

## Hardwar (Uttaranchal)

The field unit successfully demonstrated industrial malaria control at the Bharat Heavy Electricals Ltd. (BHEL). This work was expanded to control malaria in the Indian Drugs and Pharmaceuticals Ltd. (IDPL), Rishikesh and Indian Oil Corporation (IOC), Mathura. Industrial malaria control strategy has been transferred to the state government. In addition some of the outstanding contributions of the field unit are:

- A number of plants have been screened and chemicals purified with high insecticidal, repellent and antimalarial activities
- Residue analysis of insecticides in the environment demonstrating the high levels of contamination of almost all food and fibre
- Monitoring of drug resistance of *P. falciparum* and residues of antimalarials in the blood
- New techniques of residue analysis of insecticides and drugs were developed
- Discovery of an essential oil with outstanding insecticidal properties against mosquitoes

### Background

In the township of a major industrial complex, Bharat Heavy Electricals Limited (BHEL) in Hardwar, malaria cases were on the rise from 1983 to 1985 when 3,049 cases of malaria were recorded with SPR of 14.8%. There was about three-fold increase in *P. falciparum* within 2-years. The mosquito breeding habitats included low-lying areas, puddles, borrow pits and factory affluent pools in the 25 sq km premises of BHEL complex. The immediate challenge at BHEL complex was to control mosquito-genic conditions and to identify the transmission risk factors of malaria. Against this background, a field unit was opened under the IDVC project in BHEL complex in July 1986 to demonstrate control of malaria using the bioenvironmental malaria control strategy. The main objective was that once the technology of malaria control works at BHEL, it would be extended to other industrial complexes in the country.

### Activities, Progress and Achievements

#### Industrial malaria control at BHEL

The BHEL complex covers an area of 25 km<sup>2</sup> housing the main and ancillary industrial units, staff colonies and labour colonies. It has a 180-bed hospital with excellent medical facilities. The population in BHEL complex is about 70,000. *An. culicifacies*, *An. fluviatilis* and *An. stephensi* were the three known malaria vectors found in the area. *An. subpictus* was found in large numbers (31%) among all anophelines.

The main strategy to control mosquito-genic conditions was to fill and level mosquito breeding places. The civil maintenance department carried out major source reduction work by filling pits, low-lying areas, ditches, etc. with fly-ash from a coal-fired power station; construction of stand posts and proper drainage; mosquito proofing of overhead tanks; and preventive maintenance of the water supply and the sewage

system. The project staff applied expanded polystyrene (EPS) beads in underground tanks, leaking sluice valve chambers and blocked sewage manholes (Dua *et al.*, 1989), biolarvicides in water accumulation in factory scraps, blocked drains and riverbed pools (Dua *et al.*, 1993), and larvivorous fish in storm water drains, effluent ponds and drains (Dua and Sharma, 1994) for the effective control of mosquito breeding. Improved surveillance and treatment coupled with comprehensive developmental schemes were additional methods used to gain community support (Dua and Sharma, 1993). Due to these interventions, there was a sharp reduction in the density of mosquitoes and anopheline vectors (Fig. 1). Early case detection and proper treatment of cases further helped in the reduction of malaria incidence (Fig. 2). The shift to the

bioenvironmental control strategy for malaria control also led to a major reduction in the use of insecticides.

One of the highlights of this project was community involvement, including those from the low income groups and government and voluntary agencies, which made the vector control programme truly a people's movement toward self-help, health awareness, improved sanitation, and an improved environment (Dua and Sharma, 1993). In addition, many collateral benefits achieved can not be quantified in terms of monetary gains. The implementation of bioenvironmental control strategy through an integrated approach at BHEL, Hardwar has been most successful, practical and sustainable in the long run (Sharma *et al.*, 1987; Dua *et al.*, 1988, 1997).

