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THE USE OF MICROENCAPSULATED REPELLENTS AND PESTICIDES TO CONTROL OR ERADICATE VECTORS CAUSING PARASITIC DISEASES IN CATTLE IN EAST AFRICA

Final Report

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ABSTRACT

Laboratory animals have been given full protection from tsetse bites for a period greater than 10 days by dermal application of some formulations of microencapsulated natural pyrethrum. Considering the 2 hours protection conferred by currently used repellents against blood-feeders, this is a breakthrough in repellency duration. Moreover, the same type of formulation is toxic to ticks for more than 7 days, permitting us to envision the possibility of using a single agent against the two major vectors of cattle diseases in East Africa.

I. INTRODUCTION

Tsetse flies, vectors of Trypanosomyasis, and ticks, vectors of several serious livestock diseases, continue to plague the health and economy of great parts of Africa. Different approaches to controlling these vectors have been attempted over the years, including some new chemical control techniques. However, vast areas with favorable climatic and edaphic conditions for cattle production are still infected and cannot be used for raising cattle.

Full protection of cattle from tsetse fly bites is especially important when considered as treatment for slaughter cattle. In Africa, tsetse-free livestock production areas are often located far from the large cities and slaughter animals must travel for weeks through tsetse-infested areas where they may contact Trypanosomyasis. Protection from bites may replace the chemoprophylaxis treatment used under such conditions which requires more skillful application.

Previous efforts to protect people and domestic animals by repelling the tsetse flies have been disappointing. Attempts were made in the 1940's[1,2,3] and then discontinued for almost thirty years. Recently, the usefulness of some new repellents against the tsetse has been evaluated. Of nine repellents tested on human skin (including DEET, DMP, Ethyl hexanediol, 2,2-n-trimethyl pentanendiol), none gave 100% protection for more than 2 hours.[4] Impregnation of wide-mesh net jackets (ratio 0.5 g agent per g netting) conferred 100% protection to the wearer for only one day using the most effective agent, di-isopentylmalate.[5]

In practice, protection of livestock against tsetse flies and ticks by means of repellents requires a repellency duration of at least one week. An agent giving 100% protection for one week or more could replace chemoprophylactic drugs and

also acaricides as a means of protection, and might be the preferred method. To date, the only compound conferring one or two days protection against ticks and tsetse flies is natural pyrethrum.[6] However, pyrethrum decomposes very rapidly due to UV radiation from sunlight, and it also oxidizes readily. In 1975 Galun proposed attempting to increase the residual repellency of pyrethrum using microencapsulation as a possible means of reducing evaporation rate, photodegradation and oxidation, and of slowing down absorption of the material into the skin.[7]

Pennwalt Corporation scientists have reported on the advantages of microencapsulation of biologically active agents, and world-wide interest has developed in the potential for reduction of mammalian toxicity that results from the controlled release feature provided by encapsulation of highly toxic pesticides.[8] For example, microencapsulated methyl parathion is at least 12 times less toxic dermally (rabbits) than an equivalent amount of the pesticide in an emulsifiable concentrate formulation. At the same time the comparative insecticidal activity is extended up to 2-1/2 times. These concepts were demonstrated in a toxicological study in which microencapsulated methyl parathion and microencapsulated diazinon were applied to the hides of cattle.[9,10] Increased amounts of toxic pesticides were tolerated by cattle, indicative that significantly greater control of livestock pests is possible by this type of formulation.

This report contains data on the repellency and toxicity of 27 formulations of microencapsulated natural pyrethrum, with different capsule walls controlling different release rates of the repellent-pesticide. The microencapsulations were carried out by the Pennwalt Corporation and the testing against tsetse flies and ticks was carried out at the Hebrew University of Jerusalem. For comparison, non-encapsulated natural pyrethrum, microencapsulated synthetic pyrethrums and

a microencapsulated experimental repellent were also tested.

