

Development of a controlled release formulation of an indigenous insect growth regulator, DPE-28, a substituted diphenylether, for controlling the breeding of *Culex quinquefasciatus*

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Background & objectives: DPE-28, a substituted diphenyl ether (2,6-ditertiarybutyl phenyl-2',4'-dinitro phenyl ether) was reported to exhibit promising insect growth regulating activity against *Culex quinquefasciatus*, the vector of lymphatic filariasis. A controlled release formulation (CRF) of DPE-28 has been developed to control *Cx. quinquefasciatus* in its breeding habitats. Toxicity of DPE-28, safety to non-target mosquito predators and the release profile of the CRF of DPE-28 are studied and discussed.

Methods: The acute oral and dermal toxicity was tested in male and female Wistar rats as per the Organization for Economic Cooperation and Development (OECD) guidelines 425 and 402 respectively. The toxicity of DPE-28 to non-target predators was tested as per the reported procedure from this laboratory. The CRF of DPE-28 was prepared by following the reported procedure developed at this laboratory earlier. The concentration of DPE-28 released from the CRF was monitored by HPLC by constructing a calibration graph by plotting the peak area in the Y-axis and the concentration of DPE-28 in the X-axis.

Results: DPE-28 has been tested for acute oral toxicity and found to be moderately toxic with LD₅₀ value of 1098 mg/kg body weight (b.w). The results of the acute dermal toxicity and skin irritation studies reveal that DPE-28 is safe and non-irritant. DPE-28 when tested at 0.4 mg/litre against non-target mosquito predators did not produce any mortality. The release profile of the active ingredient DPE-28 from the CRF by HPLC technique showed that the average daily release (ADR) of DPE-28 ranged from 0.07 to 5.0 mg/litre during first four weeks. Thereafter the matrix started eroding and the ADR ranged from 5 to 11 mg/litre during the remaining 5 wk. The cumulative release of active ingredient showed that > 90 per cent of the active ingredient was released from the matrix.

Interpretation & conclusions: The controlled release matrix of DPE-28 was thus found to inhibit the adult emergence (>80%) of *Cx. quinquefasciatus* for a period of nine weeks. The CRF of DPE-28 may play a useful role in field and may be recommended for mosquito control programme after evaluating the same under field conditions.

Key words Controlled release formulation - diphenylether - DPE-28 - insect growth regulator - mosquito larval control

The environmental hazards posed by conventional mosquito larvicides coupled with the development of resistance in mosquito vectors to some of these insecticides warrant an alternate approach in the chemical control of vectors. The insect growth regulators (IGRs) represent a major breakthrough in insecticide chemistry and much work has been done during the past three decades. IGRs are generally classified into two groups¹ according to the mode of action. Some compounds are known to inhibit the formation of chitin, which is required for moulting from one stage into the next stage and these compounds are known as chitin synthesis inhibitors and juvenoids are the analogues of juvenile hormones that inhibit pupation.

A large number of synthetic chemicals possessing insect growth regulating property have been found to be promising in controlling insects of public health importance, such as mosquitoes, chironomid midges and pestiferous flies²⁻⁴. Compounds belonging to various chemical classes such as amides⁵, oxime ethers⁶ and carbamates⁷ have been earlier shown to possess promising insect growth regulating activity against various mosquito vectors. IGR activity against scale insects was studied with a phenoxyphenoxy carbamate⁸. A pyridyl terpenoid ether has also been studied for IGR activity against mosquitoes⁹. Pyriproxifen [4-(phenoxyphenyl)-(RS)-2-(2-pyridyloxy) ethyl ether] has been evaluated as a 0.5 granular (GR) formulation in field for controlling the breeding of *Cx. quinquefasciatus*¹⁰. Recently, a benzoylphenylurea derivative, novaluron¹¹, was reported to have promising IGR activity for controlling *Cx. quinquefasciatus* in various breeding sites.