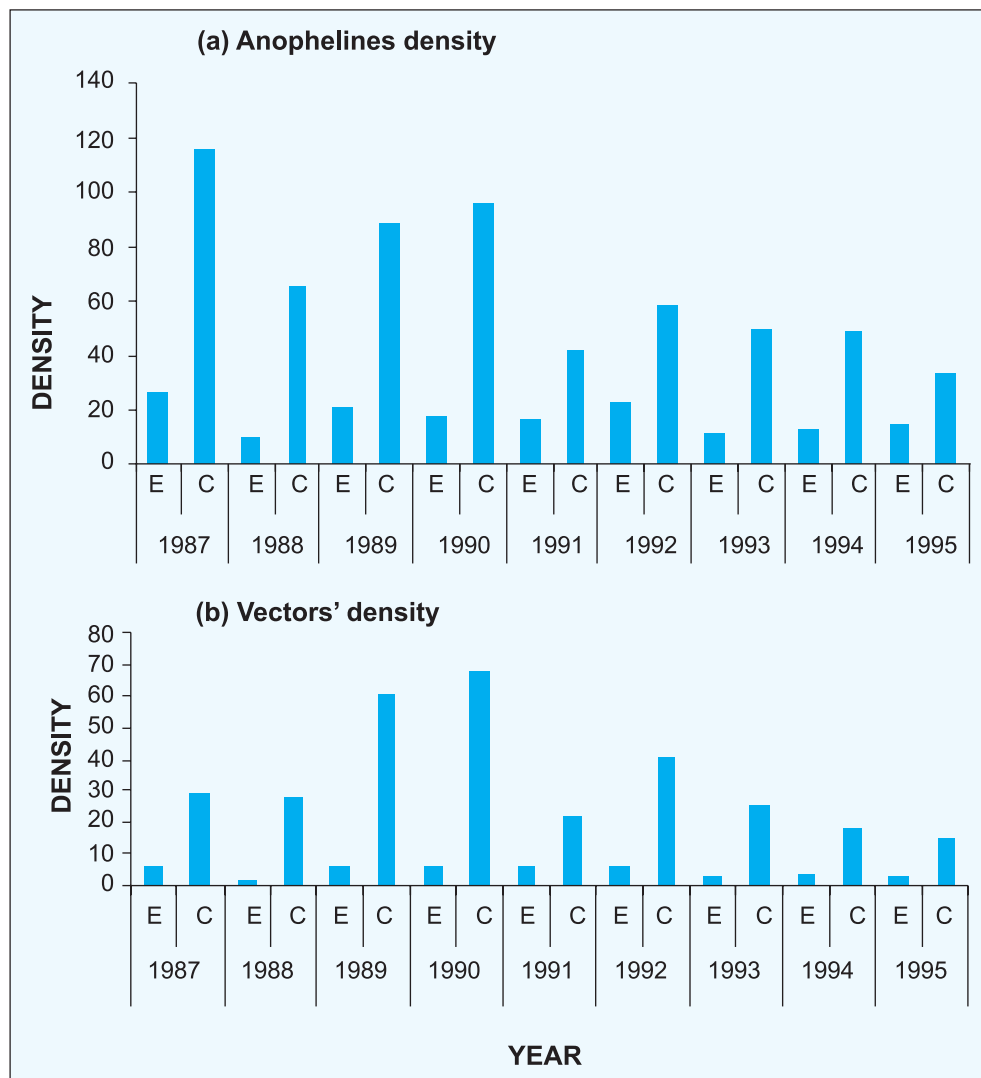


Fig. 1: Comparison of anophelines and vectors' densities in control (C) and experimental (E) areas at BHEL, Hardwar

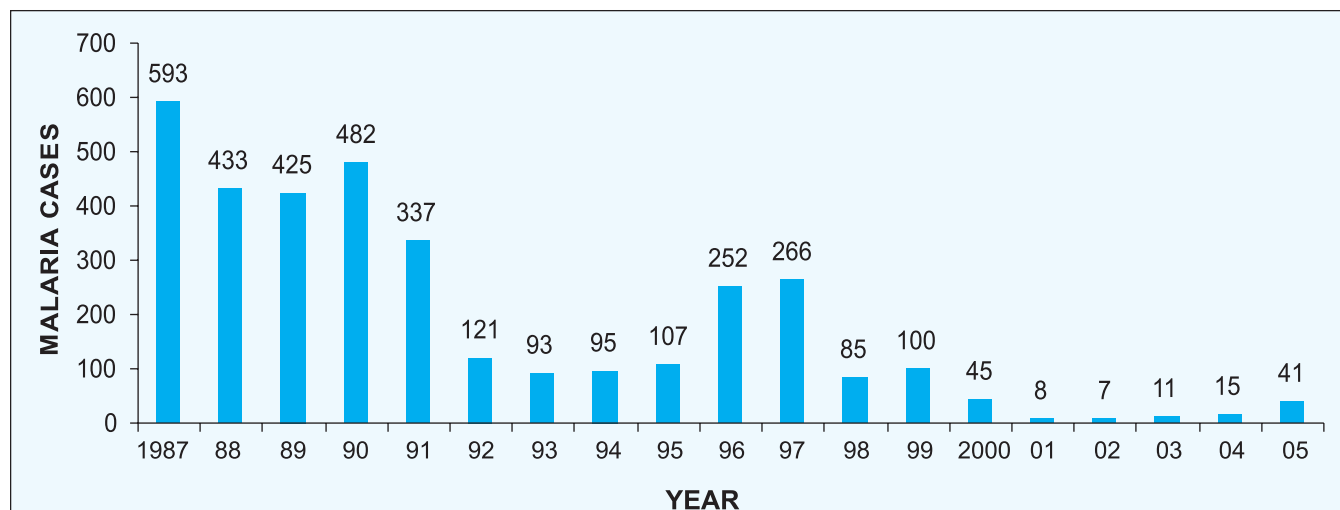


Fig. 2: Malaria incidence at BHEL complex since 1987

## Extension of the bioenvironmental control strategy

### Indian Drugs and Pharmaceuticals Limited (IDPL), Rishikesh

Considering the tremendous success at BHEL, Hardwar, the non-insecticidal integrated approach adopted for the control of malaria was extended to the IDPL complex, Rishikesh in District Dehradun. The IDPL complex is spread over an area of 15 km<sup>2</sup> and has about 25,000 population. The area was highly mosquito-genic because of innumerable natural and man-made seepage, factory effluent discharge, overhead tanks, underground tanks, open and blocked drains, borrow pits, etc. A combination of sulfalene and pyrimethamine was used to treat malaria indiscriminately regardless of the species of the parasite or sensitivity of *P. falciparum* to chloroquine (Dua *et al.*, 1991) (Table 1). The anopheline species composition was similar as that in BHEL complex. A major

mosquito breeding site of about 1 km<sup>2</sup> was eliminated by diverting the factory effluents into a drain. The intervention methods were similar to those implemented in BHEL. The study was started in 1987. Impact assessment of the interventions revealed that there has been a gradual decrease in malaria cases in this complex (Fig. 3).

### Indian Oil Corporation (IOC), Mathura

Mathura refinery township is well planned with 2,480 houses and a population of 16,000. Malaria incidence, particularly that of *P. falciparum*, was increasing and routine methods of control by larviciding, fogging and chemotherapy were not effective. Therefore, the area was taken in 1992 for integrated control strategy and > 90% reduction in malaria cases was observed after the interventions (Fig. 4).

### Consultancy services

The experience gained in BHEL, IDPL and IOC indus-

Table 1: Antimalarials used in IDPL complex, Rishikesh

Antimalarials	1985–86	1986–87	1987–88	1988–89	1989–90	% reduction in 1989–90 over 1985–86
Chloroquine	42,300	44,000	71,001	1,100	1,300	97
Primaquine	11,700	6,300	3,000	1,000	984	92
Metakelfin	6,040	8,000	4,500	2,100	307	95
Chloroquine (Injection 30 ml)	100	88	40	0	0	100

