p.m. based on 20 grams of butterfat. Apparent Kelthane residues for controls were 0.19 and 0.26 p.p.m., so that these blanks must be considered in the practical limit of detection. Standard deviation and confidence limits of each set of data from the 1 and 2 p.p.m. feeding trial were calculated and are shown in Table II.

Feeding Experiment. Results of the Kelthane feeding study are shown in Table II. Plateau values seemed to be reached within 9 days, but could not be verified statistically. The three test animals produced butterfat which had a gross Kelthane residue of 0.58 (±0.193) p.p.m., 0.59 (±0.231) p.p.m., and 0.42 (±0.078) p.p.m. compared with 0.19 (±0.081) p.p.m. for control animals. In terms of 4% milk, these residues are $^1/_{25}$ of the calculated values. Such low residues have been considered nondetectable for DDT (*10*); however, they must be considered significantly above control for the studies presented here. At the 1 p.p.m. Kelthane feeding level, residues in the butterfat were not significantly different from the controls.

Analyses of body fat at the onset of the 1 p.p.m. feeding experiment indicated that some Kelthane had been retained by the animal from the previous feeding experiment, as shown by results of 2.70 and 1.07 p.p.m. Kelthane in body fat of the experimental cows.

It appears, therefore, that an initial feeding rate of 1.0 p.p.m. Kelthane in the daily feed might not result in detectable residues of Kelthane in the milk.

Data from the Kelthane studies may be compared with those of a DDT-feeding experiment (*10*). The amount of Kelthane excreted in the milk of Holstein cows was much lower than that for a corresponding DDT-feed intake. This might be expected since Kelthane is a more polar, thus more water-soluble, compound. The fact that any Kelthane was detected at all at the 2 p.p.m. level of daily Kelthane intake was due to the greater sensitivity of the method than that for DDT. This greater sensitivity was achieved by being able to analyze up to 20 grams of butterfat sample, whereas a 5-gram sample for DDT analysis by the official method (*8, 9*) represented the practical limit.

Acknowledgment

The authors thank Rohm & Haas Co. for supplying generous samples of Kelthane and for a grant-in-aid which supported this research, and Herman Beckman and C. F. Gordon for their interest and suggestions. The technical assistance of Lucille Evans, Sophie Soo, Tom Wilson, and John Jay is gratefully acknowledged.

Literature Cited

(1) Elmer, H. S., Jeppson, L. R., *J. Econ. Entomol.* **50,** 566 (1957).
(2) Fujiwara, K., *Sitzber. Naturforsch. Ges. Univ. Rostock* **6,** 33 (1916).
(3) Gordon, C. F., Haines, L. D., J. AGR. FOOD CHEM. in press.
(4) Haines, R. G., *Mich. State Univ. Agr. Expt. Sta. Quart. Bull.* **40,** 628 (1958).
(5) Kelsey, L. P., Brayman, A. L., Trans. Peninsula Hort. Soc. **46,** No. 5, 34 (1956).
(6) Rohm & Haas Co. Laboratories, unpublished results.
(7) Rosenthal, I., Frisone, G. J., Gunther, F. A., J. AGR. FOOD CHEM. **5,** 514 (1957).
(8) Schechter, M. S., Pogorelskin, M. A., Haller, H. L., *Anal. Chem.* **19,** 51 (1947).
(9) Schechter, M. S., Soloway, S. B., Hayes, R. A., Haller, H. L., *Ind. Eng. Chem., Anal. Ed.* **17,** 704 (1945).
(10) Zweig, G., Smith, L. M., Peoples, S. A., Cox, R. J., J. AGR. FOOD CHEM. **9,** 481 (1961).

Received for review January 12, 1962. Accepted May 31, 1962.

INSECTICIDE RESIDUES IN MEAT

Residues in Body Tissues of Livestock Sprayed with Sevin or Given Sevin in the Diet

H. V. CLABORN, R. H. ROBERTS, H. D. MANN, M. C. BOWMAN, and M. C. IVEY
Entomology Research Division,
C. P. WEIDENBACH and R. D. RADELEFF
Animal Disease and Parasite Research Division, Agricultural Research Service,
U. S. Department of Agriculture, Kerrville, Tex.

