

STUDIES ON PESTICIDE RESIDUES. 1. EXCRETION OF PESTICIDES IN MILK FOLLOWING DERMAL TREATMENT OF DAIRY CATTLE WITH COUMAPHOS AND DIOXATHION

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SUMMARY

Dairy cattle were sprayed with 0.025% coumaphos, 0.050% coumaphos or 0.075% dioxathion, concentrations recommended for the control of ectoparasites of cattle, and excretion in milk was examined in samples taken during a 5-day post-treatment period.

Maximum concentration and total excretion of coumaphos in milk occurred at the first sampling, 5 hr after treatment, whereas maximum total excretion of dioxathion was observed at the third sampling 29 hr after treatment. Levels of both pesticides in milk declined rapidly and were not detectable (<0.005 p.p.m.) 70 hr after exposure. No differences were observed between the excretion patterns of coumaphos applied as wettable powder and emulsion formulations.

The concentration of pesticide in milk was shown to be a function of concentration applied, quantity of milk produced and time of sampling after exposure.

I. INTRODUCTION

The use of chemicals as contact pesticides for the control of ectoparasites of cattle is a long-established husbandry practice in northern and eastern Queensland. Since 1962 there has been a prohibition on the use of chlorinated hydrocarbon chemicals for certain livestock treatments in this State. As a result there has been a widespread acceptance by stock-owners of the organophosphorus

group of compounds as suitable substitutes. Experimental findings (Roulston and Wilson 1965) and field reports (Anon. 1962, p. 32) have demonstrated the effectiveness of these compounds for the control of the cattle tick (*Boophilus microplus* Can.).

However, no published observations are available of the magnitude and persistence of pesticides remaining in the meat and dairy products from animals treated in accordance with normal tick control measures in Queensland. In recent years an increasing awareness of this aspect of chemical pest control has been engendered by a necessary consideration of the requirements of importing countries. Maximum allowable residues of many pesticide chemicals in domestic and imported foodstuffs have been fixed by the United States Food and Drug Administration under the Federal Food, Drug and Cosmetic Act. Furthermore, a petition for a tolerance level of methoxychlor in milk (Longenecker *et al.* 1957) resulted in a decision to impose a "zero tolerance" on all pesticide chemicals in dairy products in the U.S.A. Heineman and Miller (1961) have discussed the implications these regulations could have for the dairy industry if they were rigidly implemented.

Coumaphos and dioxathion are two organophosphorus pesticides that have been used extensively in Queensland for several years. The object of the work reported in this paper was the assessment of the patterns of excretion of coumaphos and dioxathion in milk of dairy cattle exposed to treatment at levels recommended for cattle tick control.

II. MATERIALS AND METHODS

Pesticides.—Coumaphos (0,0-diethyl 0-(3 chloro-4 methyl-2-oxo-2H-1-benzopyran-7-yl) phosphorothioate) was examined as two formulations, a 16% w/v emulsion and a 47.5% w/w wettable powder, which are supplied commercially under the trade name "Asuntol". Concentrations used for spraying were 0.025% and 0.050% respectively.

Dioxathion (2:3-p-dioxane S,S-bis(0,0-diethyl phosphorodithioate)) was examined as a 30% w/v emulsion supplied commercially as "Bercotox". The spray concentration used was 0.075%.

Experimental animals.—Three groups each of three dairy cattle were treated with one of the two coumaphos preparations or with dioxathion. After treatment the animals were allowed free grazing during the test period. Differences between animals with respect to breed, weight, condition of coat and milk production were not considered in the comparison of the overall secretion patterns.

Treatment and sampling.—Cattle were sprayed with a low-pressure pump, using 9 l of fluid containing the pesticide for each animal. After treatment and just prior to the first milking the udder region was thoroughly washed to remove any pesticide adhering to the skin. Samples of milk (2.5 l) were taken from each animal before treatment and twice daily thereafter for a 5-day period. Milk yield was recorded at each milking. Milk was stored at 4°C until it could be processed. At this temperature no appreciable loss of pesticide occurs within 5 days (Timmerman *et al.* 1961).