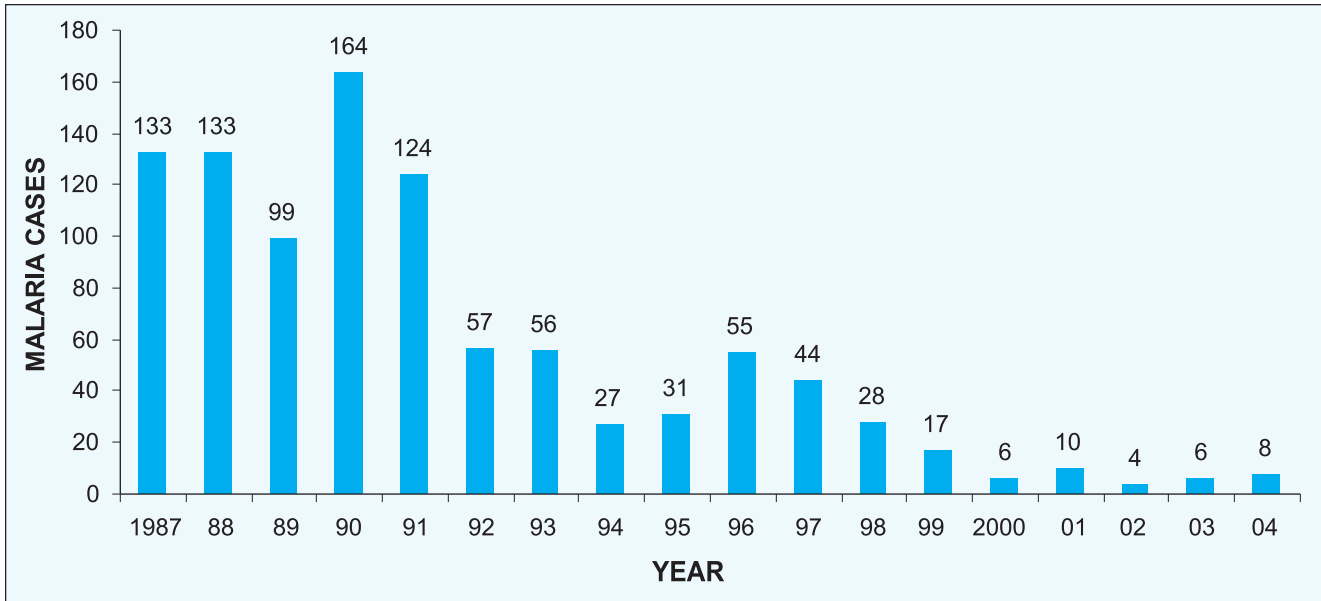


Fig. 3: Malaria cases at IDPL, Rishikesh

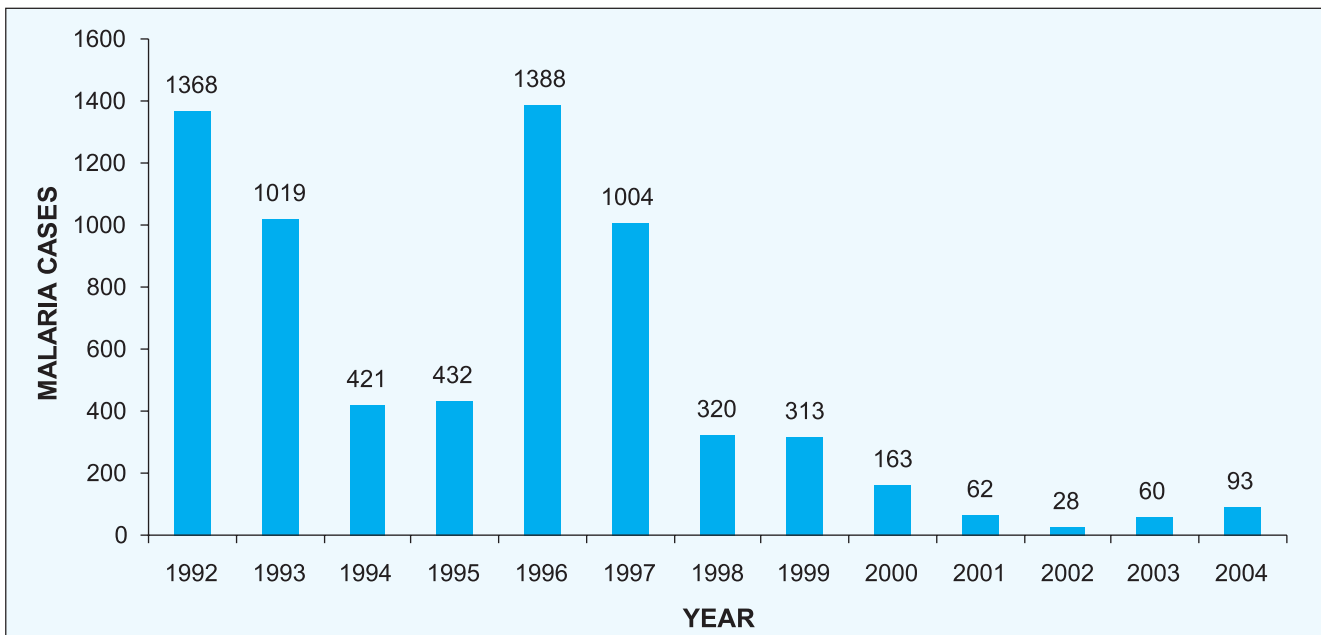


Fig. 4: Malaria cases at IOC, Mathura

trial complexes has shown that malaria control through an integrated approach is practical, sustainable in the long run, economical and reduces insecticide pollution in the environment. Following this, consultancy services were provided to the following industrial units for malaria control.

- National Thermal Power Corporation, Shaktinagar, District Sonbhadra (Dua *et al.*, 2000), U.P. (1994–96)
- National Thermal Power Corporation, Rihandnagar, District Sonbhadra, U.P. (2001 to till date)
- National Thermal Power Corporation, Talchar, Orissa (2003)
- National Thermal Power Corporation, Rai Bareilly, U.P. (2000, 2005)
- Visakhapatnam Steel Plant, Visakhapatnam, A.P. (1989)
- Heavy Engineering Corporation, Ranchi, Jharkhand (1992)
- Ordnance Factory, Tundla, U.P. (1993)

## Bionomics of *An. fluviatilis*

*An. fluviatilis* is a species complex. The bionomics of *An. fluviatilis* and its sibling species composition were studied in the foothills of Shivalik range of Uttaranchal state (Sharma *et al.*, 1995). Longitudinal study in Dehradun and Hardwar districts revealed that sibling species T and U were sympatric in the study villages (Nanda *et al.*, 1996; Singh *et al.*, 2004). Both the species were found resting indoors predominantly in cattlesheds. Blood meal analysis showed that species T and U were almost totally zoophagic (Nanda *et al.*, 1996). In incrimination studies, none of the *An. fluviatilis* specimen was found having sporozoites of any species of human malaria parasites. These observations indicated that *An. fluviatilis* is not playing any role in malaria transmission in study areas. A new cytotype has been discovered in *An. fluviatilis* population in Laksar PHC of District Hardwar. The taxonomic status, bionomics and vectorial potential of this new cytological form are currently being investigated.

