Evaluation of Cholinesterase Level in an Endemic Population Exposed to Malathion Suspension Formulation as a Vector Control Measure

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The manuscript describes a study on the blood cholinesterase (ChE) level in an exposed population at different interval of time after spraying with malathion suspension (SRES) use for kala-azar vector control in an endemic area of Bihar, India. The toxicity of a 5% malathion formulation in the form of a slow release emulsified suspension (SRES) was assessed by measuring serum ChE levels in spraymen and in the exposed population. The study showed a significant decrease in ChE levels in the spraymen ($p < 0.01$) after one week of spraying and in exposed population one week and one month after of spraying ($p < 0.01$), but was still within the normal range of ChE concentration, one year after spraying, the ChE concentration in the exposed population was the same as prior to spraying ($p > 0.01$). On no occasion was the decrease in ChE level alarming. A parallel examination of the clinical status also showed the absence of any over toxicity or any behavioural changes in the exposed population. Hence, it may be concluded that 5% malathion slow release formulation, SRES, is a safe insecticide for use as a vector control measure in endemic areas of kala-azar in Bihar, India so long as good personal protection for spraymen is provided to minimize absorption and it can substitute the presently used traditional DDT spray.

Key words: SRES-slow release emulsified suspension - ChE-cholinesterase - kala-azar - *Phlebotomus argentipes*

Kala-azar is an important public health problem in Bihar, India for over two decades. Out of an annual global estimate of 40 million cases, this Northeastern part of India alone contributes to the highest proportion. Of 343,023 cases reported in Bihar during 1991-1998, 5365 cases succumbed to the disease. Programme for control of kala-azar vector in this region is presently made by DDT spray. Reports of resistance to DDT are being reported from several parts of Bihar (Mukhopadhya et al. 1990, Basak & Tandon 1995) and hence an alternative to DDT has to be looked into. Malathion was first introduced into the National Malaria Eradication Programme (NMEP) during 1969-1970 for residual spraying in houses in Gujarat state of India to tackle the malaria problem involving a doubly resistant (to both DDT and Dieldrin) *Anopheles culicifacies* as the chief malaria vector. Since then, the malathion spraying has been carried out in a number of areas in the country where chlorinated hydrocarbon insecticides were not found to be very effective. For example, during 1988-1989, areas in the six states Maharastra, Gujarat, Andhra Pradesh, Karnataka, Haryana, and Punjab and one union territory of Dadar & Nagar Haveli were being sprayed with malathion-based insecticide protecting a population of over 39 million. Malathion slow release emulsified suspension was successfully used in the control of *Lutzomyia longipalpis* in Brazil (Oliveira Filho & Mello 1994). Since, malathion had already been used elsewhere for vector control measure, it was decided to introduce malathion as an alternative to DDT and test its efficacy as well as its toxicity, if any, in the strategic control programme in Bihar state where kala azar is endemic and a major public health problem. A pilot study was conducted in an endemic village of Patna district as test case. Malathion, O, O-dimethyl S-1, 2-bis ethoxycarbonyl phosphorodithioate, is one of the most widely used organophosphorus pesticides in India. Exposure of the general population to the pesticide may occur through the consumption of food stuffs treated incorrectly with malathion or from contact with treated areas during domestic application or by drift from aerial spraying. Like other organophophorus compounds, malathion is a known ChE inhibitor. The immediate toxic effects of malathion are well described; the long-term effects are yet to be clearly defined and are at least controversial (Banerjee et al. 1998). Studies on long-term low-level malathion exposures have shown sub-clinical effects on both central and peripheral nervous system (Banerjee et al. 1998). Hence, risk assessments of subchronic malathion exposure in the general population is of interest (Banerjee et al. 1998). During the spray period, the spraymen get continuous exposure of the insecticide. The exposure is chiefly by two routes; firstly by inhaling a small quantity during room spraying and secondly through skin absorption while handling. It is essential to protect the spraymen from excessive exposure of SRES malathion and monitor their blood/serum ChE. In the present article, the monitoring data of ChE level in spraymen and the exposed population are presented and discussed at different intervals after exposure. To assess the clinical outcome among spraymen and the exposed population, a paralled exami-

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nation of the clinical status at different interval of exposure was carried out keeping in view its future implementation as an alternate effective large scale anti kala-azar vector measure in Bihar. This paper is thus relevant to the Public Health Programme.

MATERIALS AND METHODS

Study area - Long acting malathion suspension (5%) in the form of a slow release emulsified suspension, brand name "Duration M" supplied through Brazil under a WHO/TDR project, was sprayed in an endemic village, Gulamahiyabagh, in the Patna district of Bihar state. This village is situated 17 km away from the Institute on the south bank of the river Ganges. The total population of the village was 1235 residing in 204 households. This village has been endemic for kala-azar for the last ten years or more.

Sample size for assessment of ChE level - It was anticipated that if malathion suspension reduces ChE level of spraymen and of the exposed population by no more than 25% of the original ChE level before exposure, then this insecticide could be assumed to be safe for further application. The normal range of ChE level in man is 3200-7700 U/L. For the study population the normal ChE level prior to malathion spray was first assayed on a minimum sample (n = 30) of individuals in the village. Based on this the sample size for the assessment of ChE level was calculated as 42 at $\alpha = 0.05$ type I error and $\beta = 0.10$ type II error with 90% confidence.

