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Neurobehavioural, biochemical and immunological manifestations in workers exposed to organophosphate insecticides

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Organophosphate (OP) pesticides commonly used in agriculture in India are reported to cause poisoning. The effects of acute poisoning by pesticides are well-established; however, low and long-term exposure causing ill health is controversial. Further, the existing database regarding the toxicity effects of OP pesticides (triazophos and acephate) is much limited in India. In this study, we investigated the neurobehavioral, biochemical and immunological manifestation and their possible correlation with triazophos and acephate exposure in humans. A total number of 161 employees comprising control group (n=40), maintenance group (n=50) and exposed group (n=71) working in a unit manufacturing triazophos and acephate were enrolled in the study. Control group comprised of executives, clerks, security and peon, etc. Exposed and maintenance groups included workers engaged in production, formulation, transportation and maintenance of triazophos and acephate. Demographic profile and occupational history were recorded. A battery of neurobehavioral tests was employed as per ILO (1971) recommendations to evaluate neurobehavioral performance. In a sub-set study of population, serum samples were analyzed for immunological (IgG, IgM and IgA) and thyroid function tests (T3, T4 and TSH). Neurobehavioral findings indicated that exposed group of workers had performance deficiency in digit symbol test in percentage of accuracy, compared to the other groups. A significant (P <0.05) decrease in neurobehavioral performance like finger dexterity test (accuracy %), memory test (forward and backward) and digit symbol test (total) was observed in maintenance group, as compared to control and exposed groups. Workers of exposed group showed poor performance in only tweezer dexterity test (accuracy %). Serum IgM levels showed a significant increase in exposed subjects, indicating the impairment of immune system, while thyroid function was normal in study population. The study showed a possible correlation with exposure to OP pesticides in relation to impairment in some of the neurobehavioral and immunological parameters that might be useful in assessing OP poisoning.

Keywords: Acephate, Card sorting, Digit symbol test, Finger dexterity, Hand dynamometer/steadiness test, Immunological parameters, Industrial pollution, Memory test, Nerve agents, Pesticides, Poisoning, Neurobehavioral performance tests, Thyroid function test, Triazophos, Tweezer dexterity

India is one of the most dynamic and generic pesticide manufacturers in the world with approximately 60 technical grade pesticides being manufactured by the large and medium-scale enterprises and more than 500 pesticide formulators spread all over the country (www.indiajuris.com). They manufacture significant quantities of synthetic pyrethroids, such as fenvalerate and cypermethrin, endosulphan and a range of organophosphate (OP) insecticides, such as monocrotophos, triazophos and acephate. Although the main application of pesticides is in agriculture, their use in public health programme, such as malaria or rodent control is also reported in some areas of the world[1]. Thus, a vast majority of the people, including manufacturers, formulators, end-users and community population are likely to be exposed to a plethora of pesticide chemicals. As pesticides are toxic chemicals by design, they primarily cause neurotoxic effects. Acute poisoning by pesticides is well-established; however, low-level and long-term of exposure causing ill health is still controversial[2].

Studies in human populations have identified relationships between exposure to persistent organic pollutants and neurological and behavioral disturbances in infants and children[3-6]. Although there is growing body of evidence suggesting the neurobehavioral and cholinesterase effects following acute exposure of OP insecticides[7,8], but there is paucity of information on neurobehavioral effects attributable to chronic low-level exposure to these compounds. Recently, Mackenzie *et al*.[8] presented a comprehensive review on

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neurobehavioral problems due to the low and long-term exposure to OP insecticides.

The existing available information on biomonitoring studies is mainly concerned with the exposure to combination of different type of pesticides[9-11]. In addition, little is known about immunochemical and thyroid manifestations among OP exposed workers. Earlier, we have reported immune function impairment[11], thyroid dysfunction[12], and depressed cholinesterase activity[10,11,13] in pesticide formulators exposed to combination of pesticides.

The existing database on the health survey of workers involved in the production and formulation of pesticides in the country is scanty. Therefore, in the present study, we have evaluated neurobehavioral effects and immunological profile coupled with thyroid function tests among pesticide formulators in an organized industrial unit engaged in the manufacture and formulation of OP pesticides (acephate and triazophos).