Chemical analysis.—The chilled samples were centrifuged at -5°C at 2,000 r.p.m. for 10 min. The cream layer was removed and shaken until dispersal of fat granules and separation of buttermilk occurred. After water washing, samples were processed by the petrol ether/anhydrous sodium sulphate extractive procedure described by Mills (1959) for animal tissues. The total yield of moisture-free butterfat, between 50 and 100 g, was taken for analysis. Milk fat was determined by the Babcock method (Burgess 1936) on representative sample aliquots taken at each milking.

Solvent partition between n-hexane and acetonitrile (Jones and Riddick 1952) was used in the initial separation of pesticides from the bulk of the fat.

Samples for coumaphos analysis were further purified by partition chromatography on silicic acid, using acetonitrile as the stationary phase (H. J. Schnitzerling, personal communication), final traces of fat being readily eluted with n-hexane saturated with the stationary phase. Considerable variation in activity was noted between batches of silicic acid. Chromatography of authentic coumaphos for each batch of silicic acid showed retention volumes of pesticide varying from 100 to 200 ml. In the determination of coumaphos, application was made of the characteristic ultraviolet spectra of the compound's aromatic chromophore as suggested by Kane *et al.* (1960). Although the specific extinction at 320 $\text{m}\mu$ used for measurement will not differentiate between coumaphos and its oxygen analogue, concentration of the latter in milk is less than 5% of the total organophosphorus residue (Krueger, Casida, and Niedermeier 1959). Its contribution to the total absorbance at 320 $\text{m}\mu$ was therefore ignored.

Dioxathion was determined essentially by the method of C. L. Dunn (1958*a*). Capillary column chromatography on neutral alumina was adopted to handle residues of dioxathion less than 10 μg (C. L. Dunn 1958*b*).

Recovery data, at levels corresponding to those found experimentally, and blank values were determined for both pesticides.

III. RESULTS

Results of chemical analysis are presented in Tables 1 and 2.

TABLE 1
RESIDUES OF COUMAPHOS IN BUTTERFAT AND MILK FOLLOWING SPRAY TREATMENT OF DAIRY CATTLE

Treatment	Time after Treatment (hr)	Coumaphos in Butterfat and Milk* and Total Excretion at Each Milking											
		COW 1			COW 2			COW 3			MEAN		
		p.p.m. in Fat	p.p.m. in Milk	µg Excreted per Milking	p.p.m. in Fat	p.p.m. in Milk	µg Excreted per Milking	p.p.m. in Fat	p.p.m. in Milk	µg Excreted per Milking	p.p.m. in Fat	p.p.m. in Milk	µg Excreted per Milking
Coumaphos emulsion, 0.025% ..	pre	—	—	—	—	—	—	—	—	—	—	—	—
	5	0.79	0.039	114	1.10	0.050	119	0.34	0.021	68	0.74	0.037	101
	21	0.17	0.007	34	0.39	0.013	53	0.11	0.004	29	0.23	0.009	39
	29	0.10	0.004	11	0.16	0.006	12	0.03	0.001	5	0.10	0.004	9
	45	0.07	0.003	16	0.04	0.001	5	0.14	0.006	37	0.09	0.003	19
	53	—	—	—	0.03	0.001	4	0.01	0.001	6	0.01	0.001	3
	69	—	—	—	—	—	—	—	—	—	—	—	—
	77	—	—	—	—	—	—	—	—	—	—	—	—
Coumaphos wettable powder, 0.050%	pre	—	—	—	—	—	—	—	—	—	—	—	—
	5	0.94	0.028	26	1.56	0.051	99	1.23	0.051	187	1.24	0.044	104
	21	0.59	0.024	135	1.14	0.036	146	0.61	0.019	105	0.79	0.026	129
	29	0.28	0.013	26	0.57	0.026	45	0.53	0.030	109	0.46	0.023	60
	45	—	—	—	0.21	0.004	13	0.07	0.003	20	0.09	0.003	11
	53	—	—	—	—	—	—	—	—	—	—	—	—
	69	—	—	—	—	—	—	—	—	—	—	—	—
	77	—	—	—	—	—	—	—	—	—	—	—	—

* Assessed on butterfat content determined at each milking.

— Represents a value less than the minimum detectable residue.