## Malaria relapses and chloroquine resistance

Among 725 *P. vivax* infected patients at BHEL, Hardwar, who were administration radical treatment with primaquine @ 15 mg daily for 5-days during 1987–88, the relapse rate was 6.9%. Four consecutive relapses were observed in a 19 year old female patient in spite of repeated primaquine therapy (Sinha *et al.*, 1989).

The relapse patterns of 5,541 cases of *P. vivax* malaria from four major industrial complexes, who received at least one 5-day course of primaquine at 15 mg/day was studied (Table 2). Any subject relapsing was retreated with the same course. Overall, 511

(9.2%) of the *P. vivax* cases relapsed after the first course and 99 (1.78%), 25 (0.45%) and 3 (0.05%) cases relapsed two, three and four times, respectively. Most cases of relapse occurred within 1 year of treatment. Clearly, a 5-day primaquine regimen is inadequate to control relapses among *P. vivax* cases, therefore, there is an urgent need to review the ongoing treatment strategy (Dua *et al.*, 2001).

## Chloroquine sensitivity of *P. falciparum*

The sensitivity of *P. falciparum* to antimalarials was evaluated during 1987 using the WHO 28-day extended test *in vivo* and *in vitro* tests at BHEL. Results of *in vivo* studies indicated that out of 23 *P. falciparum* cases, 9 (39%) were resistant at the RI level; 5 of these were still parasitaemic on Days 14–16, two on Day 21, and two on Days 28 and 35 (Table 3). All these cases responded to Rimodar® treatment. Eight specimens were tested *in vitro* by micro-tests; 6 were resistant to chloroquine, with minimum inhibitory concentration (MIC) ranging from 8 to 32 pmol. Epidemiological investigations revealed that, during 1987, 19 of 23 cases of *P. falciparum* (82.6%) were imported cases, 10 of which were in migrant labourers coming from the adjacent tibri area who frequently visited highly endemic areas in terai such as Tanakpur (U.P.). The remainder cases were indigenous to BHEL (Sinha *et al.*, 1989). Follow up of 113 *P. falciparum* cases at BHEL, Hardwar indicated that 29 (25.7%) were resistant (RI) by *in vivo* test and others were sensitive to chloroquine. All resistant cases responded to metakelfin (Dua *et al.*, 1997).

*In vivo* and *in vitro* susceptibility of *P. falciparum* to chloroquine were also conducted at IOC, Mathura. Eighteen of 31 cases showed resistance (MIC 8 pmol) *in vitro*. *In vivo* tests identified 13 (40.62%) strains as

**Table 2: Relapse pattern among the cases of *P. vivax* infection after 5 days of primaquine treatment**

Complex	No. of cases investigated	No. of cases showing relapses			
		Initial	Second	Third	Fourth
BHEL, Hardwar	1,498	159 (10.6)	29 (1.9)	3 (0.2)	1 (0.1)
IDPL, Rishikesh	583	42 (7.2)	4 (0.7)	1 (0.2)	0 (0)
IOC, Mathura	3,050	288 (9.4)	64 (2.1)	20 (0.7)	2 (0.7)
NTPC, Shaktinagar	410	22 (5.4)	2 (0.5)	1 (0.2)	0 (0)
Total	5,541	511 (9.2)	99 (1.8)	25 (0.5)	3 (0.5)

Figures in parentheses represent percent relapse cases.

Table 3: Results of monitoring of chloroquine resistance of *P. falciparum*

Area	Year	Test					
		<i>In vivo</i> (28-day)			<i>In vitro</i>		
		Cases	S	R	Cases	S	R
BHEL, Hardwar	1987	23	16	9 (39.0)	6	–	6
	1985–95	123	84	29 (25.7)	–	–	–
Laksar PHC, Hardwar	1999–01	37	24	13 (35.0)	76	31	45 (59.2)
IOC, Mathura	1993	32	19	13 (40.6)	31	13	18 (58.6)
Chennai	1997	60	36	24 (40.0)	30	12	18 (60.0)
NTPC, Shaktinagar	1994–96	72	56	16 (22.2)	100	48	52 (52.0)

Figures in parentheses represent percent of resistant cases; S – Sensitive; R – Resistant.

resistant and 19 (59.73%) as sensitive out of 32 strains (Dua *et al.*, 1993). *In vitro* and *in vivo* susceptibility of *P. falciparum* to chloroquine were also conducted in Chennai City. Eighteen (60%) out of 30 cases showed resistance *in vitro*. *In vivo* tests identified 24 strains (40%; 23 RI and 1 RII) as resistant and 36 (60%) as sensitive out of 60 strains (Dua *et al.*, 1997). In 1994, 1995 and 1996, 32, 22 and 18 *P. falciparum* cases from NTPC, Shaktinagar were followed successfully up to 28 days using *in vivo* chloroquine sensitivity test respectively. Of these, 24 strains (25%) in 1994, 17 (22.7%) in 1995 and 15 (16.6%) in 1996 were found resistant to chloroquine. One strain each in 1995 and 1996 had RII level resistance, while all other cases had RI level of resistance (Dua *et al.*, 2000).

### Monitoring of *in vivo* level of antimalarial drugs and its application to malaria cases

High performance liquid chromatographic (HPLC) techniques for the analysis of the antimalarial drugs and their metabolites in plasma, whole blood and blood cells of malaria patients were developed and applied in malaria cases to establish true resistance, pharmacokinetic studies and to investigate whether the subject had attained therapeutic levels.

A normal-phase HPLC method using dichloro-methane-methanol-perchloric acid (1M) (96:0:1, v/v) at a flow rate of 1 ml/min on a Nucleosil 100-7 column (250 × 8 × 4 mm) and UV detection at 254 nm has been developed to determine the concentration of sulfalene in plasma, red blood cells and whole blood after oral administration of the antimalarial drug metakelfin. The coefficient of variation was 7.1% and the extraction

recovery was 82%. Mean concentrations of sulfalene on Day 1, 7 and 15 were 49.56, 10.46 and 2.24 µg/ml in plasma, 25.02, 4.34 and 0.84 µg/ml in red blood cells and 21.12, 4.44 and 1.00 µg/ml in whole blood, respectively (Dua *et al.*, 1991).