II. MATERIALS AND METHODS

A. Preparation of microencapsulated formulations

All the microencapsulated materials reported in this paper were prepared by Pennwalt Corporation at their Technological Center located at King of Prussia, Pennsylvania. The encapsulations were based upon technology developed and patented by Pennwalt.[11] Test materials were microencapsulated by interfacial polymerization which produces a rigid but permeable capsule wall around tiny droplets of repellent/insecticide. The encapsulating composition employed was a crosslinked polyamide. An unusual degree of flexibility in preparing capsule walls for a specific controlled release rate is inherent in the Pennwalt process. Varying degrees of permeability are achieved by using preparative formulations which yield the desired variation in the wall thickness and degree of crosslinking of the polymer.[8] Each of these wall characteristics has an effect on release rate. All the microencapsulated samples prepared for this program were given a preliminary screen by Pennwalt by measuring the persistency of toxicity toward house flies (*Musca domestica*). Those possessing satisfactory controlled release characteristics were submitted for advanced testing on guinea pigs.

Natural pyrethrum was the prime candidate for encapsulation, both as is and in formulations containing piperonyl butoxide as a synergist (10 to 1 ratio of synergist to pyrethrum). One sample tested was an encapsulated synergized natural pyrethrum in which the capsule wall was made by a different experimental process and consisted of a blend of waxes.

Microcapsules ranging in size from 30-50 μm were dispersed in an aqueous medium, into which a thickener was added. Final concentrations of the natural pyrethrum ranged from 0.6% to 2.2%.

Synthetic pyrethroids were also included in the advanced screenings. Those used were bioresmethrin, resmethrin, and permethrin.

An experimental repellent, reported to show good repellency against biting flies, was supplied by Dr. Terry P. McGovern, USDA-SEA, Beltsville, Maryland. This repellent, a cyclohexane carbamate derivative, was tested as is and in a microencapsulated formulation.

Each sample submitted for tests was rated for release characteristics. Such a rating was based upon empirical data obtained from previous tests. No direct determination of release by chemical analysis was performed on the test samples.

B. Repellency Tests

1. Tsetse Flies

The tsetse flies used were *Glossina morsitans*. *Glossina* pupae were made available through the kind courtesy of the Tsetse Research Laboratory, School of Veterinary Science, Langford, Bristol. Pupae were kept at 28°C at 70% humidity.

Ten to twelve hatched flies were placed in nylon mesh cages (14 x 8 x 4 cm) and kept at 24-25°C in a humid incubator. Repellency tests were carried out with hungry flies, teneral flies 48 hours after emergence, or non-teneral flies 48 hours after the

last blood meal. Five cc of the formulation was applied to semi-shaved bellies of guinea pigs and spread evenly over an area of 16 x 9 cm^2. The same guinea pig treatment was used for evaluating repellency and toxicity against both tsetse flies and ticks. Testing was carried out at 28°C and 70% humidity. The treated guinea pig was immobilized on its back, using a specially designed restrainer table, and fly cages were placed on the treated bellies on their large surface, and exposed to the repellent for 5 minutes. The cages were stored uncovered in a dark room. This avoided the accumulation of noxious vapors that might have occurred from the common practice of covering. Immediately on removal of the cage, the number of engorged flies was recorded and all the flies were transferred to a clean cage. The number of knocked-down flies was recorded 1/4 hour later. One-half hour later the cage was placed on an untreated control animal and additional feeding was noted, an indication of the residual effect of the repellent. Mortality was recorded 24 hours following testing. We observed that pyrethrum protected the guinea pigs for 5-6 days, and standardized the testing procedure, beginning testing at the 6th day following treatment and testing daily until feeding was observed. Formulations which gave full protection for 9 days and more were tested again using flies of different ages, including the most resistant tsetse stage, pregnant females.[12,13]

2. Ticks

The ticks used were adult female *Ornithodoros tholozani* starved for over two years. Testing was carried out under the conditions described above,

using the same restraining table for the guinea pigs as described but with a special apparatus for testing tick repellency and toxicity harnessed to the guinea pig. This consists of a stiff rubber sheet with two holes of 5 cm fitted with brass cylinders with bottom flanges each holding a tightly-fitted glass cylinder for holding the ticks, projecting slightly from the bottom according to the method of Bar Zeev and Gothilf.[14] This apparatus is strapped tightly to the guinea pig belly. Ornithodoros adults do not climb on glass and are therefore in contact with the treated belly area for the whole period of exposure, 10 minutes for repellency testing, 6 hours for toxicity testing. Repellency testing was begun 2 days following application, and was carried out daily using different batches of ticks, ten ticks per batch, and continued until feeding was observed. The number of attached and of engorged ticks was recorded. Mortality was recorded one week after exposure since delayed mortality seemed to occur. Only formulations conferring 7 or more days of protection against tsetse were tested for toxicity to ticks.