ENTOMOLOGICAL STUDIES have shown Sevin (1-naphthyl *N*-methylcarbamate) to be a promising insecticide for the control of ticks (*1*), horn flies [*Haematobia irritans* (L.)] (*4*), and lice (*5*) on livestock, and Gyrisco *et al.* reported that the compound was effective against the gypsy moth caterpillar [*Porthetria dispar* (L.)] (*2*). These possible uses of Sevin on livestock and forage make residue studies necessary on meat and milk. Roberts and coworkers (*6*) described a study on Sevin in which residues were determined in milk from dairy cows fed Sevin in the diet, and in milk from dairy cows and in fat of beef cattle sprayed with the insecticide. Gyrisco and coworkers (*2*) also reported on the residues in milk after feeding high levels of Sevin to dairy cattle. No residues of Sevin were reported by either group. To obtain more information on residues in other tissues of cattle and in the tissues of other farm animals after spraying with or feeding Sevin, a study was planned by the U. S. Department of Agriculture in cooperation with Union Carbide Chemicals Co. In this study, residue determinations were made on seven kinds of tissue from cattle, sheep, goats, and hogs sprayed with Sevin, and on the same tissues from beef cattle fed Sevin in the diet. All tissues were analyzed for Sevin and for 1-naphthol, a likely metabolite. Liver and kidney tissues were also analyzed for conjugates of 1-naphthol.

Experimental Techniques

Treatment of Animals. Spray treatments were applied to one group each

Cattle, sheep, goats, and hogs were sprayed four times in 2 weeks with a 1.0% suspension of Sevin, and Hereford steers were fed 50 and 200 p.p.m. of Sevin in the diet for a period of 27 days. Animals were slaughtered at 1 and 7 days after spraying, and at the end of the feeding period. Residues of Sevin, 1-naphthol, and conjugates of 1-naphthol were not detected in the body tissues of any sprayed animals 7 days after spraying, except in the fat and brain of a goat. No residues were detected in tissues of cattle fed Sevin in the diet.

Table I. Summary of Sevin Feeding Test

Level of Feed Contamination (P.P.M.)	Total Feed Consumed (Pounds)	Total Sevin Consumed (Mg.)
0	226.12	0
	273.00	0
50	304.50	6,896.9
	238.81	5,409.0
	279.11	6,321.8
	276.00	6,251.4
200	226.00	20,475.6
	262.00	23,737.2
	316.00	28,629.6
	316.00	28,629.6

Table II. Recovery Rates of Sevin, 1-Naphthol, and Conjugates from Animal Tissues

	Per Cent Recovery		
Tissue	Sevin[a]	1-Naphthol[a]	Conjugates[b] (1-Naphthol)
Omental fat	95	96	...
Muscle	92	86	...
Liver	66	67	60
Heart	76	53	...
Kidney	72	52	78
Brain	81	83	...

[a] 30 μg. added to 25 grams of tissue.
[b] 20 μg. added to 10 grams of tissue.

Table III. Sevin Residues in Tissues of Cattle, Sheep, Goats, and Hogs After Four Sprayings with 1.0% Sevin

Time After Spraying	P.P.M. Sevin in Tissues[a]						
	Omental fat	Renal fat	Muscle	Liver	Heart	Kidney	Brain
			CATTLE				
1 Day	0.57	0.54	0.13	0.00	0.05	0.10	0.10
7 Days	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
			SHEEP				
1 Day	0.21	0.10	0.07	<0.04	0.07	0.04	0.15
7 Days	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
			GOATS				
1 Day	0.38	0.24	0.18	<0.04	<0.04	0.06	23.0
7 Days	0.70, 0.90	0.12, 0.25	<0.04	<0.04	<0.04	0.04	0.30
			HOGS				
1 Day	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04

[a] Values shown are net values from which readings of control samples have been subtracted.

of steers, sheep, goats, and hogs. There were three animals in each group: two were sprayed and one served as a control. The treated animals were sprayed to the point of runoff with a 1.0% water suspension from a 50% wettable powder twice a week (Monday and Thursday) for 2 weeks. Twenty-four hours after the last spraying, the control and one treated animal from each group were sacrificed, and samples of omental and renal fat, liver, kidney, heart, brain, and muscle were collected. One week after the first slaughter, the second treated animal from each group, except the hog group, was sacrificed, and tissue samples were collected. It was not necessary to sacrifice the second treated hog since no residues had been found in the first set of tissue samples.

In the feeding test, two levels of feed contamination were used. One group of four Hereford steers was fed a diet containing 50 p.p.m. of technical Sevin, and a second group of four was fed a diet containing 200 p.p.m. Two animals were used as controls. Sevin was dissolved in acetone at a concentration to allow 1 ml. of solution to contaminate 1 pound of feed to the desired level. The feed was weighed and the Sevin added to it a short time before feeding. All feed given the test animals during the 27-day feeding period was contaminated. A summary of the total amount of feed and Sevin consumed by each animal is presented in Table I. Omentectomies were performed on two animals in each group of four before the beginning of the test and on the same animals after 2 weeks of feeding. Twenty-eight days after feeding started, one control and the two animals from each treated group that had not been sampled by the omentectomy method were sacrificed and samples of tissue collected. The remaining animals were not subsequently sacrificed since no residues were found.