A normal-phase HPLC method using dichloro-methane-methanol-1 M perchloric acid (100:9:0.4, v/v/v) at a flow rate of 0.8 ml/min on a Zorbax-Sil column with fluorescence detection has been developed for the separation of quinine and quinidine from other antimalarials. Within-day and day-to-day coefficients of variation averaged 0.74 and 7.56%, respectively. The extraction recovery of quinine from plasma, serum, red blood cells and whole blood (filter paper) was 88.13, 87.12, 78.0 and 77.5%, respectively. The method is capable of separating quinine from dihydroquinine, a compound usually found as an impurity in authentic quinine samples. The method has been used for the determination of quinine in plasma, serum, red blood cells and whole blood (filter paper) of six healthy and 20 *falciparum* cases. The average quinine concentration in *P. falciparum* cases was three to four times higher than in that of healthy volunteers. Quinine was absorbed much less in red blood cells than in plasma or serum (Dua *et al.*, 1993).

A reversed-phase HPLC method using acetonitrile-methanol-1M perchloric acid-water (30:9:0.8:95, v/v/v/v) at a flow of 1.5 ml min<sup>-1</sup> on u-Bondapak C<sub>18</sub> column with UV (254 nm) detection has been developed for the separation of sulphadoxine, sulfalene and sulphamethoxazole from other antimalarials. Calibration curves were linear in the range 0.5–100 µg ml<sup>-1</sup>. The limit of quantification was 50 ng ml<sup>-1</sup>. Within-day and day-to-day coefficients of variation averaged 2.1 and

6.45%, respectively. The extraction recovery of sulphadoxine from plasma, red blood cells and whole blood was 90.28, 92.05 and 94.69% respectively. The method has been used for the determination of sulphadoxine concentrations in plasma, red blood cells and whole blood of eight healthy and 50 *P. falciparum* malaria cases after administration of two tablets of fansidar. Mean sulphadoxine concentration in plasma was higher than red blood cells or whole blood. Sulphadoxine concentration in plasma and whole blood of *P. falciparum* malaria cases was significantly higher as compared to healthy volunteers, while it was the same in red blood cells. Sulphadoxine was absorbed much less in red blood cells than in plasma of whole blood (Dua *et al.*, 1994).

A reversed-phase HPLC method using acetonitrile-methanol-(1 M) perchloric acid-water (30:9:1:95, v/v/v/v) at a flow-rate of 1.5 ml/min on a u-Bondapak C<sub>18</sub> column with UV detection at 254 nm was developed for the separation of primaquine, its major metabolite carboxyprimaquine and other metabolites such as N-acetyl-primaquine, 4-hydroxyprimaquine, 5-hydroxyprimaquine, 5-hydroxy-6 methoxyprimaquine, dimethyl-primaquine and 6-methoxyprimaquine, and also other antimalarials. The calibration graphs were linear in the range 0.025–100 µg/ml for primaquine and 4–1000 µg/ml for carboxyprimaquine. The within-day and day-to-day coefficients of variation averaged 3.65 and 6.95%, respectively for primaquine and 3.0 and 7.52% respectively for carboxyprimaquine in plasma. The extraction recoveries for primaquine and carboxyprimaquine were 89 and 83% respectively. The mean carboxyprimaquine concentration was much higher in plasma and blood cells of *P. vivax* patients than that of in plasma from healthy subjects. The carboxyprimaquine level was also higher in blood cells than plasma, whereas the primaquine concentration was same in both groups (Dua *et al.*, 1996).

A case of *P. vivax* malaria resistant to chloroquine was found from IOC, Mathura. The infection failed to respond to 2 cycles of standard chloroquine therapy. The concentration of chloroquine was monitored using HPLC. The plasma and whole blood chloroquine concentrations were 260 and 106 µg/l, respectively, while 15 µg/l plasma concentration is considered lethal to *P. vivax* (Dua *et al.*, 1996).

A reversed-phase HPLC method using acetonitrile-methanol- (1 M) perchloric acid-water (25:9:0.8:95, v/v/v/v) at a flow-rate of 1.0 ml min<sup>-1</sup> on LiChrospher 100

RP 18 column (250 × 4 min; 5 µm) with UV (254 nm) detection has been developed for the determination of sulfalene in plasma and blood cells after oral administration of the antimalarial drug metakelfin. Calibration curves were linear in the range of 0.5–100 µg ml<sup>-1</sup>. The limit of quantification was 50 ng ml<sup>-1</sup>. Within-day and day-to-day coefficients of variation averaged 3.84 and 5.31%, respectively. Mean extraction recoveries of sulfalene from plasma and blood cells were 87.21 and 84.65%, respectively. Mean concentrations of sulfalene in plasma of *P. falciparum* cases on Days 2 and 15 were 44.58, 14.90 µg ml<sup>-1</sup> respectively, while in blood cells concentrations of sulfalene were 7.77 and 0.75 µg ml<sup>-1</sup> respectively after oral treatment with two tablets (1000 mg) of metakelfin. Significant difference was recorded on Day 2 for sulfalene concentration in blood cells of healthy and *P. falciparum* cases ( $t = 9.49$ ;  $p < 0.001$ ) (Dua *et al.*, 1998).

A normal phase HPLC method using dichloromethane-methanol-perchloric acid (1M) (100:9:1.2 v/v/v) at a flow of 1 ml/min on a uPorasil column and UV detection at 343 nm has been developed to determine the concentration of chloroquine and its major metabolite desethylchloroquine in plasma and blood cells. The limit of quantification was 20 mg ml<sup>-1</sup>. Within-day and day-to-day coefficients of variation for chloroquine averaged 2.3 and 2.76%, respectively. Mean extraction recoveries of chloroquine from plasma and blood cells were 91.05 and 90.5%, respectively. The plasma and blood cells concentrations within 3 h of administering dose reached above therapeutic chloroquine concentration to clear *P. vivax* parasites. The red blood cell concentrations were higher than those in plasma in all samples. Terminal half-life for chloroquine in plasma and blood cells were 136.4 and 168.4 h respectively (Dua *et al.*, 1999).

