QUEENSLAND DEPARTMENT OF PRIMARY INDUSTRIES
DIVISION OF ANIMAL INDUSTRY BULLETIN No. 121

STUDIES ON PESTICIDE RESIDUES. 2. CARBARYL RESIDUES IN THE BODY TISSUES AND MILK OF CATTLE FOLLOWING DERMAL APPLICATION

By I. S. HURWOOD, B.Sc.

SUMMARY

Cattle were sprayed with carbaryl (1-naphthyl N-methyl carbamate) and samples of fat, muscle, liver and kidney were taken from animals slaughtered at 1, 3 or 7 days after single or multiple spray treatments. Carbaryl was found in all body tissues examined 1 and 3 days after exposure. There was an initial preferential concentration of the pesticide in the fat, but levels of carbaryl in fat, muscle, liver and kidney were comparable by the third day. Carbaryl was not detected in samples taken 7 days after treatment.

The concentration of carbaryl in the milk of dairy cattle given a single spray treatment tended to remain constant during the first 2 days after treatment, then to fall rapidly. Carbaryl was not detected in milk obtained at the seventh milking 79 hr after treatment.

I. INTRODUCTION

Carbaryl (1-naphthyl N-methyl carbamate), probably the most widely used of the carbamate pesticides and exhibiting cholinergic activity similar to that of the organophosphorus compounds, possesses relatively low mammalian toxicity compared with many of the latter group (Carpenter *et al.* 1961). Entomological studies have shown that in Queensland carbaryl is effective in the control of cattle tick (*Boophilus microplus* (Canes.)) (Roulston and Wilson 1965) and buffalo fly (*Haematobia exigua* (de Meij.)) (Nolan and Annand 1963).

Initial residue studies (Gyrisco *et al.* 1960) indicated that carbaryl was not excreted in the milk of dairy cattle fed a ration containing up to 450 p.p.m. of this compound. Similarly, no residues were detected in the milk of dairy cattle sprayed with 0·5% carbaryl wettable powder to the point of run-off (Roberts *et al.* 1960; Eheart, Turner, and Dickinson 1962). However, Camp *et al.* (1963) showed that application of 1 quart of 1·0% spray resulted in the persistence of carbaryl residues in excess of 0·025 p.p.m. for 24 hr after treatment. The persistence of residues of carbaryl in the body tissues of cattle, sheep, pigs and goats after multiple spraying with 1·0% carbaryl has been reported (Claborn *et al.* 1963). In their work rapid elimination of pesticides occurred from all tissues, with no residues detected 7 days after final exposure.

Conflicting observations on residues of carbaryl in milk following dermal treatment prompted the work on the pattern of excretion of carbaryl in milk reported in this paper. Additional data on the persistence of carbaryl residues in body tissues of cattle slaughtered at periods up to 7 days after treatment are also presented.

II. MATERIALS AND METHODS

(i) *Pesticide.*—Spray fluid containing 0·3% carbaryl was prepared from a 45·0% colloidal dispersion concentrate ("Sevin" cattle dip) by heating the formulation to a clear melt and then mixing it into the required volume of water.

(ii) *Treatment and sampling.*—Each application of carbaryl to an animal was 2 gal of the 0·3% fluid applied as a spray from a low-pressure pump.

In one experiment, six of 14 grazing steers received a single application of carbaryl and a further six animals were sprayed three times at 2-day intervals. Two untreated animals were used as controls. Groups of two animals from each treatment regimen were slaughtered at 1, 3 or 7 days after the final treatments. Samples of omental and perirenal fat, liver, kidney and diaphraghm muscle were collected from the control and experimental animals and stored at —20°C until they were analysed.

In a second experiment three dairy cattle being milked twice daily received a single application of carbaryl. At the milking immediately before spraying and twice daily thereafter for a 5-day post-treatment period, milk yield was recorded and samples were taken for butterfat and carbaryl determination. To avoid direct contamination of milk samples by pesticide spray deposits, the udder region was thoroughly washed before each milking. Milk samples (1,500 ml) for residue determination were stored at 4°C for periods not exceeding 3 days to facilitate handling during laboratory processing. Under these conditions no appreciable loss of carbaryl from stored milk occurs (Timmerman *et al.* 1961).

(iii) *Sample preparation.*—Samples of fatty tissue were finely comminuted and then macerated with methylene chloride and anhydrous sodium sulphate in a top-drive macerator. After filtration through Whatman No. 41 paper, the solvent was evaporated to obtain 50 g of moisture-free fat. A 50-g portion of liver, kidney and muscle was sampled without refinement.

The butterfat fraction was separated from wholemilk by the method previously described in these studies (Hurwood 1966). The total recovery of moisture-free butterfat (50–100 g from 1,500 ml milk) was taken for analysis. Aliquots (1,000 ml) of defatted milk were freeze-dried before analysis.

