Toxicity of piperonyl butoxide to *Boophilus microplus*

SYNERGISM studies with the cattle tick *Boophilus microplus* have been effected by enclosing larvae in packets impregnated with olive oil solutions of the test chemicals and recording mortality after 48 h[1,2]. In these conditions larvae exposed continuously to 0.4% piperonyl butoxide were apparently unaffected. Subsequently, in studies to determine the sensitivities of the mixed function oxidase systems in organophosphorus (and carbamate) resistant and susceptible strains of *B. microplus*, larvae were immersed in aqueous colloids[3] of piperonyl butoxide. Concentrations greater than 0.02% proved to be toxic and an LC_{50} of 0.044% at 24 h after treatment was determined. This unexpected result aroused interest in the acaricidal potential of piperonyl butoxide. Although it is generally regarded as a nontoxic synergist[4], some workers have claimed it is toxic to houseflies at fairly high dosage[5] but others found it to be nontoxic[6]; toxicity to the mite *Acarus siro* (formerly *Tyroglyphus farinae*) has been recorded[7,8].

We decided that testing of the material alone and mixed with carbaryl, against parasitic stages of the tick in the field, was warranted. The mixture was tested because of the well known synergism of carbaryl and other carbamate insecticides by piperonyl butoxide against resistant houseflies. This synergistic action is believed to be due to piperonyl butoxide reducing the abnormally rapid rates of oxidative degradation of the carbamates by resistant flies[9]. The ticks used in our tests, however, were of the Biarra strain[10], and are resistant to carbaryl because of low sensitivity of their acetylcholinesterase to inhibition by carbaryl, while their detoxication systems are normal[11].

Cattle naturally infested with all stages of Biarra ticks were sprayed with aqueous emulsions of piperonyl butoxide formulated with one third its volume of Triton X-100. (*B. microplus* is a one-host tick with a cycle of approximately 21 d, and infested cattle normally carry mixed populations of larvae, nymphs and adults.) Cattle were also sprayed with a commercial wettable powder formulation of carbaryl at 0.3% active ingredient, and with mixtures of carbaryl and the piperonyl butoxide formulation. Counts of semiengorged females 4.5 mm to 8 mm in length were made at intervals up to 21 d after treatment and a survival index was calculated from counts of ticks on untreated control animals[12]. The results in Table 1 show that piperonyl butoxide is lethal to all stages of the tick when used at relatively high concentrations. The acaricide carbaryl allowed 28% survival of resistant Biarra ticks compared with 1% survival expected with susceptible Yeerongpilly ticks (W. J. R., unpublished data). With the addition of piperonyl butoxide at concentrations $\geq 0.3\%$, however, less than 1% of the resistant strain survived.

As much lower concentrations of piperonyl butoxide have been shown to inhibit the degradation of carbaryl in laboratory tests on larvae (C. A. S., unpublished data), this type of protective action doubtless contributed to the effectiveness of the spray mixtures. The lethal effect of piperonyl butoxide itself may also have contributed to the enhanced toxicity of the mixtures and it is not certain, therefore, whether piperonyl butoxide acts with carbaryl predominantly as a synergist or mainly as a toxicant participating in some type of joint action.

It seems likely that the toxic action of piperonyl butoxide resulted from inhibition of a mixed function or closely related oxidase, the recognised type of target of piperonyl butoxide[13]. This indicates that the cattle tick is vitally dependent on some part of this system. In support of this we have preliminary evidence that another methylenedioxyphenyl compound sulfoxide, which inhibits cattle tick mixed function oxidase, is also toxic.

The immediate significance of these findings is that an acaricide which had failed against a resistant strain has been restored to full effectiveness in the field by the addition of a compound which has no significant mammalian toxicity[14]. A more important aspect in the long term could be that from this class of compound a practical acaricide could emerge.

Table 1 Field control of the Biarra strain of cattle tick with sprays containing piperonyl butoxide or carbaryl or mixtures of the two chemicals

Spray Composition (%) Carbaryl	Piperonyl butoxide	Cattle per treatment	Survival index* on days† after treatment 1	2	5	7	9	12	14	16	19	21	Mean 1–21 days
–	0.03	4	99	64	92	132	52	105	86	42	36	38	75
–	0.3	4	53	28	3	16	33	40	33	14	4	1	23
–	3.0	4	95	27	0.8	0.7	1	5	4	1	0.4	0.4	14
0.3	–	2	39	11	3	39	92	16	19	11	9	40	28‡
0.3	0.03	2	35	22	3	4	33	61	41	24	5	1	23
0.3	0.3	2	1	0	0	0.7	5	0.7	0.4	0.8	0.8	1	1
0.3	3.0	2	3	0	0	0.7	4	0.3	0	0	0	0	1

*Calculated as follows: (Tick count on treated animals/Tick count on control animals) × (Pretreatment count on control animals/Pretreatment count on treated animals) × 100.
On control cattle daily totals of more than 200 semiengorged females were recorded before and after treatment.
†Stages of tick at treatment were: adult females, day 1–8; nymphs, day 9–15; larvae, day 16–21.
‡In similar conditions less than 1% of the acaricide-susceptible Yeerongpilly ticks survived (W. J. R. unpublished data).

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