A survey was conducted to find chloroquine concentration profile in the community of Mewat region, District Gurgaon (Haryana). Plasma chloroquine and desethylchloroquine concentrations were determined in 55 *P. falciparum* and two *P. vivax* patients, and 29 persons whose blood slides were negative for malaria parasite before giving any treatment. Mean chloroquine concentrations in cases with *P. falciparum* and no malaria parasites were 0.018 and 0.016 mg/ml, respectively. Chloroquine to desethylchloroquine ratio was between 2 and 3 in both groups. Only 10 malaria parasite negative cases out of 29 had plasma chloroquine concentrations above 0.016 µg ml<sup>-1</sup> required for

malaria chemoprophylaxis. Chloroquine (CQ) was undetectable in plasma samples of 8 out of 55 *P. falciparum* cases, and it was below the therapeutically effective concentration of  $0.016 \mu\text{g ml}^{-1}$  suggesting improper treatment, while in 29 *P. falciparum* cases, parasitaemia recurred despite required chloroquine concentration confirming chloroquine resistant status. Irregular prophylaxis and lack of proper treatment was one of the major causes of malaria outbreak in this area (Dua *et al.*, 1999).

Concentrations of CQ and desethylchloroquine (DCQ) in blood cells and plasma from CQ sensitive and resistant cases were determined after 2 and 7 days initiation of treatment by HPLC. On Day 2, the mean CQ concentrations in the samples collected from the sensitive cases were higher than those in the samples from the resistant patients in plasma ( $0.47 \text{ v } 0.32 \mu\text{g ml}^{-1}$ ,  $p < 0.02$ ) and particularly in the blood cells ( $1.51 \text{ v } 0.46 \mu\text{g ml}^{-1}$ ,  $p < 0.001$ ). By Day 7, however, the CQ concentrations in the two groups were similar. Although, the mean ratio of the CQ to DCQ concentrations was significantly higher in the blood cells from the sensitive group than in those from the resistant cases ( $p < 0.01$ ), the CQ/DCQ ratios for the plasma were similar for the two groups. Similarly, the mean ratio between the blood cell concentration of CQ on Day 2 and the concurrent plasma concentration was also relatively higher ( $p < 0.001$ ) in the sensitive group (Dua *et al.*, 2000).

Whole blood concentrations of chloroquine, sulphadoxine and quinine were determined at different intervals and at the time of parasite recrudescence after completion of treatment with the respective drugs to confirm the status of drug sensitivity. A case of multi-drug resistant *P. falciparum* malaria was found in Sonapur, Assam where recrudescence occurred despite taking standard oral treatment with chloroquine, sulphadoxine/pyrimethamine and quinine sequentially. Whole blood concentrations of chloroquine, sulphadoxine and quinine at the time of recrudescence were  $0.35 \mu\text{g/ml}$  (Day 7),  $18 \mu\text{g/ml}$  (Day 14), and  $0.009 \mu\text{g/ml}$  (Day 14), respectively. Therefore, monitoring of drug-resistant *P. falciparum* malaria and its proper treatment should be intensified to check the spread of multi-drug resistant strains in other parts of the country (Dua *et al.*, 2003).

The pharmacokinetics of chloroquine was studied in Indian tribal and non-tribal healthy volunteers and

patients infected with *P. falciparum* malaria after a single dose of 600 mg chloroquine. Mean area under the curve (AUC), half-life ( $T_{1/2}$ ) and peak concentration ( $C_{\text{max}}$ ) in tribal *P. falciparum* patients were  $18.79 \pm 5.82 \mu\text{g/h/ml}$ ,  $115.94 \pm 57.71 \text{ h}$ , and  $435 \pm 135.17 \text{ ng ml}^{-1}$  respectively, while of non-tribal *P. falciparum* patients were  $17 \pm 5.6 \mu\text{g/h/ml}$ ,  $76.15 \pm 8 \text{ h}$  and  $454 \pm 19 \text{ ng ml}^{-1}$  respectively. Pharmacokinetic parameters did not differ between tribal and non-tribal subjects in healthy volunteers or *P. falciparum* patients. However, the time to reach maximum concentration ( $T_{\text{max}}$ ) was 8 h in tribal subjects and 4 h in non-tribal subjects. Mean ratio of area under the curve (AUC) of chloroquine to desethylchloroquine in *P. falciparum* patients of tribals were higher ( $4.26 \pm 1.34$ ) than that of non-tribal subjects ( $3.41 \pm 0.66$ ) suggesting reduced chloroquine metabolism in tribal subjects. However, the difference was statistically non-significant ( $t = 1.35$ ,  $p < 0.5$ ). Delayed  $T_{\text{max}}$  and impaired chloroquine metabolism may be associated with general health problems such as malnutrition, anaemia and parasitic infestations of tribal population in India (Dua *et al.*, 2002).

A normal-phase HPLC method using dichloro-methane-methanol-1 M perchloric acid (100:10:0.9 v/v) at a flow rate of  $1.0 \text{ ml min}^{-1}$  on a LiChrospher Si column with UV (254 nm) detection has been developed for the determination of amodiaquine and its metabolites desethylamodiaquine and bisdesethylamodiaquine in plasma. The limit of quantification was 5 ng/ml. Mean within-day and day-to-day coefficients of variation were 4.10 and 6.27% for amodiaquine, 3.43 and 4.80% for desethylamodiaquine and 3.53 and 5.23% for bisdesethylamodiaquine, respectively. Mean extraction recovery of amodiaquine, desethylamodiaquine and bisdesethylamodiaquine from plasma were 82.48, 74.50 and 69.65% respectively. Chloroquine and its metabolite desethylchloroquine, quinine, sulphadoxine and primaquine do not interfere in the detection of amodiaquine, desethylamodiaquine and bisdesethylamodiaquine in plasma (Dua *et al.*, 2004).