Collection of Storage of Tissues. Extreme care was exercised in the collection of samples to prevent mechanical contamination. Immediately after collection, samples were placed in polyethylene bags, quick frozen, and stored in the deep-freeze until analyzed. All samples were analyzed for 1-naphthol within 48 hours after collection.

Chemical Methods. The method used for the determination of Sevin, 1-naphthol, and conjugates of 1-naphthol in animal tissues was basically the same as that described in Union Carbide Chemicals Co. Laboratory Manual a "Chemical Method No. 30 UlA15," modified by Johnson (*3*). Some minor changes were made in the authors' laboratory to improve recoveries. A brief description of the procedures follows.

1-NAPHTHOL. Twenty-five grams of chopped tissue was blended with 75 grams of anhydrous sodium sulfate and 200 ml. of methylene chloride. The solution was decanted and filtered through a folded filter into a 500-ml. separatory funnel. The residue was blended with another 200-ml. portion of methylene chloride, which was filtered into the same funnel. The combined extracts were extracted with 50- and 30-ml. portions of 0.5*N* sodium hydroxide. The methylene chloride extract was separated and held for the Sevin determinations described below.

The alkaline extracts were drained into a 125-ml. separatory funnel, acidified with 7 ml. of concentrated hydrochloric acid, and extracted with two 25-ml. volumes of methylene chloride. The combined extracts were dried with 5 grams of anhydrous sodium sulfate and decanted onto a 25 × 19 mm. column of Florisil which had been prepared to contain 3.5% water determined by titration with Karl Fischer reagent. The

Table IV. Residues of Chemically Bound 1-Naphthol (Conjugates) in Unwashed Kidney Tissue of Sprayed Animals

Animal	P.P.M. 1-Naphthol in Kidney Tissue[a]	
	1 Day after spraying	7 Days after spraying
Cattle	1.50	0.12
Sheep	0.34	0.26
Goats	4.00	0.10
Hogs	<0.02	<0.02

[a] Values shown are net values from which readings of control samples have been subtracted.

solvent from the column was collected in a 250-ml. Erlenmeyer flask. The column was washed with 40 ml. of water-saturated methylene chloride, which was collected in the same flask. A drop of diethylene glycol was added to the flask and the solvent evaporated to dryness under reduced pressure at approximately 10° C.

One milliliter of 0.1*N* alcoholic sodium hydroxide was added and the flask rotated to allow complete contact with the walls. Nine milliliters of glacial acetic acid was then added, followed by 1 ml. of a fluoborate solution (0.025 gram of *p*-nitrobenzenediazonium fluoborate dissolved in 5 ml. of methanol and 20 ml. of glacial acetic acid), which was added with continuous swirling. The solution was transferred to a 16-mm. cuvette and the absorbance read immediately at 475 mμ, with acetic acid as a blank.

SEVIN. The methylene chloride extract from the 1-naphthol procedure described above was transferred to a 500-ml. Erlenmeyer flask and dried with anhydrous sodium sulfate. The solution was filtered through a folded filter into a 1000-ml. Erlenmeyer flask and the filter washed with methylene chloride. The solvent was removed by distillation through a three-ball Snyder column to a volume of 5 or 10 ml., and the remaining solvent evaporated at room temperature with a jet of air. The residue was transferred to a 500-ml. separatory funnel with 200 ml. of Skellysolve F and extracted twice with 25-ml. portions of acetonitrile. The acetonitrile extract was concentrated to 5 ml. by distillation through a Snyder column, and the remaining solvent was removed by adding and evaporating two 15-ml. portions of Skellysolve F.

The residue was dissolved in 25 ml. of water-saturated methylene chloride and passed through a Florisil column, prepared as described above for 1-naphthol. The eluted solvent was collected in a 250-ml. Erlenmeyer flask. The column was washed with an additional 100 ml. of solvent, collected in the same flask. A drop of diethylene glycol was added and the solvent evaporated to dryness under reduced pressure.

To the residue, 2 ml. of 0.1*N* alcoholic sodium hydroxide was added and the flask rotated to allow complete contact with the walls. After 2 minutes, 18 ml. of glacial acetic acid was added. Then, 1 ml. of the fluoborate solution was added with constant swirling. The solution was quickly filtered through a plug of glass wool into the 16-mm. cuvette and the absorbance determined at 475 mμ with acetic acid as a blank.