(iv) *Chemical analysis.*—Carbaryl was initially extracted by solvent partition using n-hexane and acetonitrile (Jones and Riddick 1952). All concentrating steps were effected using a Kuderna-Danish evaporator fitted with a 3-ball Snyder column to minimize loss of carbaryl.

Carbaryl was determined spectrophotometrically using the p-nitrobenzene diazonium fluoborate coupling method (Anon. 1958). The modifications suggested by Claborn *et al.* (1963) to improve recovery of pesticide were followed. Recoveries of added carbaryl in the range of 0·5–5·0 μg were 79 to 91%. The limits of detection of carbaryl in samples of biological origin were calculated by the method of Bates (1964).

Fat determinations in milk were carried out by the Babcock method (Burgess 1936).

III. RESULTS

Results of chemical analysis, corrected for percentage recovery and blank value, are presented in Tables 1 and 2.

TABLE 1

RESIDUES IN BODY TISSUES FOLLOWING SINGLE AND MULTIPLE SPRAY TREATMENT OF CATTLE WITH 0·3% CARBARYL

Treatment	Interval Between Final Treatment and Slaughter (days)	Carbaryl (p.p.m.)*				
		Liver	Kidney	Diaphragm Muscle	Omental Fat	Perirenal Fat
Single application	1	0·03	0·04	0·05	0·07	0·17
	3	0·05	0·05	0·04	0·10	0·16
	7	ND	ND	ND	ND	ND
3 applications at 2-day intervals ..	1	0·17	0·24	0·09	0·52	0·92
	3	0·16	0·10	0·07	0·14	0·10
	7	ND	ND	ND	ND	ND

* Values are the mean for 2 animals

ND = No detectable residue in 50g tissue

TABLE 2

MEAN CARBARYL RESIDUES IN BUTTERFAT AND MILK OF THREE DAIRY CATTLE FOLLOWING A SINGLE SPRAY TREATMENT WITH 0·3% CARBARYL*

Time after Treatment (hr)	Carbaryl in Butterfat (p.p.m.)	Carbaryl in Milk** (p.p.m.)	Total Carbaryl per Milking (μg)
5	1·4	0·075	15·5
	(1·0–1·7)	(0·066–0·083)	(12·9–20·4)
21	0·5	0·021	11·3
	(0·4–0·6)	(0·015–0·029)	(8·0–13·3)
29	0·6	0·045	11·6
	(0·6–0·7)	(0·034–0·053)	(8·4–15·1)
45	0·7	0·034	14·6
	(0·7)	(0·027–0·035)	(13·8–15·3)
53	0·6	0·035	8·6
	(0·5–0·6)	(0·028–0·044)	(8·1–8·9)
69	0·1	0·004	2·2
	(0·09–0·1)	(0·004–0·005)	(2·1–2·3)
77	ND	ND	ND
93	ND	ND	ND
117	ND	ND	ND

* Figures in parenthesis indicate range for three animals.

** Assessed on butterfat content determined at each milking.

ND = no detectable residue of carbaryl in butterfat from 1,500 ml milk.

The method of extraction used prior to chemical analysis of residual carbaryl excluded any contribution from either 1-naphthol or its conjugated derivatives. Carbaryl residues expressed as parts per million (p.p.m.) in liver, kidney, muscle, omental and perirenal fat in Table 1 are the mean of two animals in each group. No individual tissue levels in excess of 1.0 p.p.m. carbaryl were found. Analysis of similar tissues from the untreated controls showed an apparent level of 1·0 μg carbaryl per 50 g of tissue, resulting in a limit of detection of 0·02 p.p.m.

The excretion of carbaryl in milk expressed as p.p.m. in butterfat, p.p.m. in wholemilk and also total excretion at each milking are shown in Table 2. Carbaryl was found in the butterfat from all milk samples taken up to 69 hr after treatment. Apparent carbaryl (2 μg in 50 g butterfat) in pretreatment samples resulted in a limit of detection of 0·002 p.p.m. in wholemilk. The defatted milk fraction (1,000 ml) showed no detectable residues of carbaryl in samples taken 5, 29 and 53 hr after treatment.

IV. DISCUSSION

The data presented indicate that pesticide residues resulting from carbaryl applied as a spray to cattle are rapidly eliminated from body tissues. It was noticeable in multiple-treated animals that carbaryl was initially absorbed preferentially into fatty tissue but was removed at least partially unchanged within 3 days.

This is supported by the relative persistence of residues of carbaryl in liver and kidney during this period of elimination from depot fat. The lowest level of pesticide was recorded in the muscle. Residues of carbaryl in singly treated animals were in most samples only slightly above the limit of detection, but levels in excretory tissue increased slightly from day 1 to day 3. Carbaryl was not detected in any sample taken 7 days after treatment. The absence of residues in tissues 7 days after treatment has previously been observed where cattle received four sprays of 1·0% carbaryl and were sacrificed 1 and 7 days after final exposure (Claborn *et al.* 1963).