Sampling procedure - A stratified random sampling method was adopted for selecting individuals from each age group i.e. 0-5, 5-15, 15-25, 25-35, above 35.

Blood collection procedure - Informed consent was obtained for taking the blood samples. Venous blood (2 ml) was collected from spraymen (n = 6) and from a sample of the population exposed in the study area before the malathion spray and one week after spray to assess the immediate effect. The collected blood samples were immediately stored in the icebox and transported to the laboratory for processing. One month and 12 months after spraying blood samples were again collected from the same individuals to assess medium and long-term effects of malathion. In the course of blood collection in the field, some individuals voluntarily came forward for the test, which resulted in an increase of the sample size.

Working conditions of the spraymen - The spraymen wore masks and gloves during spraying of the malathion suspension to avoid the absorption of insecticide. After spray work was over they washed their hands with soap and detergents for personal protection.

Reagent - The serum ChE level was monitored adopting colorimetric procedure based on the Ellman reaction using Sigma Diagnostic Cholinesterase (BTC) reagent, US.

Statistical analysis - All the experiments were carried out in duplicate and the average was used for determination of the mean ± SD 95% confidence range of the ChE level. Z-statistic was used to estimate the alteration of the mean ChE level between the value before spray and after 1 week, before spray and after 1 month, and before spray and after 1 year. Student's paired t-test was used for comparing ChE levels of spraymen between before spray and after 1 week. Analysis of variance (ANOVA) was also used to see the difference in mean ChE level of exposed population for all occasions the F-ratio being calculated by 2-sample comparison of variance arising within samples and between samples. EPI-Info, Ver.-6 was used for all statistical analysis.

RESULTS

Serum ChE level among the spraymen and exposed population was assessed at different points of time. The mean ChE level of spraymen after 1 week was approximately 83% of its original value i.e. prior to spray. Thus the immediate effect showed a statistically significant ($p < 0.01$, t-statistic) decrease in ChE level (Table I), but it was within normal range. The mean ChE level of the exposed population before spray, 1 week after, 1 month after also showed a decrease in ChE level (Table II) that was as statistically significant ($p < 0.01$, Z-statistic) but within normal range. On these occasions the mean ChE level

TABLE I

Immediate effect of malathion paint formulation on the cholinesterase (ChE) level of sprayman

ChE level(U/L) (Mean SD)

Group	Before spray	After 1 week	p value [a]
Spraymen (n = 6)	7033.67 ± 1768.01	5824.17± 1461.04	< 0.01

a: paired student's t-statistics

TABLE II

Effect of malathion paint formulation on cholinesterase (ChE) level (U/L) of exposed population

Point of observations	n	ChE level (mean ± SD)	95% CI of ChE	p-value [a]	ChE level (%)
Before spray	56	6278.89 ± 1660.86	5853.76-6704.2		
After 1 week	56	4971.26 ± 1777.04	4516.07-5425.9	< 0.01	79
After 1 month	64	5136.55 ± 19.057	4683.31-5590.7	< 0.01	82
After 1 year	42	6250.43 ± 2093.22	5627.40-6872.3	> 0.01	100

a: Z-statistic for comparing between a/b, a/c, a/d

was 79% and 82% of the ChE level prior to spray. No significant difference was observed in mean ChE level one year after spraying as compared to before spray ($p > 0.01$, Z-statistic). Again analysis of variance (ANOVA) was used (Table III). The difference of the mean ChE levels observed showed significance ($p = 0.000007$, F-ratio = 9.41 at 3,214 d.f.).

TABLE III
Analysis of variance (ANOVA)

Source of variation	d.f.	Mean sum of square	F-statistic	p-value
Between samples	3	32212174.0	9.41	0.000007
Within samples	214	3423405.74		

DISCUSSION

The present investigation was designed to evaluate the toxicity of a slow release malathion suspension formulation in an exposed population at different times of observation. Epidemiological studies suggest that exposure to organophosphate pesticides can induce effects on both the central and peripheral nervous system either after acute intoxication or as a result of low level long term exposure (Banerjee et al. 1998). Clinical studies have indicated that long-term low-level exposures to organophosphates induce significant changes in neurobehavioral patterns and constitute a potential health hazard for occupational workers (Banerjee et al. 1998). Attempts have been made to select exposure levels which did not produce overt toxicity to the exposed individuals.

In the present study a slow release malathion suspension formulation showed a decrease in ChE level in the spraymen just after exposure but this was well within normal range. After exposure the ChE level was found to be 82% of the normal ChE level of spraymen. Similarly, exposed individuals in the study area did not reveal any toxic level of ChE. After 1 and 4 week of exposure the ChE level reduces by 21% and 18% respectively in these exposed individuals. Their clinical status was examined during the study at different interval of time but no major abnormalities was observed. A few exposed individuals complained of cough with bronchitis during the spray period but after medication they were cured and there were no reports of any death to the exposed individuals over a period of one year. After one year of exposure the normal ChE of the exposed individuals had returned to the normal level and their clinical examination did not showed any major abnormalities. So on the basis of the findings of the present study, it can be concluded that the malathion formulation may be indicated as safe for use as a vector control measure and can be safely applied in the endemic area of kala-azar in Bihar so long as there is good personal protection for the spraymen during application.

The control of *Phlebotomus argentipes* the vector of kala-azar by SRES malathion is not only less cumbersome than DDT which requires two rounds for three consecutive years but it is also cost effective (Kishore et al. 2002).

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