Earlier, the structure-activity relationships in diphenylethers for insect growth regulating activity against mosquitoes were studied and one of the compounds, DPE-28 (2,6-ditertiarybutyl phenyl-2',4'-dinitro phenyl ether) was found to be highly effective against *Cx. quinquefasciatus* with EC₅₀ and EC₉₀ (concentration to inhibit 50 and 90% of adult emergence) values of 2.2×10^{-3} and 1.16×10^{-2} mg/litre respectively¹². It was earlier reported¹³ that the treatment of mosquito eggs with an EC₅₀ dose of DPE-28 caused mortality while an EC₉₀ dose completely ceased adult emergence. There was a significant reduction in the fecundity and fertility rates of emerged mosquitoes when treated with EC₅₀ dose of DPE-28. Studies on the effect of the juvenoid, DPE-28 on the biology and behaviour of *Cx. quinquefasciatus* showed that the developmental duration, sex ratio,

mating success and blood feeding were considerably affected by the exposure of larvae and pupae to this compound¹⁴. The present investigation deals with studies on the toxicological data, safety to various non-target organism and development of an environment-friendly controlled release formulation¹⁵ and the release kinetics of the active ingredient from the matrix under simulated conditions.

Material & Methods

Synthesis of the diphenylether DPE-28: The diphenylether, DPE-28 was synthesized by a procedure reported from this Centre earlier¹².

Toxicological studies on DPE-28: Toxicological studies on DPE-28 were carried out at International Institute of Biotechnology and Toxicology (IIBAT), Padappai, Tamil Nadu after obtaining the approval of the Ethical Committee of IIBAT. The acute oral toxicity of DPE-28 was tested in female Wistar rats by following the Organization for Economic Cooperation and Development (OECD) guideline 425¹⁶. In main test dose progression 1.75, 5.5, 17.5, 55, 175, 550 and 2000 mg/kg b.w. doses were selected as per the guideline. Following dosing, the rats were observed for 14 days for mortality and clinical signs of toxicity. Bodyweight gains of the survived animals were noted weekly. A gross necropsy was performed on all animals that died during the study and on all survivors on day 14.

The acute dermal toxicity of DPE-28 was tested in male and female Wistar rats as per the OECD guideline 402¹⁷. Appropriate weight of the test substance was evenly applied at the dose of 2000 mg/kg b.w. to the clipped area of skin of the animals. Following dosing, the rats were observed for 14 days for mortality and clinical signs of toxicity. Bodyweight gain was noted. All animals were necropsied at the end of the 14-day observation period.

The primary skin irritation potential of DPE-28 was tested in female New Zealand white rabbits as per OECD guideline 404. A quantity of 0.5 g of the test substance was moistened with minimum volume of distilled water so as to prepare a paste. The paste thus prepared was evenly applied to the clipped area of the skin of the rabbits under a gauze patch. At the end of 4 h, the gauze patch was removed and application site was wiped with water without altering the integrity of the epidermis to remove the residual test substance. The experiment was repeated to confirm the non-irritant response of the test substance by using two additional animals. The animals were observed for erythema

and oedema at 60 min, 24, 48 and 72 h following the removal of the gauze patch.

Effect on non-target organism: The effect of DPE-28 at 0.4 mg/litre (> 30 times the EC_{90} value of the compound to *Cx. quinquefasciatus*) was studied on non-target mosquito predators like, fish (*Gambusia affinis*), water bug (*Diplonychus indicus*) adults, dragon fly (*Anisoptera* species) nymphs, water scorpion (*Nepa* species) adults and tadpole (*Rana hexadactyla*) by following the reported procedure from this laboratory¹⁸. One predator was released into 250 ml disposable cup containing 100 ml tap water. Only one predator was used so as to avoid cannibalism. Ten replicates were performed for the test concentration along with four replicates of untreated controls. The predators were observed for mortality after every 24 h exposure period for 72 h.

Controlled release formulation of DPE-28: A 20 per cent emulsifiable concentrate (EC) was made by using xylene as solvent and tween 80 as emulsifier and used for development of a controlled release formulation. An aqueous (20%) slurry of sodium carboxymethylcellulose (NaCMC) (Commercial grade, as a 10% aqueous slurry gives a viscosity of 65 dPa at 28°C) was made homogeneous by stirring with 1 per cent expanded polystyrene beads as floating agent and required quantity of 20 per cent EC. The quantity of EC was adjusted in such a way that each pellet would contain 1 g of the active ingredient (ai). The slurry was transferred to special moulds for making the pellets crosslinking with an aqueous solution of aluminium sulphate (LR grade, 0.05M) with pH adjusted of 3.2 using 50 per cent NaOH for 48 h (Effective crosslinking period). The gelling medium may be reused for next crosslinking. The pellets were washed with water to remove the remaining gelling medium and dried either at room temperature for one to two weeks or at 50-55°C in a vacuum oven for 1 - 2 days. Controlled release matrices without DPE-28 were also made to serve as control matrices.

