International Journal of Cosmetic Science **10**, 23–28 (1988)

Effectiveness of macrogol and talcum base formulations of a new insect repellent N,N-diethyl phenyl acetamide

S. PRAKASH,‡ S. KUMAR, M. V. S. SURYANARAYANA, R. K. SHARMA* and K. M. RAO Defence Research & Development Establishment, Gwalior-474002, India and *Institute of Nuclear Medicine and Allied Sciences, Probyn Road Delhi, India

‡ Correspondence to Dr Shri Prakash, Scientist 'B', Entomology Division, Defence Research & Development Estt, Gwalior-474002, India.

Received 16 October 1987

Key words: DEPA, DMP, insect repellent, *Aedes aegypti*, macrogol ointment, gas chromatographic analysis.

Synopsis

The effectiveness of macrogol and talcum base formulations of a new multi-insect repellent N,N-diethyl phenyl acetamide (DEPA) was studied against *Aedes aegypti* mosquitoes in the laboratory. The efficacy was compared with widely used repellent in India, viz. Dimethylphthalate. Twenty-five per cent DEPA incorporated macrogol ointment and talcum base formulation offered more than 8 h and 6 h protection, as compared to DMP for 3 h and 1 h 30 min, respectively. The ointment was found to be stable as more than 99% DEPA was recovered by gas liquid chromatography after 6 months of storage at room temperature. The repellent formulations have not shown any gross adverse effect to the skin of albino rabbits.

Résumé

L'efficacité d'un nouvel agent repousseur d'insectes (le N,N-diethyl phenyl acetamide) dans des formulations à base de macrogol et du talc

L'efficacité des bases formulées à partir de macrogol et de talc pour un nouvel insecticide, N,N-diethyl phenyl acetamide (DEPA) a été étudiée contre les moustiques *Aedes aegypti* en laboratoire. Cette efficacité a été comparée avec un insecticide couramment utilisé en Inde, le Dimethyl phthalate. 25% de DEPA incorporé à une base formulée à partir de pommade de macrogol et de talc a permis une protection respectivement de plus 5 h et de plus de 8 h comparativement au DMP pour lequel la protection était respectivement de 3 h et 1 h 30. La pommade s'est avérée stable car plus de 99% de DEPA ont été identifiés par GLC après 6 mois de stockage à température ambiante. Les formulations d'insecticides n'ont révélé aucun effet adverse sur la peau des lapins albinos.

INTRODUCTION

Repellents are of particular importance for civil and military personnel for protection against a variety of dangerous and disease-bearing insects. At present no broad spectrum, long-lasting insect repellent is available for topical application on human skin. The widely used repellent, dimethylphthalate (DMP), provides protection for 2–3 h only and diethyl-m-toluamide (m-DEET), though effective against various species of insects, is costly in India as the starting material required for synthesis needs to be imported. So the ideal repellent formulation for skin with respect to cosmetic acceptability, protection offered, non-toxic non-irritant and cost effectiveness is to be explored.

N,N-diethylphenylacetamide (DEPA) has been reported as a long-lasting, non-irritant, non-toxic, cost-effective and multi-insect repellent (1–6). The present paper deals with the repellent

efficacy of macrogol ointment and talcum base formulation of the new compound, DEPA, against *Aedes aegypti* mosquitoes and the efficacy of this compound was compared with dimethylphthalate presently being used by the Indian Armed Forces. The skin irritancy of these formulations has also been studied in albino rabbits in the laboratory.

MATERIALS AND METHODS

Preparation of macrogol ointment

The macrogol ointment was essentially based on US pharmacopeal preparations with some modifications. Ten to 25% active ingredient of repellent DEPA/DMP was incorporated in formulation. Six per cent of essential oils (citronal:citronella oil) were added in the ratio of 1:2. The following grades of polyethylene glycol of USP standard were used in the formulations.

Polyethylene glycol 400–53 parts;
600–15 parts;
4000–32 parts.

The polyethylene glycol (PEG) of various molecular weights were weighed and heated to 65°C. Volatile essential oil and the desired concentration of repellent were added to the molten base, which was stirred and allowed to cool to a homogeneous mass.

Talcum base formulations

Different concentrations of DEPA/DMP (10–25%) were incorporated with the following ingredients of IP/BP/USP standard:

talcum–36 parts;
kaolin–15 parts;
precipitated chalk–15 parts;
light magnesium carbonate–5 parts;
magnesium stearate–5 parts;
calcium stearate–4 parts;
citronal/citronella oil, 1:2– 5 parts;
zinc oxide–5 parts;
boric acid–10 parts.

Formulations without repellent were prepared and used as control.

Laboratory evaluation of repellent formulations

The desired concentration of test compound was applied to the back of human hands to an approximate area of 150 cm^2. The treated surface of the hand was exposed to 200 5–7-day-old unfed female *Aedes aegypti* mosquitoes in a $75 \times 60 \times 60$ cm test chamber. Minimum effective dose (MED) and protective time (PT) were determined as per the method described by Sharma *et al.* (7).

