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## Sensitivity and accuracy of a bio-assay for the determination of the concentration of residual pesticide in natural water bodies

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Determination of pesticide levels in natural water bodies requires complex chemical methods not always available in tropical countries where such control programmes are implemented. Apart from chemical methods, effectiveness of treatment can be estimated by mortality of target and non-target species. But the latter criteria do not permit a good appraisal of dispersion and residual activity of pesticide. We propose here a simple and cheap bio-assay adaptable to water-borne vector or intermediate host control programmes. Results obtained with the bio-assay were compared with those obtained with gas liquid chromatography, a standard chemical method.

Key words: Pesticide, bioassay; Temephos; Vector control

### Material and Methods

We used two formulations of temephos (Abate<sup>R</sup>, Cyanamid): Abate 200-EC, a 20% (v/v) emulsifiable concentrate and Abate 5-CG, a 5% (v/v) clay-based granular form. Both were applied on the same day in similar ponds containing about 5 m<sup>3</sup> of water. 5 ml of the liquid form and 187 g of the granular form were applied per m<sup>3</sup>.

The bio-assay was based on the toxicity of temephos to *Aedes aegypti* larvae. A standard value of the LD<sub>50</sub> (=0.5 p.p.b.) was determined, as described by W.H.O. (1975), before the bio-assays and confirmed after them. Third instar mosquito larvae were dispatched in batches of 30 individuals. Each batch was placed in a 200 ml disposable cup of distilled water with various dilutions of pesticide. Four batches were tested with each dilution. All results were recorded after 24 h contact. The mortality rate was corrected by Abbott's formula (Abbott, 1925) and plotted on log-probit graph paper.

The toxicity of the treated pond water was checked by a similar method. After treatment, samples of pond water were diluted and four batches of 30 larvae were placed in 4 × 200 ml samples of each dilution. 1/2, 1/4, 1/5, 1/50, 1/100, 1/200, 1/400,

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1/500, 1/1000 and 1/2000 dilutions of pond water sampled were used. Controls consisted of both pure untreated pond water and the distilled water employed for dilutions. After 24 h exposure, the mortality rates were plotted on log-probit graph paper. The  $LD_{50}$  in term of dilutions of pond water was expressed in terms of the standard  $LD_{50}$  for temephos determined as described in the previous paragraph. The pond water was sampled every two days after the day of application, the first samples being taken three hours after temephos application.

The chemical determination of temephos was performed with the same samples of pond water used for the bio-assays. The method used was gas liquid chromatography (g.l.c.).

A Philips PU 4500 gas chromatograph apparatus equipped with a flame ionisation detector and a glass column packed with 2% SE 30 on Chrom Q, 100–120 mesh, I.D. = 4 mm, 1.5 m length, carrier gas nitrogen, passed through an oxygen trap (Oxysorb<sup>R</sup>) and moisture trap (molecular sieve) was used. Flow rates were: nitrogen  $33 \text{ ml.min}^{-1}$  at a pressure of 1.2 atm, hydrogen  $30 \text{ ml.min}^{-1}$  at a pressure of 1.4 atm, air =  $300 \text{ ml.min}^{-1}$  at a pressure of 0.8 atm. Oven temperature was regulated to  $260^\circ\text{C}$  and the detector injector temperature to  $270^\circ\text{C}$ . The volume injected onto the column was  $0.5 \mu\text{l}$ . Retention time was about 20 min. Calculations were made using peak-height. Values used for calculation were the mean values of four consecutive injections.

500 ml of pond water was extracted with freshly distilled hexane ( $3 \times 80 \text{ ml}$ ). The organic layers were recombined, washed with 20 ml of saturated NaCl solution, then dried in anhydrous  $\text{Na}_2\text{SO}_4$ . The sodium sulphate was then washed with 40 ml hexane. The organic layers were recombined and vacuum distilled. The residue was dissolved in the minimum volume of diethyl ether and transferred with a pipette into a high ml conical tube fitted with a teflon-lined screw cap. This operation was repeated twice to ensure near total recovery of the residue. The solvent was evaporated and the residue dissolved ultrasonically in  $200 \mu\text{l}$  of benzene. The sample was then ready for g.l.c. and stored at  $-20^\circ\text{C}$ . Known amounts of technical grade temephos, dissolved in a few ml of acetonitrile, were added to 500 ml of distilled water and extracted as described above. Recovery from extraction ranged from about 95% at 2000 p.p.b. to about 70% at 50 p.p.b., the lowest concentration.