HPLC analysis for determining the concentration of DPE-28: HPLC system (ThermoFinnigan, CA) composed of Spectra System P4000 solvent delivery system, Spectra System AS3000 autosampler and Photodiode array (PDA) detector SN4000 was utilized. The output signals were monitored with ChromQuest 4.0 Chromatography workstation. HPLC analysis for DPE-28 was carried out under the following chromatographic conditions – acetonitrile / water (70:30) with 0.01 per cent phosphoric acid adjusted to

pH 3.2 and at a flow rate of 1.0 ml/min. A Supelcosil C8 column (15 cm x 4.6 mm) of 3 μ m particle size was used for the analysis. Analysis was done at UV 270 nm and 40°C.

Release of DPE-28 from the controlled release matrices: One controlled release matrix containing DPE-28 was released in 1.0 litre of water taken in a 2-litre beaker. Five replicates were set up for matrices with DPE-28 and five replicates were set up with matrices without DPE-28 to serve as control. Water was changed at daily intervals. Everyday 500 ml of water was withdrawn for conducting bioassay (two replicates with 250 ml for each replicate) against early third instar larvae of *Cx. quinquefasciatus* obtained from the cyclic colony maintained at the Centre. About 15 ml of water sample was withdrawn from each replicate, extracted with 25 ml of dichloromethane in a 50 ml separating funnel and the bottom layer was withdrawn and dried over anhydrous magnesium sulphate. This was filtered through a Whatman filter paper No.1 and taken in a 50 ml test tube and the solvent was removed in a water bath. The residue was taken up in 1 ml of acetonitrile (HPLC grade) and taken in the HPLC sample injection vial.

Results & Discussion

Acute oral toxicity: None of the animals died in groups treated with DPE-28 with 175 and 55 mg/kg b.w. for acute oral toxicity during the study period, whereas four animals died in groups treated with 2000 mg/kg b.w. The survived animals showed normal body weight gain on days 7 and 14 compared with the zero day body weight. Gross pathology examination conducted on survived animals at the end of the experimental period and animals that died during the study did not reveal any macroscopic lesion that could be attributed to the toxicity of the test material, DPE-28. Based on the experimental results, estimated acute oral LD_{50} of DPE-28 for Wistar rat (female) was 1098 mg/kg b.w.

Acute dermal toxicity: No clinical signs of toxicity or mortality were observed in animals treated with DPE-28 at the dose of 2000 mg/kg b. w. throughout the observation period. All the animals showed a normal trend in the body weight gain on days 7 and 14. No compound related lesions were observed under gross pathological examination. From the experimental results, acute dermal LD_{50} of DPE-28 for Wistar rat was considered as >2000 mg/kg b.w.

Primary skin irritation test: None of the animals exhibited any skin reaction at any of the time points

of observation. Thus, the primary skin irritation index was calculated as zero. Hence it is concluded that DPE-28 was non-irritant to the skin of New Zealand white rabbits under the experimental conditions tested.

The results of the acute oral toxicity of DPE-28 to Wistar rats suggest that this compound can be considered as a moderate toxic material based on the classification of World Health Organization¹⁹. The results of the dermal toxicity and skin irritation studies reveal that this compound is safe and non-irritant.

Effect on non-target organisms: DPE-28 when tested at 0.4 mg/litre against mosquito predators, fish (*Gambusia affinis*), water bug *Diplonchus indicus* (adults), dragon fly (*Anisoptera* species) nymphs, water scorpion (*Nepa* species) adults and tadpole (*Rana hexadactyla*) did not produce any mortality. DPE-28 was found to be non toxic to all the five predators at a concentration of 0.4 mg/litre which is more than 30 times its EL_{90} value to the target mosquito vector, *Cx. quinquefasciatus*.

Standardization of DPE-28 and release profile of DPE-28 from the CRF by HPLC: HPLC analysis for DPE-28 revealed that there was a peak for DPE-28 with a retention time of 5.84 min (Fig. 1). A calibration curve (Fig. 2) was constructed by plotting the peak area in the Y-axis and the concentration of DPE-28 in the X-axis. The unknown concentrations were obtained by solving the linear regression equation for the calibration curve $y = 22044x + 34582$.