### Search for new antimalarials

Five compounds formed by peroxydisulphate oxidation of primaquine were isolated using chromatographic methods (Dua *et al.*, 1998), characterised (Sinha and Dua, 2004) and evaluated for antimalarial activity *in vitro*. One compound, 6-methoxy-5, 8-bis-(*u'*-amino-*l'*-methylbutylamino)-quinoline, was found to have good gametocytocidal activity against *P. yoelli*

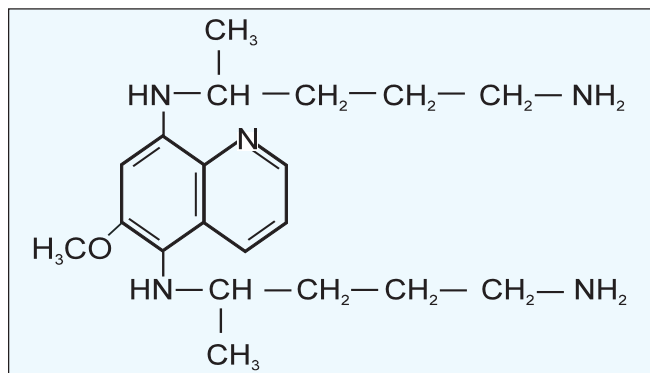


Fig. 5: Structure of compound having good gametocytocidal activity against *P. yoelli* infected mice

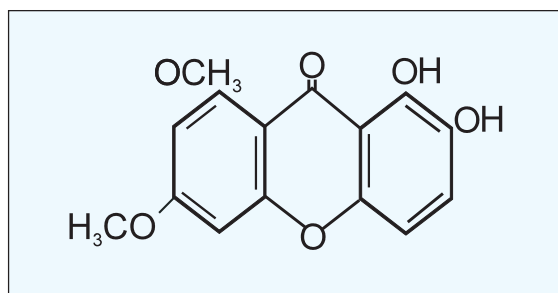


Fig. 6: Structure of 7,8-dihydroxy-1,3-dimethoxy-xanthone

infected mice at 10 mg/kg dose *in vivo* (Dua *et al.*, 2002) (Fig. 5). This compound has been patented [Indian Patent No.189970 (3280/Del/98)].

Four xanthenes were isolated from the roots of *Andrographis paniculata* plant and evaluated for antiparasitic activity using *in vitro* test. One compound showed substantial activity ( $IC_{50} = 5 \mu\text{g/ml}$ ). This compound (7, 8 dihydroxy-1-3-dimethoxy xanthone) gave 70% reduction in parasitaemia in *P. berghei* infected mice at the dose of 30 mg/kg (Dua *et al.*, 2004) (Fig. 6).

Eight fractions were isolated from the seeds of *Azadirachta indica* (neem) by using solvent partition and florisil column chromatographic methods. Three fractions were obtained from seed cake, out of which fractions code A-1 and A-2 showed  $IC_{50}$  values of 4.8 and 5.0  $\mu\text{g/ml}$  against *in vitro* grown *P. falciparum*. Similarly, five fractions were isolated from *A. indica* oil and two fractions code A-5 and A-6 showed significant *in vitro* antimalarial activity with their  $IC_{50}$  values of 2.25 and 2.30  $\mu\text{g/ml}$  respectively. The other fractions showed moderate to low antimalarial activities (Dua *et al.*, 2003).

## Prospecting for botanical pesticides

Studies showed that *Lantana camara* flower extract exhibited repellent properties against *Aedes* mosquitoes. Methanol extract of *L. camara* isolated in coconut oil provided 1 h 45 min protection against the bite of *Ae. albopictus* in laboratory. An application of 5 ml of this extract on human volunteers gave 94.5% protection against *Aedes* mosquitoes for 1 h following application and provided 69.9% biting reduction of *Aedes* mosquitoes for the total follow-up of 6 h of the application on human volunteers (Dua *et al.*, 1996). Purified fraction isolated by solvent partition and column chromatography method resulted in significant enhancement of repellency of *Lantana* flower fraction. HR-2(5) fraction isolated by column chromatographic method significantly increased the repellency. The mean protection time was 3 h 45 min against *Ae. albopictus* in the laboratory. One application of this fraction gave 100% biting protection for a period of 2 h against *Aedes* mosquitoes and can provide 75% protection at 7 h post-application of the fraction against *Aedes* mosquitoes (Dua *et al.*, 2003).

Neem oil has been investigated as a potential mosquito repellent. Neem oil (2%) mixed in coconut oil provided 96–100% protection from anophelines, 85% from *Aedes*, 37.5% from *Armigeres*, whereas it showed wide range of efficacy from 61–94% against *Culex* spp (Sharma *et al.*, 1995). The application of neem cream on exposed body parts @ 2 g/person dose showed 78% (range 65–95%), 89% (range 66–100%) and 94.4% (range 66–100%) protection against *Aedes*, *Culex* and *Anopheles* mosquitoes, respectively. Significant difference was observed between neem cream treated and untreated persons for *Aedes* mosquitoes ( $p < 0.001$ ). Application of neem cream was found to be a safe and suitable alternative to insecticide impregnated coils for personal protection against mosquitoes and one application was 68% effective for four hours (Dua *et al.*, 1995).

In 116 fractions screened for insecticidal properties against *An. stephensi* and *Ae. aegypti* mosquitoes, 11 showed antilarval activity with their  $LC_{50}$  value  $< 250$  ppm, while four fractions showed adulticidal activity. Two fractions (codes NBDB(5)048A12P-04a and NBDB(5)048A12P-04b) have antilarval and adulticidal action against *An. stephensi* and *Ae. aegypti* mosquitoes.

A fraction (code MRCHAR 03-05P1) isolated from the

plant belonging to Garhwal region of northwest Himalayas showed excellent adulticidal activity against *An. stephensi*, *An. culicifacies*, *Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus*. The invention has been filed for a patent. Larvicidal activity of *Hibiscus abulmoschus* Linn. (Malaceae) against mosquitoes was evaluated. Aqueous extract of this plant showed LC<sub>50</sub> values of 52.3, 52.6, 43.8, 181.2 and 184 ppm against *An. culicifacies*, *An. stephensi*, *Cx. quinquefasciatus*, *Ae. aegypti* and *Ae. albopictus* mosquitoes, respectively.

### Monitoring insecticide residues in environment

Despite of remarkable insecticidal properties of HCH and DDT for the control of vector-borne diseases, they became major environmental pollutants due to their chemical nature and extensive use in agriculture and public health. Therefore, studies have been carried out to determine these residues in different environmental components. HCH residues were found in the rain water from Hardwar which was due to its extensive use during “Ardh Kumbh” congregation in 1992 and correlated with the amount used (Fig. 7).

