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Dialkyl Phosphates in Urine Samples from Pesticide Formulators Exposed to Disulfoton and Phorate

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Organophosphorus (OP) compounds are widely used as insecticides. When humans are exposed to these insecticides, alkyl phosphate metabolites are excreted into the urine, and plasma and erythrocyte acetylcholinesterase (ChE) levels may be depressed. Eight alkyl phosphates may be identified in urine of persons exposed to OP insecticides. These metabolites are dimethyl phosphate (DMP), diethyl phosphate (DEP), dimethyl thiophosphate (DMTP), diethyl thiophosphate (DETP), dimethyl dithiophosphate (DMDTP), diethyl dithiophosphate (DEDTP), dimethyl phosphorothiolate (DMPTh), and diethyl phosphorothiolate (DEPTh). Exposure to some OP insecticides may be determined by measurement of urinary alkyl phosphates (MORGAN et al. 1977, SHAFIK et al. 1973).

Metabolites of the OP insecticides, disulfoton (O,O-diethyl S-{2-(ethylthio)ethyl} phosphorodithioate) and phorate (O,O-diethyl S-{(ethylthio)methyl} phosphorodithioate), were quantitated in daily urine specimens. Plasma and erythrocyte ChE levels were also measured in blood samples obtained weekly from pesticide formulators. The formulators worked with disulfoton for the first 25 weeks of this study, and with phorate for the next ten weeks. The purpose of this study was to determine the specific dialkyl phosphates excreted following exposure to disulfoton and phorate, and to compare the increase in urinary alkyl phosphates to the depression of plasma ChE as indicators of OP exposure.

MATERIALS AND METHODS

Participants. Eleven employees of a pesticide formulating plant voluntarily participated in this study. Only three formulators (RF, RB, RT) who had worked at the plant more than two years were available for study during the entire nine month period. The remaining formulators were hired after the study began. All formulators were males between the ages 22 and 52. Eight formulators (GM, JO, RP, JM, JK, CC, BW, RC) had no previous occupational exposure to OP insecticides.

Reagents. Analytical grade HCl, nanograde acetone and hexane, reagent grade NaCl and NaOH were used. N-amyl-N'-nitro-N-nitrosoguanidine was obtained from Aldrich Chemical Company, Milwaukee, WI, and was used to prepare the diazopentane reagent as described by SHAFIK et al. (1973). A fortified sample of urine

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containing DMP, DEP, DMTP, DETP, DMDTP, and DEDTP was obtained from the Iowa Epidemiologic Studies Program (ESP), University of Iowa, Oakdale, IA, and was analyzed once for every 20 urines tested. CG-400 ion exchange resin, 100-200 mesh, was obtained from Rohm and Haas, Philadelphia, PA. Acetylcholine iodide was obtained from Eastman Kodak Company, Rochester, NY, and was dissolved in distilled water to produce a 0.11 N solution (0.7510 g acetylcholine iodide and 25 mL distilled water).

Alkyl phosphate procedure. Urine specimens were collected each weekday morning from each formulator. Samples (100-150 mL) were collected in polypropylene disposable containers and immediately refrigerated at the formulating plant. Each week the specimens were transported to the laboratory where they were kept frozen until the alkyl phosphates could be determined.

All urine samples were extracted using a slight modification of the procedure described by LORES & BRADWAY (1977). An ion-exchange column was prepared by mixing 1 g of CG-400 resin in 10 mL 0.1 N HCl to form a slurry. This slurry was added to a 9 inch disposable pipet that was plugged with a small amount of glass wool. The column was rinsed with 5 mL 0.1 N HCl followed by 50 mL distilled water. Two ml of urine, instead of 1 ml as suggested by LORES & BRADWAY, were treated with 10 mL acetone to remove alkyl phosphates from some interferring compounds. The urine and acetone were combined in a 15-mL glass stoppered centrifuge tube, mixed well, and centrifuged at 650 g for ten min. The supernatant was transferred to the column and the residue remaining in the centrifuge tube was extracted with 2 mL acetone. Following centrifugation, this supernatant was also added to the column. The column was allowed to drain as much as possible (usually 5 min) prior to transferring the resin to a 15 x 150 mm culture tube. The empty column was rinsed with 1 mL acetone and the rinse was added to the culture tube. The CG-400 resin was acidified with 0.05 mL 6 N HCl and allowed to stand for one h with only an occasional shaking of the resin. The diazopentane reagent was slowly added to the resin until a faint yellow supernatant was obtained. This usually required about 1 mL of the diazopentane reagent. The treated resin was vortexed every ten min for one h. The supernatant was transferred to a 13-mL graduated centrifuge tube, and the resin was washed with small portions (2-3 mL) of hexane until a total of 10 mL was obtained. Following centrifugation (650 g, 10 min), the supernatant was transferred to another 13-mL graduated centrifuge tube and concentrated to 2 mL with a stream of dry air. This solution was then analyzed by gas chromatography with a flame photometric detector (SHAFIK et al. 1973).