## Results and Discussion

Figs. 1 and 2 compare respectively the results of the EC-200 formulation titration and those of the 5-CG formulation titration by bio-assay and g.l.c. With both g.l.c. and bio-assay the relationship of  $\log C$  to time appeared to be linear. The EC-200 formulation correlation rates were  $-0.984$  ( $P < 10^{-3}$ ) for bio-assay and  $-0.97$  ( $P < 10^{-2}$ ) for g.l.c., whereas the 5-CG formulation correlation rates were  $-0.983$  ( $P < 10^{-3}$ ) for bio-assay and  $-0.997$  ( $P < 10^{-3}$ ) for g.l.c. The similarity of the slopes for each series of samples indicates the reliability of bio-assay. However, the g.l.c. determination lines were shifted to slightly higher values. A possible explanation is that the pond water contained algae and colloidal materials which were able to bind or to dissolve some of the temephos. Hexane was able to extract the whole temephos whereas mosquito larvae would be mainly sensitive to temephos in the aqueous phase.

Bio-assay sensitivity is as low as 10 p.p.b. and residual toxicity could be detected

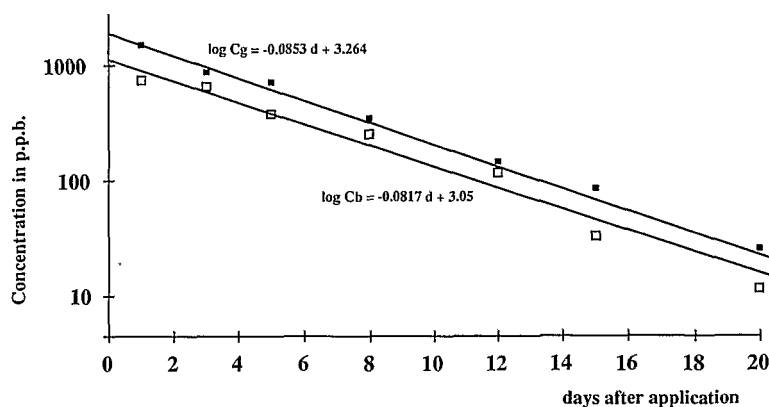


Fig. 1. Comparison between gas liquid chromatography (g.l.c., ■) and bio-assay (□) tests for determination of the concentration of residual 20% emulsifiable concentrate temephos in pond water.  $C_g$  = concentration determined by g.l.c. and  $C_b$  = concentration given by bio-assay.

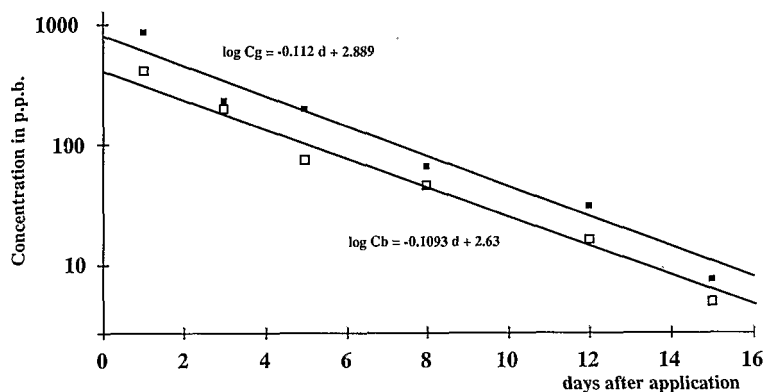


Fig. 2. Comparison between gas liquid chromatography (g.l.c., ■) and bio-assay (□) tests for determination of the concentration of residual 5% clay-based granular temephos in pond water.  $C_g$  = concentration determined by g.l.c. and  $C_b$  = concentration given by bio-assay.

even below this concentration but with a lower accuracy. The sensitivity of the g.l.c. method used was limited to about 50 p.p.b. mainly because of nearby interfering peaks.

The bioassay is practical in field conditions. *A. aegypti* larvae are easy and cheap to breed. This method allows determination of the concentration of pesticide in natural water bodies, but its accuracy and its sensitivity is related to the mosquito stock and particularly to its  $LD_{50}$  value. Nevertheless the use of this bio-assay gives a valuable appraisal of the effectiveness of a pesticide treatment, as well as its dispersion and residual activity.

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