Skin lipid level was used as a non-invasive technique for monitoring HCH and DDT body burden under field conditions. Skin lipid and whole blood samples were collected from occupationally exposed individuals and

the general population and analysed to determine a possible correlation between lipid and blood concentrations of HCH and DDT residues. Significant correlations were observed for  $\beta$ -HCH ( $r = 0.5594$ ,  $p < 0.01$ ), total HCH ( $r = 0.4862$ ,  $p < 0.05$ ) and  $p,p'$ -DDE ( $r = 0.8092$ ,  $p < 0.001$ ) for occupationally exposed individuals. However, for the general population, significant correlations were observed for  $\gamma$ -HCH ( $r = 0.4250$ ,  $p < 0.05$ ),  $\beta$ -HCH ( $r = 0.5019$ ,  $p < 0.01$ ), total HCH ( $r = 0.5388$ ,  $p < 0.001$ ),  $p,p'$ -DDE ( $r = 0.6275$ ,  $p < 0.001$ ),  $p,p'$ -DDT ( $r = 0.7260$ ,  $p < 0.001$ ) and total DDT ( $r = 0.7856$ ,  $p < 0.001$ ). Correlation of HCH and DDT residue between skin lipid and whole blood from paired samples implies that the collection of skin lipid as a non-invasive sampling method for halogenated hydrocarbons has a potential application as an indicator of body burden particularly in general population. However, skin lipid may not be a suitable bioindicator for occupationally exposed population because dermal exposure occurs in work place and skin lipid may contain chemicals representative of occupational exposures but not related to body burden which is evident by the low correlation of exposed workers (Dua *et al.*, 1998).

A filter paper method for the determination of HCH and DDT residues in whole blood has been developed using finger-prick blood dried on filter paper. The method is simple, storage and transportation of samples is easy, and social acceptability is high (Dua

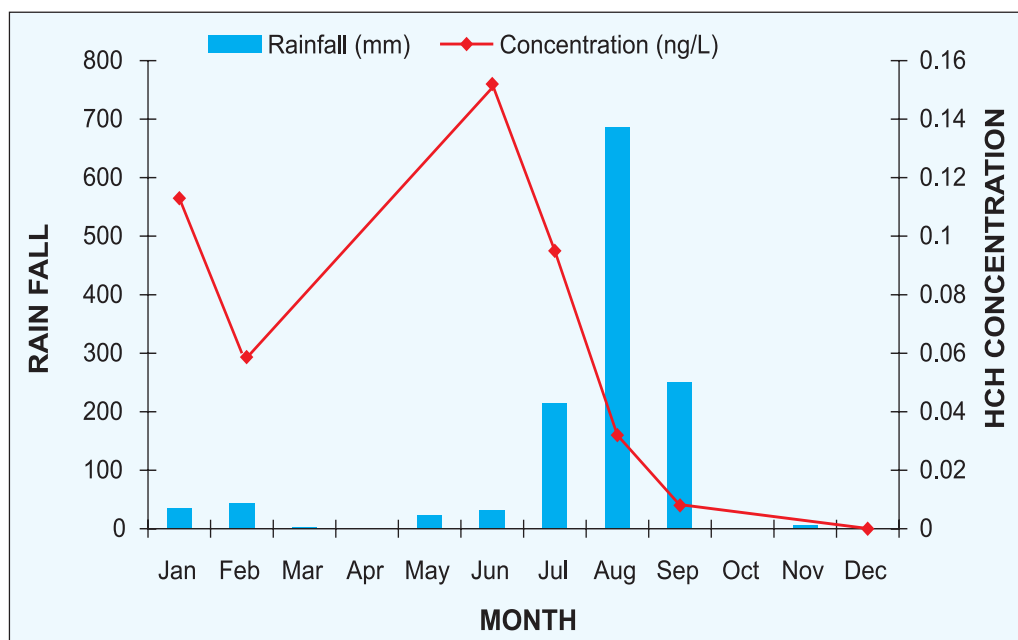


Fig. 7: Concentration of HCH residues in rain water in Hardwar during Ardh Kumbh congregation in 1992

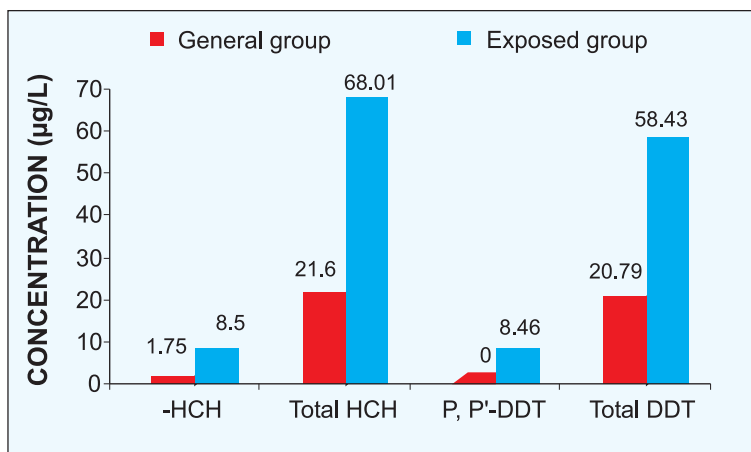


Fig. 8: Comparison of HCH and DDT residues in whole blood from general and occupationally exposed population

*et al.*, 1996). The method was applied to monitor the concentration of HCH and DDT in occupationally exposed and general population and the results are given in Fig. 8.

Water samples of five lakes situated on the hilly terrain of Nainital were analysed for residues of organochlorine insecticides. Results indicated a moderate level of contamination of all the five lakes with HCH residues and a moderate to high level of contamination with DDT residues. DDT contamination in water from all lakes exceeded the maximum permissible limit of DDT (1.0 µg/L) for drinking water (Dua *et al.*, 1998).

Larvivorous fish *G. affinis* was used to reduce DDT contamination in water, sediment and edible fish from rural ponds of India. Statistically significant difference was recorded in water and sediment from ponds without fish and ponds with *Gambusia*. Mean DDT level in food fish taken out of ponds with composite fish (food

fish and *Gambusia*) was lower than the ponds with food fish alone. *Gambusia* played a major role in reducing DDT contamination in water, sediment and food fish. Significant correlations of DDT concentration in water-fish, water-sediment and sediment-fish were observed from all categories of ponds except ponds with composite fish. DDT concentrations in water and fish from many ponds were above maximum permissible limits (Dua *et al.*, 1999).

### Training programme and technical support provided by IDVC field unit, Hardwar

The field unit imparted training to laboratory technicians, microscopists, engineers, architects, medical entomology students, international scientists, etc. The field unit organised several training courses, group meetings, workshops, seminars, exhibitions and health camps.