Threshold levels of alkyl phosphates were established after analyzing 24 urine samples collected from three formulators prior to the formulation of desulfoton and phosate. The threshold levels (Table 1), defined as two standard deviations above the mean, were used for comparing alkyl phosphate levels in pre- and

post-formulation urine samples. No DMDTP (limit of detection = 0.004 ppm) was observed in the pre-formulation urine samples, therefore no threshold value was established.

TABLE 1

Mean and upper threshold levels of urinary alkyl phosphates measured in pre-formulation urine specimens.

Metabolite	Mean(ppm)	Threshold(ppm)
DMP	0.040	0.14
DEP	0.050	0.13
DMTP	0.180	0.32
DETP	0.040	0.12
DEDTP	0.010	0.06
DMPTh	0.004	0.01
DEPTh	0.008	0.06

Acetylcholinesterase procedure. Plasma and red cell ChE levels were determined using a modification of the NABB & WHITFIELD (1967) method as described in the Manual of Analytical Methods for Analysis of Pesticides in Humans and Environmental Samples (EPA 1980). This titrimetric method required the use of a pH Stat that was linearized with pH 4.01 and pH 8.00 buffers and standardized with primary standard potassium acid phthalate.

The normal ranges in moles/min/mL for ChE levels determined by this procedure are 2.00-7.00 for plasma ChE, and 8.00-17.00 for red cell ChE. Formulators were considered poisoned if the plasma or red cell ChE levels were below 2 or 8 μmoles/min/mL, respectively.

RESULTS

The percentage of urine specimens from the formulators that had specific alkyl phosphate levels above the threshold levels during the formulation of disulfoton and phorate is summarized in Table 2. The predominant alkyl phosphates found in urine during disulfoton formulation were DEP, DEPTh, and DETP. The percentage of urine samples from ten formulators who had DEP, DEPTh, and DETP above the threshold levels were 56, 34, and 19%, respectively. DEDTP and the four dimethyl metabolites exceeded the threshold levels in only 11% or less of the urines analyzed. The excretion of alkyl phosphates varied considerably among the individual formulators during disulfoton formulation. For example, all urines from CC and only 23% of urines from RF had DEP above the threshold. Sixty-eight % of 313 urines collected daily from ten formulators during disulfoton formulation contained one or more alkyl phosphates above the threshold level. The range of diethyl phosphates in urine samples during desulfoton formulation were DEP (.01-4.40 ppm), DETP (.01-1.57 ppm), DEDTP (<.01-.05ppm) and DEPTH (<.01-.55 ppm).

TABLE 2

Percentage of urine specimens from formulators that had specific alkyl phosphate levels above the threshold level during the formulation of disulfoton and phorate.

Pesticide Formulated	Formulator	Number Specimens	DEP	DETP	DEDTP	DEPTh	DMP	DMTP	DMPTh
Disulfoton	RF	57	23	10	0	14	7	16	10
	RB	57	65	38	0	35	0	12	5
	RT	56	32	11	2	20	0	11	7
	GM	52	78	25	0	40	25	7	4
	JO	28	68	43	4	18	12	4	4
	RP	9	44	2	0	33	0	0	0
	JM	18	94	44	0	83	0	6	0
	RK	14	78	14	0	57	0	7	14
	CC	16	100	50	0	100	0	0	0
	RC	6	67	0	0	50	0	0	0
Total		313	56	19	1	34	6	9	6
Phorate	RF	23	61	35	13	61	0	13	17
	RB	45	96	67	4	82	2	9	16
	RT	16	94	31	0	50	6	25	6
	JM	52	96	77	2	86	8	8	6
	RK	44	98	70	14	89	7	7	9
	CC	18	100	33	11	56	11	11	5
	BW	5	80	60	0	100	20	0	0
	RC	19	100	53	0	79	0	5	16
Total		222	93	60	6	78	5	9	10