III. RESULTS AND DISCUSSION

A. Tsetse Flies

Formulations of nonencapsulated natural pyrethrum at a concentration of 0.50 mg/cm^2 gave guinea pigs complete protection from tsetse bites for 6 days (Table 1 - 4515-118). Repellency was not increased by synergizing the pyrethrum, since it conferred only 5 days protection from tsetse bites (Table 1 - 4515-113). Microencapsulating natural pyrethrum did increase the duration of repellency in some of the formulations.

Fifteen of the 27 formulations did not show increased repellency duration. These had minimal effective doses (M.E.D.s) ranging between 0.013-0.10 mg/cm^2, a wide range, possibly indicative of a wide range in the rate of release. Twelve of these formulations did show increased repellency duration, with the best formulations (4632-105-7 and 4632-105-2), giving a protection time of 10-15 days. Both of these preparations had low M.E.D.s and were not synergized. Treatments were repeated after 8 months storage, and showed the same duration of protection as before, indicating that these microencapsulated materials have a long shelf life.

Tsetse flies and ticks are vectors of serious diseases, thus we want to protect people and livestock from the pathogens they carry, rather than merely decrease irritation caused by their bites. Therefore we selected the most severe criterion for repellency, 100% protection, although a preparation giving somewhat less protection in the field would still significantly reduce the damage caused by these pests. In many of the formulations, a high protection level, over 90%, persisted for some days after the first-bite record, extending the period of actual protection beyond that cited in Table 1. However, we concentrated on 100% protection within the scope of the present research, and deterioration of protection with time was not followed up. Again, there is good reason to believe that a high level of protection would persist for some time.

A high mortality of flies was observed when repellency tests were carried out during the first five days following treatment. After five days, few flies died,

even when the repellent continued to confer full protection. This high early mortality necessitated delaying determining the M.E.D., usually done immediately following drying of the repellent, to 48 hours following application, in order to distinguish between the toxic and repellent effects of pyrethrum. Although the mortality of flies tested after the first five days was lower, the flies were affected by exposure to the repellent and did not feed on untreated animals offered to them ca. 1/2 hour later. A longer recovery period, hours, is apparently needed. More teneral flies died than pregnant ones, while repellency appeared to be equally effective on both groups.

Examination of the hair of treated animals under the dissecting microscope showed that the microcapsules were clumped and did not adhere well to the hair. Spreader-sticker adjuvents should improve the residual effect of these formulations.

Some of the synthetic pyrethrins did not confer protection from tsetse bites when tested only 2 days following treatment, despite their very high toxicity, as previously reported by Hadaway.[13] These include microencapsulated resmethrin, bioresmethrin, or permethrin as well as non-encapsulated bioresmethrin. Both non-encapsulated and microencapsulated cyclohexane carbamate or microencapsulated methyl parathion did not deter biting even when used in a high concentration of 50 mg/cm^2.

B. Ticks

Pyrethrum is also an effective acaricide and if the application of microencapsulated pyrethrums can protect cattle from infestations of ticks its use can become much more attractive from the economic point of view. We therefore tested the same formulations for their effects as a tick repellent or toxicant. Starved adult

females of Ornithodoros tholozani are repelled for shorter periods than the tsetse flies. However, ticks killed within 24 hours of their attachment to the host do not transmit their pathogens which require several days of activation to be virulent. Hard ticks attach and feed for periods of days, and toxicants can act over a long-term period. We therefore tested the effects of the formulations during a 6 hour contact period and found that many of the preparations gave 100% mortality. Ticks have become resistant to most of the available acaricides, therefore frequency of dipping or spraying has been increased to once or twice weekly.