Materials and Methods

The study was conducted in a unit of an organized sector engaged in production and formulation of OP insecticides triazophos and acephate. Study population comprised 161 male workers, except three females in control group and the mean age ranged 28.45 to 35.02 yrs. The subjects were divided into three groups: Group I: Control group (n=40) employees of administration department e.g. managers, executives, clerks, typists, stenographers, peon, etc.; Group II: Maintenance group (n=50) comprising mechanics, electricians, engineers, fitters and helpers, etc.; and Group III or Exposed group (n=71) comprising workers and supervisors associated in production and formulation of OP insecticides.

The study was approved by the Ethical Committee of the Institute. The objectives of study were explained to the participants and their informed consent was obtained. There was specific work schedule (8 h shift-duty) for exposed and maintenance group workers, while the control group subjects had 8 h general duties without any shift. Normal hygiene measures like washing of hands before meals and taking bath after the work-shift and use of protective devices (apron gloves, helmet, industrial boots masks, etc) were being practiced. Each subject was interviewed for their demographics, smoking habits, alcohol intake, medical history, past and present illness, work conditions and exposure to other chemicals (Tables 1-3). Their detailed occupational history (past and present) was recorded on pre-designed proforma.

Table 1—Demographics characteristics of study population

Characteristics	Status	Control (n=40)	Maintenance (n=50)	Exposed (n=71)
Education/ Literacy status	Illiterate	-	1 (2.0)	-
	1^{st} – 4^{th} Std	-	1 (2.0)	-
	5^{th} – 10^{th} Std	5 (12.5)	23 (46.0)	12 (16.9)
	11^{th} - 12^{th} Std	-	13 (26.0)	14 (19.7)
	College	35 (87.5)	12 (24.0)	45 (63.4)
Sex	Male	37 (92.5)	50 (100)	71 (100)
	Female	3 (7.5)	-	-
Smoking status	Smoker	5 (12.5)	4 (8.0)	6 (8.5)
	Non Smoker	35 (87.5)	46 (92.0)	65 (91.5)
Chewing Status	Non-chewer	30 (75.0)	26 (52.0)	40 (55.7)
	Chewer	10 (25.0)	24 (48.0)	31 (44.3)
Diet	Vegetarian	34 (85.0)	34 (68.0)	54 (76.1)
	Mixed	6 (15.0)	16 (32.0)	17 (23.9)
Alcoholism	Yes	6 (15.0)	2 (2.0)	2 (5.8)
	No	34 (85.0)	48 (98.0)	69 (94.2)
Socio-economic status#	Low	-	-	1 (1.4)
	Lower middle	11 (27.5)	28 (56.0)	33 (46.5)
	Upper middle	9 (22.5)	13 (26.0)	21 (29.6)
	Upper	20 (50.0)	9 (18.0)	16 (22.5)
Marital status	Married	32 (80.0)	39 (78.0)	48 (67.6)
	Unmarried	8 (20.0)	11 (22.0)	23 (32.4)

#Kupuswami Scale; Figures in parenthesis indicates percentage

Table 2—Distribution of subjects according to the age and duration of Job

Group	Sample size (number)	Age (years) Mean ± SD	Duration of Exposure (years)			
			≤1.0	1.1-2.0	2.1-5.0	>5.0
Control	40	35.02 ± 9.98	4	15	10	11
Maintenance	50	31.38 ± 9.08	18	5	15	12
Exposed	71	28.45 ± 6.81	29	10	16	16

Table 3—Age group wise distribution of study subjects

Age group (Years)	Control (40)	Maintenance (50)	Exposed (71)	Total	Chi square significance
Up to 25	8 (20.0)	17 (34.0)	31 (43.7)	56 (34.8)	
26-35	13 (32.5)	19 (38.0)	29 (40.8)	61 (37.9)	
36-45	13 (32.5)	9 (18.0)	10 (14.1)	32 (19.9)	P =0.013
above 45	6 (15.0)	5 (10.0)	1 (1.4)	12 (7.5)	
Total	40	50	71	161	

Figures in parenthesis indicates percentage

Neurobehavioral tests

The following neurobehavioral tests were administered to assess possible neurobehavioral deficits among workers exposed to pesticides in their occupation.