Skin irritancy of magrogol ointment and talcum base formulation

Studies on skin irritancy following repeated application of 25% macrogol ointment and talcum base formulation was essentially based on the method described by Draize *et al.* (8) with slight modifications. Male albino rabbits weighing 1.8 to 2.0 kg were divided into groups of five each. 100 mg of ointment or talcum powder was applied to shaven areas (2.2 cm^2) of different groups daily for 7 days. Observations on erythema and oedema were recorded by three independent

evaluators during 7 days of application and 7 days following discontinuation of treatment, as per the method described by US Code of Federal Regulations (9).

Gas chromatographic analysis of repellent formulation

Since DEPA-incorporated macrogol ointment was found most effective in the studies, gas chromatographic analysis (GLC) of DEPA incorporated ointment for stability studies was carried out by the following procedure.

Sample preparation

A 1-g portion of the formulation was dissolved in 100 ml solution of 100 g of sodium chloride/100 ml. The mixture was swirled and extracted (5 min vigorous shaking) with 25 ml of chloroform. The addition of sodium chloride helps to minimize the tendency of chloroform aqueous mixture to form emulsions. The aqueous phase was further extracted with two additional 25-ml portions of chloroform and finally made up to 100 ml with chloroform for GLC analysis.

GLC analysis was achieved on a Perkin Elmer model 3920 B with flame ionization detector. The column was constructed of 6 ft × 2 mm ID stainless steel column packed with 5% stabilized DEGS (Analabs, USA) on 100–120 mesh gas chrom.

The chromatographic conditions were as follows: column temperature 210°, injection port temperature 220° and detector temperature 250°, carrier gas (nitrogen) flow rate, 30 ml/min.

The repellent concentrations were calculated by comparing the peak height obtained for the formulation sample with the peak height for a standard.

RESULTS AND DISCUSSION

A comparison of minimum effective dose (MED) and protective time (PT) for various concentration of DEPA and DMP in ointment and talcum base formulations are given in Table I. The results indicate that macrogol ointment and talcum base formulations of DEPA are more effective as compared to DMP when tested against *A. aegypti* mosquitoes in the laboratory. The effectiveness was concentration-dependent. Macrogol ointment containing 25% DEPA showed protection for more than 8 h as compared to 3 h in respect of DMP.

Yap (10) reported the effectiveness of a soap formulation containing 20% DEET and 0.5% permethrin, offering protection for 4 h against various species of mosquitoes. It clearly indicates that macrogol ointment containing 25% DEPA is more effective than the soap-based DEET and permethrin combined formulation. The protection offered by 25% DEPA base macrogol ointment was the same as that offered by stearic acid base ointment prepared by us (7). However, the authors are of the opinion that macrogol ointment may be specially suitable for use in cold and dry climates.

Dust formulation of insecticides/repellents have been used topically for the control of insects of public health importance (11). The present study indicates that talcum base 25% DEPA formulation offered protection for 5 h as compared to 1 h 30 min by DMP against *A. aegypti*. The talcum base formulation in addition to its normal antiperspirant properties will also act as a mild insect repellent, especially in hot and humid climates.

After close examination of the skin of albino rabbits, it was found that DEPA/DMP incorporated ointment and talcum did not cause any gross adverse effect to the skin of rabbits in respect of erythema, oedema and dryness and the score was observed as 0. Besides, the active

ble I. Repellency of DEPA and DMP incorporated ointment and talcum base formulations to *Aedes aegypti*

	Macrogol ointment				Talcum base			
	DEPA		DMP		DEPA		DMP	
	MED g/150 cm^2	PT (h)	MED g/150 cm^2	PT (h)	MED g/150 cm^2	PT (h)	MED g/150 cm_2	P (h
10	0.1	220	0.25	90	0.1	225	0.225	5
15	0.075	330	0.20	120	0.075	250	0.20	6
20	0.05	390	0.15	150	0.05	255	0.175	8
25	0.025	480	0.1	180	0.025	310	0.15	9

MED = minimum effective dose; PT = protection time.

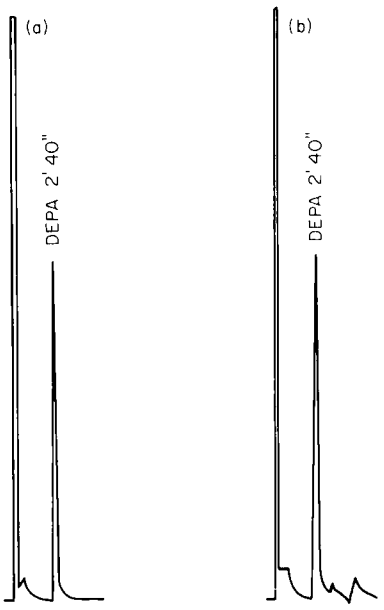

Figure 1. (a) Typical GLC of DEPA; (b) formulation sample.

ingredient, DEPA, used in the formulation does not show any adverse effect on the skin of human volunteers following topical application in trials.