TABLE 2
RESIDUES OF DIOXATHION IN BUTTERFAT AND MILK FOLLOWING SPRAY TREATMENT OF DAIRY CATTLE

Treatment	Time after Treatment (hr)	Dioxathion in Butterfat, Milk* and Total Excretion at Each Milking											
		COW 7			COW 8			COW 9			MEAN		
		p.p.m. in Fat	p.p.m. in Milk	μ g Excreted per Milking	p.p.m. in Fat	p.p.m. in Milk	μ g Excreted per Milking	p.p.m. in Fat	p.p.m. in Milk	μ g Excreted per Milking	p.p.m. in Fat	p.p.m. in Milk	μ g Excreted per Milking
Dioxathion emulsion, 0.075%	pre	—	—	—	—	—	—	—	—	—	—	—	—
	5	3.33	0.130	534	2.30	0.104	204	1.13	0.049	261	2.25	0.094	358
	21	1.00	0.051	669	1.21	0.044	363	1.61	0.058	599	1.24	0.051	544
	29	1.29	0.095	797	2.22	0.091	612	3.18	0.111	982	2.23	0.099	797
	45	0.92	0.043	508	1.37	0.051	476	1.53	0.055	805	1.27	0.050	600
	53	0.08	0.005	33	—	—	—	—	—	—	—	—	—
	69	—	—	—	—	—	—	—	—	—	—	—	—
	77	—	—	—	—	—	—	—	—	—	—	—	—

* Assessed on butterfat content determined at each milking.
— Represents a value less than the minimum detectable residue.

Values are not shown to the completion of the fifth day of sampling, since by the 69-hr sample residues were not detectable by the methods used. Residues of coumaphos, expressed as parts per million (p.p.m.) in butterfat, p.p.m. in wholemilk and also total pesticide excreted at each milking are shown in Table 1. There was minimum detectable residue of 1.0 μg for coumaphos, representing a lower limit of detection of the order of 0.001 p.p.m. in milk on the basis of a 50-g butterfat sample.

Residues of dioxathion expressed as p.p.m. in butterfat, p.p.m. in wholemilk and also total pesticide excreted at each milking are shown in Table 2. Because of a high and variable blank, the minimum detectable residue of dioxathion was 5.0 μg . This represented a lower limit of detection of the order of 0.005 p.p.m. in milk. The results presented have been corrected with respect to blank and recovery values.

IV. DISCUSSION

On the basis of pesticide distribution studies on wholemilk (Krueger, Casida, and Niedermeier 1959), it was accepted that virtually all organophosphorus pesticide residue is located in the fat fraction. Residues of coumaphos and dioxathion were quantitatively measurable in milk sampled 5 hr after treatment. Maximum excretion of coumaphos resulting from treatment with a 0.025% emulsion or a 0.050% wettable powder occurred at this milking. At succeeding milkings a rapid and progressive decline in the excretion of coumaphos in milk was observed. Similar observations have been reported in the United States, where dairy cattle exposed to coumaphos emulsion as 0.75% and 0.50% sprays showed greatest concentration of pesticide in milk 5 hr after treatment (Claborn *et al.* 1960; Radeleff and Claborn 1960). Exposure to coumaphos emulsion resulted in lower residues in milk compared to wettable powder treatment. This was attributed to the lower concentration (0.025%) recommended by the manufacturer for the emulsion formulation and was unlikely to be related to physical differences in the formulations.

Concentration of dioxathion in milk was at or near its maximum at the first sampling at 5 hr after exposure, but unlike residual coumaphos, the level of excretion did not vary significantly during the two days following treatment. Fluctuation in concentration during this period reflected differences in milk yields and in butterfat values occurring between morning and afternoon sampling. There was a progressive increase in the total excretion of dioxathion for 30 hr following treatment. Here total pesticide excreted rather than concentration in milk would be a better guide to the residual characteristics of dioxathion.

This relative persistence of maximum rate of elimination in milk and the considerable variation observed between animals in the pattern of excretion have been previously demonstrated in cattle sprayed with 0.5% and 0.1% dioxathion emulsion (Schranz 1956).

Coumaphos residues in milk did not exceed 0.050 p.p.m. for a 0.025% spray or 0.060 p.p.m. for a 0.050% spray. The highest concentration of dioxathion in milk was 0.130 p.p.m. By the sixth milking, 69 hr after treatment, no residues of coumaphos or dioxathion were detectable in milk, irrespective of the concentration or type of formulation used.

Results indicate that dairy products derived wholly from milk taken from cattle during the 3 days following field exposure to the pesticides examined would contain residues detectable by standard chemical procedures and could be rejected in circumstances where a "zero tolerance" is imposed.

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