The release profile of the active ingredient DPE-28 from the controlled release matrix giving the average daily release (ADR) in mg/litre, cumulative release of the active ingredient at weekly intervals is given in Fig. 3. From the results, it was inferred that

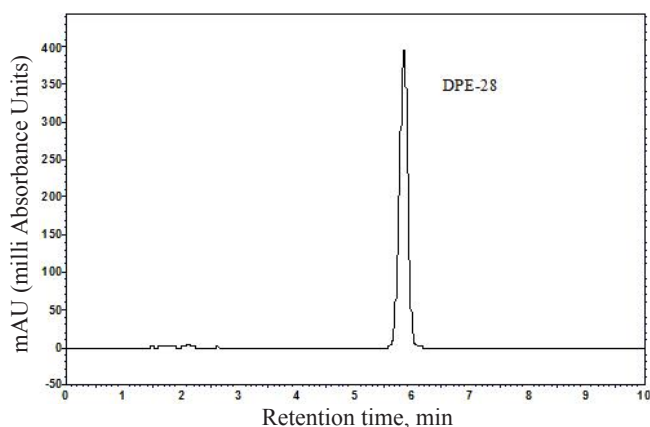


Fig. 1. HPLC chromatogram of DPE-28.

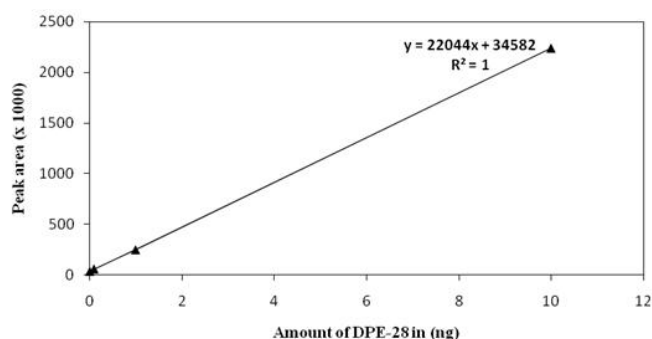


Fig. 2. Calibration graph for the estimation of DPE-28.

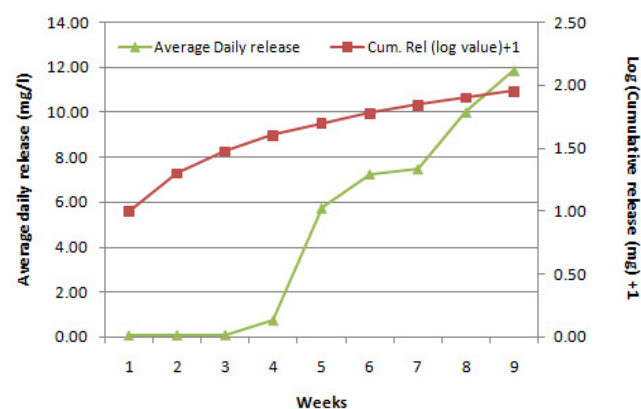


Fig. 3. Release profile of DPE-28 from the controlled release matrix.

the average daily release of DPE-28 ranged from 0.07 to 5.0 mg/litre during first four weeks. Thereafter the matrix started eroding and the ADR ranged from 5 to 11 mg/litre during the remaining 5 wk. The matrix thus could inhibit the adult emergence (>80%) of *Cx. quinquefasciatus* during the period of nine weeks. The cumulative release of the active ingredient showed that > 90 per cent of the active ingredient was released from the matrix. This observation of a CRF exhibiting inhibition in adult emergence for a period of more than two months could be compared to the efficacy of methoprene, a widely used juvenile hormone mimic, formulated as 30-day slow release Altosid pellets for controlling mosquitoes in underground storm water drainage catch basins. The results showed that methoprene applied at 3.5 g of the pellet per basin could effectively control mosquitoes for a month under field conditions²⁰. Similar observation was also made during the development of controlled release matrices for triflumuron, an IGR which were stable for 14 to 16 wk²¹. Thus, the use of controlled release formulations will be more practical and desirable and may make the Programme more cost-effective.

The treated larvae and pupae showed various abnormalities. Larvae were usually dechitinized, elongated and attached to the previous moult. Mortality in the larval-pupal intermediate was prominent with DPE-28. The pupae formed after treatment became pale resulting in mortality. Considering the effect of the CRF of DPE-28 in inhibiting the adult emergence of *Cx. quinquefasciatus* for about nine weeks, its safety to non target aquatic predatory organisms tested and the other toxicological data, the CRF of DPE-28 may play useful role in field and may be recommended for mosquito control programme after evaluating the formulation in controlling *Cx. quinquefasciatus* in its breeding habitats.

Conflicts of interest: Authors declare that there is no conflict of interest.

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