During chromatographic analysis triplicate determinations were made for each formulation yielding average recovery for DEPA of 99.95% with a relative mean deviation of 0.85.

The recoveries of the added standard for all the formulations indicate that there was no interference from other ingredients present in the sample. Typical chromatograms showing DEPA and extracted DEPA from the formulations are given in Figure 1 a and b, respectively.

To test for stability at room temperature, standard formulations were stored for 6 months at room temperature. The results of an assay on this formulation indicated 99.3% of the initial concentration of DEPA remained. In addition, the DEPA has been found stable for more than 2 years under different temperature conditions.

The cost analysis has indicated that DEPA is much cheaper than DEET because the starting material is indigenously available, and in respect of repellency it is equally or more effective than DEET against mosquitoes and other insects of public health importance (2, 3). Keeping in view the merits of DEPA described and the toxicological data published elsewhere (4, 5), the Drug Controller of India has issued a manufacturing licence for use of DEPA for topical application on human skin which will be available commercially very soon.

CONCLUSION

The efficacy of a new multi-insect repellent, N,N-diethylphenyl acetamide (DEPA) incorporated macrogol ointment and talcum formulation was studied against *A. aegypti* in the laboratory. The effectiveness was compared with dimethyl-phthalate (DMP). Macrogol ointment containing 25% DEPA offered protection for 8 h as compared to 3 h by DMP. Talcum base formulation of DEPA showed 5 h protection as compared to 1 h 30 min in respect of DMP.

These formulations did not show any gross adverse effect to the skin of albino rabbits following topical application. Stability studies showed that 99.3% DEPA of the initial concentration remains after 6 months of storage at room temperature.

ACKNOWLEDGMENT

The authors are thankful to Dr P. K. Ramachandran, Director, Defence R. & D. Establishment, Gwalior for keen interest in the work.

REFERENCES

1. Kalyanasundaram, M. A preliminary report on the synthesis and testing of mosquito repellents. *Ind. J. Med. Res.* **76** 190–194 (1982).
2. Santosh Kumar, Shri Prakash, Sharma, R. K., Jain, S. K., Kalyanasundaram, M., Swamy, R. V. and Rao, K. M. Field evaluation of three repellents against mosquitoes, blackflies and land leeches. *Ind. J. Med. Res.* **80** 541–545 (1984).
3. Mathur, K. K., Gupta, G. P., Kaushik, M. P., Sikdar, A. K. and Rao, K. M. Laboratory evaluation of Diethyl phenyl acetamide and other insect repellents against rat flea *Xenopsylla cheopis*. Rothchild (Siplinopters. Publicidae). *Ind. J. Med. Res.* **83** 466–470 (1986).
4. Rao, S. S., Shri Prakash, Santosh Kumar, Kaveeshwar, U., Bhattacharya, B. K., Jaiswal, D. K. and Rao, K. M. Toxicologic studies of an insect repellent N,N-diethyl-phenylacetamide. *Ind. J. Med. Res.* **85** 626–633 (1987).
5. Shri Prakash, Rao, S. S., Santosh Kumar & Rao, K. M. Studies on the effect of N,N-diethyl phenyl acetamide—an insect repellent on toxicity and reproduction in rats. *Ind. J. Pharm. Sci.* Nov-Dec, 220–222 (1987).
6. Kalyanasundaram, M., Amalraj, D., Parley, K. P., Nisha, G. and Suigatha, C. S. Synthesis of substituted amides for repellency against mosquitoes. *Curr. Sci.* **55(5)** 266–268 (1986).
7. Sharma, R. K., Jain, S. K., Santosh Kumar and Rao, K. M. Evaluation of some insect repellent formulations. Part I. Repellency against mosquitoes. *Ind. J. Hosp. Pharm.* **21** 26–29 (1984).
8. Draize, J. H., Woodward, G. and Calvery, H. O. Methods for the study of irritation and toxicity of substances applied topically to the skin and membranes. *J. Pharm. Exp. Ther.* **82** 377–390 (1984).
9. Code of Federal Regulations, USA, title, 16 (1976) Part 1500. 41. In *Advances in Modern Toxicology*, Vol. 4, pp. 196–197. *Dermatotoxicology and Pharmacology*. Hemisphere Publishing Corporation, Washington (1977).
10. Yap, H. H. Effectiveness of soap formulations containing DEET and permethrin as personal protection against outdoor mosquitoes in Malaysia. *J. Am. Mosq. Control. Assoc.* **2(1)** 63–67 (1986).
11. Nassif, M., Brooke, J. P., Hutchinson, D. B. A., Kamel, O. M. and Savage, E. A. Studies with permethrin against body lice in Egypt. *Pest. Sci.* **11** 679–684 (1980).