The excretion of carbaryl in milk persisted for at least 69 hr after spraying. The greatest concentration appeared at the first milking, 5 hr after exposure, but the level of pesticide tended to remain constant until the fifth milking, 53 hr after exposure. During this period, minor fluctuation in the concentration of carbaryl in milk could be largely attributed to differences in milk yield and in fat content between morning and afternoon milking. The amount of carbaryl excreted in milk remained constant until the fourth milking, 45 hr after exposure, and then fell rapidly. This pattern of excretion appears to be associated with the observed short-term storage of carbaryl in depot fat and its rapid release during the 2 or 3 days following treatment.

The observed excretion of carbaryl for at least 69 hr following dermal treatment suggests a more persistent transfer of this pesticide to milk than published data had indicated. Roberts *et al.* (1960) sprayed dairy cattle with 0·5% carbaryl and were unable to detect residues in 100-ml samples of milk at any time after treatment. Applications of 1·0% spray used by Camp *et al.* (1963) resulted in measurable levels of carbaryl in 400-ml milk aliquots for 24 hr following treatment. In each of these experiments the chemical method for the estimation of carbaryl was similar to that used in the present studies, the major difference being in the aliquot of milk analysed. Aliquots of 1,500 ml used in the current work give a potential 4 to 15-fold increase in sensitivity over previously published data.

The results show that if slaughter cattle are sprayed at the recommended rate of application and held from 3 to 7 days before slaughter there should be no detectable residues in the meat. If a "zero tolerance" is to be achieved for milk, a rejection period of 3 days after treatment suggested by these studies may be shown to be inadequate if larger samples or more sensitive methods of analysis are employed.

V. ACKNOWLEDGEMENTS

The author wishes to thank Mr. D. C. Stocks for technical assistance in the laboratory. Fat percentages in milk were provided by the Dairy Research Branch of the Department of Primary Industries.

REFERENCES

ANON. (1958).—Determination of Sevin Residues—Union Carbide Chemicals Co. A Laboratory Manual. Chemical Method 30/U 1A15.

BATES, J. A. R. (1964).—Residue data—definitions and presentation. *Chem. Ind. (Lond.)*: 1591.

BURGESS, L. A. (1936).—The Babcock test. *Qd Agric. J.* 46:633.

CAMP, H. B., BUTTRAM, J. R., HAYS, K. L., and ARTHUR, B. W. (1963).—Sevin residues in milk from dairy cows following dermal applications. *J. Econ. Ent.* 56:402.

CARPENTER, C. P., WEIL, C. S., PALM, P. E., WOODSIDE, M. W., NAIR, J. H. III, and SMYTH, H. F. Snr. (1961).—Mammalian toxicity of 1-naphthyl N-methylcarbamate (Sevin insecticide). *J. Agric. Fd Chem.* 9:30.

CLABORN, H. V., ROBERTS, R. H., MANN, H. D., BOWMAN, M. C., IVEY, M. C., WEIDENBACH, C. P., and RADELEFF, R. D. (1963).—Residues in body tissues of livestock sprayed with Sevin or given Sevin in the diet. *J. Agric. Fd Chem.* 11:74.

EHEART, G. F., TURNER, E. C., and DICKINSON, JEAN (1962).—Residues of Sevin in whole milk from sprayed and dusted cows. *J. Econ. Ent.* 55:504.

GYRISCO, G. G., LISK, D. J., FERTIG, S. N., HUDDLESTON, E. W., FOX, F. H., HOLLAND, R. F., and TRIMBERGER, G. W. (1960).—The effects of feeding high levels of Sevin on residue, flavor and odor of the milk of dairy cattle. *J. Agric. Fd Chem.* 8:409.

HURWOOD, I. S. (1966).—Studies on pesticide residues. I. Excretion of pesticides in milk following dermal treatment of dairy cattle with coumaphos and dioxathion. *Qd J. Agric. Anim. Sci.* 23:555.

JONES, L. R., and RIDDICK, J. A. (1952).—Separation of organic insecticides from plant and animal tissues. *Anal. Chem.* 24:569.

NOLAN, J., and ANNAND, A. M. (1963).—An *in vitro* comparison of the effectiveness of D.D.T. and Sevin against buffalo fly. *Aust. Vet. J.* 39:227.

ROBERTS, R. H., JACKSON, J. B., WESTLAKE, W. E., ACKERMAN, A. J., and CLABORN, H. V. (1960).—Residue studies of livestock sprays containing Sevin. *J. Econ. Ent.* 53:326.

ROULSTON, W. J., and WILSON, J. T. (1965).—Chemical control of the cattle tick *Boophilus microplus* (Can.). *Bull. Ent. Res.* 55:617.

TIMMERMAN, J. A., DOROUGH, H. W., BUTTRAM, J. R., and ARTHUR, B. W. (1961).—*In vitro* stability and recovery of insecticides from milk. *J. Econ. Ent.* 54:441.

(Received for publication September 21, 1966)

The author is an officer of the Biochemistry Branch, Division of Animal Industry, Department of Primary Industries, and is stationed at the Animal Research Institute, Yeerongpilly, Brisbane.