The microencapsulated systems presented here may be important in Africa in conferring protection from tick-borne diseases in addition to the tsetse fly. We therefore believe that the effectiveness of these systems as a means of protecting cattle from these two major pests should be tested in the field in Africa.

IV. ACKNOWLEDGEMENT

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Table 1. Natural Pyrethrum - Repellency to Tsetse Flies and Ticks

Sample	Identity	Release Rate[a]	Test Concentration (mg/cm^2)	Repellency (days)		Mortality %			MED (mg/cm^2)[e]
				Tsetse[b]	Ticks	Tsetse	Ticks[c]	Ticks[d]	
4632-97-1	Non-synergized, encapsulated	M	0.90	9	5		30	90	.09
97-2	"	S	0.90	7	3		30		.052
97-3	"	S	0.80	7(9)	< 2		30		.027
105-1	" "	F	0.50	< 6	< 2		80		.013
105-2	" "	F	0.52	11	> 2		100	80	.026
105-3	" "	F	0.52	8	3		60	60	.026
105-4	" "	F	0.52	9	3		70	60	.052
105-5	" "	F	0.42	6	< 2		60		.026
105-6	" "	F	0.50	8	< 2		100		.026
105-7	" "	M	0.60	10(15)	< 2		100	80	.014
4515-118	Non-synergized, not encapsulated	-	1.05	6			-	-	.052
"	" " "	-	0.52	6	< 2		100		-
4632-104-1	Synergized, encapsulated	F	0.55	6	3		100		.013
104-2	" "	F	0.52	< 6	< 2		100	50	.027
104-3	" "	F	0.52	7(9)	< 2		100	50	.10
104-4	" "	F	0.52	< 6	< 2		80		.10
104-5	" "	F	0.52	< 6	< 2		80		.05
104-6	" "	F	0.56	6	< 2		90		.10
104-7	" "	F	0.46	6	< 2		80		.10
4683-42-1	Synergized, encapsulated	M	0.56	6	< 2	90	0		.10
43-1	" "	M	0.42	7(9)	< 2	100	100	90	.04
43-2	" "	M	0.52	< 5	< 2	100	50		.027
45-1	" "	S	0.38	7	< 2	100	100	100	.014
45-2	" "	S	0.38	< 6	< 2		60		.08
46-1	" "	M	0.35	< 6	< 2		0		.052
46-2	" "	S	0.46	< 6	< 2		0		.10
47-1	" "	S	0.52	7	< 2		10	90	.052
4659-52	" "	M	0.52	7	< 2		0	100	.052
4632-97-5	Synergized, encapsulated[f]	M	0.35	7	< 2		50		.027
4515-113	Synergized, not encapsulated	-	0.52	5	2		100		.010

a. Estimated rate of release based on earlier findings with capsules of similar wall characteristics: S = slow release; M = moderate release; F = fast release.

b. Numbers in parenthesis indicate maximal protection period, while otherwise minimal protection period is given.

c. Ten minute exposure two days after application.

d. Six hour exposure seven days after application.

e. Minimum dosage required to give 90% protection against tsetse.

f. Wax wall.

Table 2. Synthetic Insecticides - Repellency to Tsetse Flies and Ticks

Sample	Identity		Release Rate[a]	Test Concentration (mg/cm^2)	Repellency (days)		Mortality
					Tsetse	Ticks	Ticks[b]
4683-59-1	Bioresmethrin	Encapsulated	S	0.52	2	2	100
4632-97-4	Resmethrin	"	M	1.0	2	2	60
4632-123-1	Permethrin	"	M	0.20	2	2	90
4632-123-2	Permethrin	"	M	0.90	2	2	70
4632-123-3	Methyl Parathion	"	M	0.52	2		
4632-97-6	Cyclohexane carbamate derivative	"	M	4.5	2	2	0
4683-49	Bioresmethrin	Not encapsulated	-	0.52	2	2	100
4632-97-1	Cyclohexane carbamate derivative	" "	-	0.35	2	3	10

a. Estimated rate of release based on earlier findings with capsules of similar wall characteristics: S = slow release; M = moderate release; F = fast release

b. Ten minute exposure two days after application.