Finger dexterity

Removing rivette from a hole in the upper part of the board with the preferred hand and at the same time removing one washer from a vertical stack on a peg with the other hand, assembling the washer on the rivette and inserting the assembly into the corresponding hole in the lower part of the board (Time=180 s). Score: No. of assembly inserted

Tweezer dexterity

The apparatus comprised of a steel board having 100 holes (1 cm deep and 1.5 mm in diameter arranged in 10 rows). The subject was supposed to put cylindrical metallic pins (2.5 mm long and 1.5 mm in diameter) with the help of a tweezer into the holes as fast as he could do in 180 s.

Card sorting

This test assesses abstract reasoning ability and the ability to shift cognitive strategies in response to changing environmental contingencies.

Hand steadiness test

Hand steadiness test was used to measure the finger tremor where subject was to insert a stylus into a hole from 6 cm diameter to 0.5 cm diameter without touching the wall of hole.

Hand dynamometer test

The test was used to measure the hand strength in Kg.

Mental control

Designated as mental control, the recitation of word lists and arithmetic progressions is often used for a cursory examination of attention and concentration.

Forward and backward memory test

In the forward test, the subject has to recall the numbers in same order, spoken by experimenter, while in backward memory test, the subject has to recall the numbers in backward order, spoken by experimenter.

Digit symbol

This test requires the subjects to write the appropriate symbol under each digit correctly as quickly as possible within 3 min, following a digit and a symbol sequence given in the upper part of the test sheets.

All the tests were administered in a noiseless, comfortable environment with minimal distractions.

Biochemical tests

Blood samples of these subjects from cubital vein were also obtained with the help of vacutainer (BD Vacutainer, Plymouth, PL6 7BP, UK). Serum was obtained by centrifuging blood sample at 5000 rpm for 5 min and stored at −20°C till the time of analysis. In a sub-set of samples, thyroid function tests (T3, T4 and TSH) were estimated by radio-immunoassay (RIA) using commercially available diagnostic kits obtained from M/s Immunotech, Beckman Coulter, Co., Czech Republic. The immunologlobulins (IgG, IgM and IgA) were estimated by turbidometric method employing kits obtained from M/s Transasia Biomedical Ltd. as per the procedure described by the manufacturer.

Statistical analysis

Data presented as mean ± SE. All statistical analysis was performed using SPSS 15 software. Analysis of variance (ANOVA) was used for comparison among groups, followed by group-wise comparisons by Dunnett's T3 test. Linear regression was used for studying various relationships among continuous variables. Two-tailed tests were used for all comparisons with level of significance as 5% (P <0.05).

Results and Discussion

Demographic characteristic and distribution of study subjects

Demographic profiles of the study population and distribution of subjects and occupational history of the workers are summarized in Tables 1-3. As shown in the Table 1, education status of the study population (control, maintenance and exposed) was from standard 1st to college levels, except one worker from maintenance group, who was uneducated. All the workers in study group were male, except three workers who were female in control group. Most of the workers were non-smokers, but 40% of the workers reported a current tobacco chewer status. Administration group had higher percentage of smokers, but the fraction of workers reporting a tobacco chewer status was smaller in administrative group. Similarly, administrative group had significant percentage of alcohol consumers (15.0%), as compared to production (2.8%) and maintenance groups (4.0%). Socio-economic status of the workers revealed that maximum number (50%) of upper class workers belonged to the control group, while maximum number lower class workers belonged to the maintenance group. Majority of the workers were married. Distribution of subjects of various groups in relation to age and duration of job showed the mean age of the study groups ranging from 28.45 to 35.02 years (Table 2). About 85% of the production workers, as compared to 52.5% of the administrative and 72% of maintenance workers were below the age of 35 years (Table 3), indicating that comparatively younger people were employed in production department as formulators.

Neurobehavioral effects

Functional integrity of the brain function of study population was assessed by neurobehavioral test (NBT) and the results are shown in Table 4. NBT was applied to all the three groups of workers to evaluate pesticide effects on tweezer dexterity, finger dexterity, hand steadiness, card sorting (design and face value), hand dynamometer, mental control, memory (forward and backward) and digit symbol test. The data were analyzed statistically on the test performance of all the three groups. While analyzing the data, three factors (total score, error and percentage of accuracy) were considered to see the effect of pesticides on the neurobehavioral test performance.