CONJUGATES OF 1-NAPHTHOL. Forty grams of chopped liver or kidney tissue was blended with 160 ml. of water. A 50-gram sample of the blended material was weighed into a 125-ml. Erlenmeyer flask, 10 ml. of concentrated hydrochloric acid was added, and the mixture was refluxed for 10 minutes. The hot mixture was filtered through a fast, folded filter into a 500-ml. separatory funnel, and the residue and filter were washed with 30 ml. of water. The filter paper and residue were blended twice with 150-ml. portions of methylene chloride, and the solvent was filtered into the separatory funnel containing the aqueous extract. After shaking, the layers were allowed to separate, and the methylene chloride was transferred to a 500-ml. Erlenmeyer flask and dried with anhydrous sodium sulfate. The solution was filtered and concentrated to 25 ml. by evaporation at reduced pressure, decanted onto a 100 × 19 mm. Florisil column, and eluted with 40 ml. of water-saturated methylene chloride. All of the solvent passed through the column was collected in a 125-ml. separatory funnel and extracted twice with 50- and 30-ml. portions of 0.5*N* sodium hydroxide. The alkaline extract was collected in a 125-ml. separatory funnel, acidified with 7 ml. of concentrated hydrochloric acid, and then extracted with two 25-ml. portions of methylene chloride. The methylene chloride extracts were collected in a 200-ml. Erlenmeyer flask, a drop of diethylene glycol was added, and the solvent was evaporated to dryness at reduced pressure. The color was developed as described for 1-naphthol above, beginning with the last paragraph.

For comparison, per cent recoveries of Sevin and 1-naphthol, when known amounts were added to the various tissues, are shown in Table II. In the determination of recoveries of conjugates, 1-naphthol was added to the tissues.

Results

Sevin Residues. Residues of Sevin found in tissues following the spray treatments are shown in Table III. Small residues were found in tissues of cattle, sheep, and goats 1 day after spraying. At 7 days, these residues were eliminated by cattle and sheep, and only small residues remained in the fat and brain of goats. No residues of Sevin were found in tissues of hogs 1 day after spraying, and there were no detectable residues in tissues of cattle at the end of the 27-day feeding period.

1-Naphthol Residues. There were no residues of 1-naphthol in any tissues of sprayed animals, except that 0.34 p.p.m. was found in the brain of the goat slaughtered 1 day after spraying. This was the same sample that contained 23.0 p.p.m. of Sevin (Table III). Residues of 1-naphthol were not detected in any tissues of cattle fed Sevin in the diet.

Conjugates of 1-Naphthol. No residues of chemically bound 1-naphthol were found in liver tissues from either sprayed or fed animals. As shown in Table IV, small amounts of conjugates were found in unwashed kidney tissues of sprayed cattle, sheep, and goats. None was detected in hog kidney, nor in kidney tissue from cattle fed Sevin in the diet. Washing the kidney tissue of sprayed cattle in running water reduced the amount of bound 1-naphthol from 1.5 p.p.m. to 0.02 p.p.m., which indicated that the conjugates were present in excretory wastes rather than stored in kidney tissue.

Discussion

The data presented indicate that any residues resulting from dermal applications of Sevin to cattle, sheep, and hogs are quickly eliminated from the tissues. Feeding Sevin to cattle at 50 and 200 p.p.m. in the diet likewise caused no detectable residues of Sevin or 1-naphthol in tissues.

Data on residues in tissues of the sprayed goats are difficult to evaluate. In spite of precautions taken in the collection of samples, the analyses of the brain taken 1 day after spraying and omental fat taken 7 days after spraying suggest that these samples were mechanically contaminated. It would be difficult to justify the increase in residue in omental fat taken 7 days after spraying over that taken at 1 day, and also the difference in omental fat and renal fat taken from the same animal. Likewise, it does not seem reasonable that a high residue of Sevin would be stored in the brain of a goat and not be stored in the brains of a calf and a sheep that had received the same treatment. Conclusive statements regarding these residues must await acquisition of more data.

Literature Cited

(1) Drummond, R. O., Moore, B., Warren, J., *J. Econ. Entomol.* **52,** 1220 (1959).
(2) Gyrisco, G. G., Lisk, D. J., Fertig, S. N., Huddleston, E. W., Fox, F. H., Holland, R. F., Trimberger, G. W., J. AGR. FOOD CHEM. **8,** 409 (1960).
(3) Johnson, D. P., Union Carbide Chemical Co., private communication, 1960.
(4) Johnson, W. T., Longford, G. S., *J. Econ. Entomol.* **53,** 477 (1960).
(5) Moore, B., Drummond, R. O., Brundrett, H. M., *Ibid.*, **52,** 980 (1959).
(6) Roberts, R. H., Jackson, J. B., Westlake, W. E., Ackerman, A. J., Claborn, H. V., *Ibid.*, **53,** 326 (1960).

Received for review January 26, 1962. Accepted May 22, 1962.