One-way analysis of variance was carried out to compare the different NBT parameters, followed by appropriate post-hoc tests like least significant difference test, Tuckey test and Games-Howell test. Tuckey test showed that exposed group had relatively lower performance in tweezer dexterity (total score), as compared to maintenance group (39.58 ± 1.09 *vs*. 41.71 ± 41.71), but showed significant decrease in tweezer dexterity accuracy (accuracy %: 91.30 ± 1.160 *vs*. 94.54 ± 0.770, P <0.05). Further, finger dexterity (accuracy %: 89.67 ± 1.210) and hand dynamometer (5.89 ± 0.140) were significantly lower in maintenance group, when compared with control group (accuracy %; 93.47 ± 1.01, P <0.05) and hand steadiness (5.19 ± 0.16, P <0.01).

The post-hoc test showed significantly lower performance in maintenance group in card sorting (accuracy %; 98.79 ± 0.22) and mental control 45” (20.43 ± 1.55), as compared to control group performance in card sorting (Face) (accuracy %;

Table 4—Neurobehavioural test performance of study population

Descriptive	Control		Maintenance		Exposed		ANOVA	
	N	Mean	N	Mean	N	Mean	F	Sig
Tweezer Dext (total score)	40	42.13±1.24	49	41.71±0.990	71	39.58±1.090	1.612	0.203
TD Accuracy (%)(a)	40	93.01±0.99	49	94.54±0.770	71	91.30±1.160	2.520	0.084
TD Error (%)	33	8.48±1.03	40	6.69±0.820	67	9.22±1.200	1.292	0.278
Finger dext (total score)	40	29.05±0.99	49	29.76±0.840	71	30.58±0.830	0.737	0.480
FD Accuracy (%) (a)	40	93.47±1.01	49	89.67 ±1.210**	71	90.99±0.870	2.890	0.059
FD Error (%)	29	9.01±1.07	44	11.51±1.230	65	9.84±0.880	1.195	0.306
Hand steadiness (b)	40	5.19±0.16	49	5.89±0.140*	71	5.58±0.120	5.204	0.006
Card sorting (design) (c)	39	41.31±1.35	49	41.61±1.120	71	41.41±0.920	0.017	0.983
Card Sorting Accuracy (%)	39	98.93±0.35	49	98.95±0.240	71	98.42±0.390	0.782	0.459
CS Error (%) per	10	3.94±0.69	15	3.31±0.270	27	3.86±0.660	0.238	0.789
Card Sort(Face)	39	116.51±0.41	49	118.14±1.350	71	115.96±0.310	2.209	0.113
CSF (accuracy %) (a)	39	99.38±0.16	49	98.79±0.220	71	98.93±0.160	2.374	0.096
CSF(error)	14	1.72±0.24	25	2.37±0.260	41	1.85±0.200	1.802	0.172
Hand dynamometer	40	39.65±1.18	49	39.29±1.130	71	40.08±0.770	0.182	0.833
Mental control (Total)30”	40	8.95±0.58	49	9.35±0.400	71	9.08±0.290	0.227	0.797
Mental control (error)	3	1.33±0.33	13	1.38±0.180	13	1.46±0.240	0.052	0.949
Mental control (total) 45”(a)	40	16.23±1.00	49	20.43±1.550	71	18.73±1.040	2.442	0.090
Mental control (error)	39	2.38±0.52	49	2.94±0.52	71	3.30±0.47	0.775	0.462
Memory forward (b)	40	6.08±0.18	49	4.96±0.230#	71	5.79±0.1	11.073	0.001
Memory backward (b)	40	4.73±0.16	49	3.47±0.220#	71	4.37±0.13	13.075	0.001
Digit symbol total (b)	40	66.15±1.82	49	58.0±1.730#	71	65.24±1.24	7.794	0.001
DST accuracy% (c)	40	98.75±0.51	49	96.79±0.660	71	96.66±0.67	2.666	0.073
DST Error % (c)	40	1.25±0.51	49	3.22±0.660	71	3.34±0.67	2.666	0.073
MC 30” (accuracy %)	40	99.05±0.546	49	96.81±0.83	71	97.74±0.64	2.12	0.123
MC 45” (accuracy%)	39	90.36±2	49	88.83±1.92	71	87.85±1.67	0.436	0.647

(a) Comparison is done by least significant difference test; (b) Tuckey post-hoc test; and (c) comparison by Games Howell post-hoc test ± - Standard deviation

Table 5—Levels of Immunological profile and thyroid hormones in study subjects

Parameters	Control group (n=12)	Maintenance group (n=11)	Exposed group (n=28)
IgA (mg/dL)	281.88±19.27	291.39±11.68	272.42±15.51
IgG (mg/dL)	1455±55.32	1473.86±56.55	1374.10±30.68
IgM (mg/dL)	160.13±10.12	182.09±10.72	194.22±7.82*
T_3 (nmol/L)	2.36±0.14	2.32±0.14	2.42±0.10
T_4 (nmol/L)	138.58±7.29	127.82±9.11	131.11±5.60
TSH (mIU/L)	3.14±0.55	4.08±1.36	3.06±0.41

Data presented as mean ± SE; **P* <0.05

99.38 ± 0.16, *P* <0.05) and mental control 45” (16.23 ± 1.00, *P* <0.05). Significantly lower performance was also observed in the post-hoc test in maintenance group in memory (forward) (4.96 ± 0.23), memory (backward) (3.47 ± 0.22), digit symbol (total) (58.0 ± 1.73), as compared to control and exposed groups. Although digit symbol (accuracy %; 96.79 ± 0.66) was significantly lower, digit symbol (error %; 3.22 ± 0.66) was significantly higher in maintenance group, when compared to control group. The post-hoc test showed that maintenance group had significantly lower performance in card sorting (accuracy %; 98.79 ± 0.22) and mental control 45” (20.43 ± 1.55), compared to control group performance in card sorting (Face) (accuracy %; 99.38 ± 0.16, *P* <0.05), mental control 45” (16.23 ± 1.00, *P* <0.05). The post-hoc test maintenance group also showed significantly lower performance in memory (forward) (4.96 ± 0.23), memory (backward) (3.47 ± 0.22), digit symbol (total) (58.0 ± 1.73), compared to control and exposed groups. But, digit symbol (accuracy %; 96.79 ± 0.66) was significantly lower and digit symbol (error %; 3.22 ± 0.66) was significantly higher in maintenance group, when compared to control group.

In this study, the exposed group of workers indicated significant differences in poor percentage in tweezer dexterity (accuracy %) test, but no significant differences were found in total score in the same test, indicating speed of the work was not affected. In one of the studies, tremor has been found to be related to exposure to multiple pesticides[14], but not to OP insecticides[15,16]. Grip strength has not been found to be related to exposure to fumigants[17,18] or multiple pesticides[19]. Although most studies have reported deficits in one or more tests of cognitive function while different tests are reported to be affected in different studies. However, in a few studies, no relationship of OP exposure has been found to any test[20-22]. However, Kamal and Hoppin[23] reported increased prevalence of neurologic symptoms and changes in neuro-behavioural performance in moderate pesticide exposure that reflect cognitive and psychomotor dysfunction.

Thyroid function and immunoglobulin function

In a sub-set of present study population, immunological profile and thyroid function were assessed by measuring immunoglobulins and thyroid hormones, respectively. A significant increase (*P* <0.05) in the level of serum IgM in exposed group was observed, when compared to control group (Table 5). Our earlier study[11] on pesticides formulator has indicated a positive correlation between serum IgM and IgA (r=0.407; *P* <0.05), indicating immune-modulation in pesticide-exposed population. Immuno-modulation has also been reported in workers exposed to pesticides in agricultural environment with decrease in odd ratio for IgM. It is now being increasingly clear that long-term and low-dose exposure to pesticides may cause immune-suppression[24]. Earlier, we have also reported[13] a significant decrease in RBC’s cholinesterase (ChE) activity (*P* <0.05) in exposed subjects of this study population, indicating appreciable exposure to their working environment. Thyroid hormones profile was in normal range in all study groups, however, levels of TSH was relatively increased in maintenance group, but was statistically insignificant (Table 5). Our earlier study[12] has also shown significant elevation in TSH level in pesticide formulators. However, small sample size in the present study might be one of the factors for insignificant level of TSH. Earlier, thyroid disease among women exposed to pesticides in agriculture work has also been reported[25].

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