The predominant alkyl phosphates found in urine during phorate formulation were DEP, DEPTh, and DETP (Table 2). The percentage of urine samples from eight formulators that had DEP, DEPTh, and DETP above the threshold level were 93, 78, and 60%, respectively. DEDTP and the dimethyl metabolites exceeded the threshold levels in 10% or less of the urines analyzed. Ninety-eight % of the 222 urine specimens collected during phorate formulation contained one or more alkyl phosphates above the threshold level. The range of diethyl phosphates in urine samples during phorate formulation were DEP (.02-5.14 ppm), DETP (.08-4.08 ppm), DEPTP (<.01-.43 ppm), and DEPTh (.04-2.13 ppm).

Although elevated DEP, DETP, and DEPTh levels were identified in urine during disulfoton formulation, the plasma and erythrocyte ChE levels showed no significant reduction. During disulfoton formulation, all plasma ChE levels were greater than 3 μmoles/min/mL, and 78% were greater than 4 μ moles/min/mL. During phorate formulation, two formulators had depressed plasma ChE levels. The plasma ChE levels were lower during phorate formulation than during disulfoton formulation. Twelve % of the plasma ChE levels were 3 μ moles/min/mL or less during phorate formulation; whereas, no plasma ChE levels below 3 μ moles/min/mL were observed during disulfoton formulation. Individual variations in the plasma ChE levels during the formulation of the two OP insecticides were evident. For example, during disulfoton formulation, 20% of JM's plasma ChE levels were less than 4 μ moles/min/mL; whereas, 91% were less than this level during phorate formulation.

DISCUSSION

Each pesticide formulator was not exposed to the same amount of OP insecticide during the entire 35 weeks of the study. Although new employees were first assigned jobs with minimum exposure, they generally exhibited a higher level of diethyl phosphates than the formulators with more experience. The formulators were provided with protective equipment, including work clothes, gloves, boots and cartridge respirators, but based on the elevation of urinary alkyl phosphates and depression of plasma ChE levels, the protective equipment was inadequate or, more likely, not properly used.

One formulator (CC) experienced neurological symptoms and had a plasma ChE of 1.7 μmoles/min/mL following exposure to phorate while cleaning a large mixing tank. The symptoms included headache, dizziness, nausea, sweating, and weakness. CC's DEP level averaged 1.6 ppm the week prior to cleaning the tank and 3.5 ppm the week following this exposure to phorate. Another formulator (RC) had significantly reduced ChE levels during the study. His plasma ChE dropped from 5.1 to 2.5 μmoles/min/mL, and his red cell ChE dropped from 18.0 to 9.5 μ moles/min/mL during the nine weeks that he worked with phorate. The approximate 50% reduction in both plasma and red cell ChE was accompanied by a

50-fold increase in his DEP level. His DEP level rose from 0.07 ppm to 3.5 ppm while working with phorate.

The source of the apparent low levels of dimethyl phosphates in urine may be related to the environment within the formulating plant. During the previous year, small quantities of methyl parathion and naled were formulated. The formulation of these dimethyl oP insecticides resulted in the unavoidable contamination of the working environment. One or more of the dimethyl phosphrates were detected in each of the urine samples analyzed, however, the household levels of DMP, DMTP, and DMPTh were exceeded in only 10% or less of the urine samples. A metabolite with the same retention characteristics as DMDTP was obtained from 11% of the urine samples during formulation of disulfoton, and 42% of the urines during formulation of phorate.

Diethyl OP insectices, such as disulfoton and phorate, are broken down to the diethyl metabolites, whereas the dimethyl OP insecticides, such as methyl parathion and dimethoate, produce the dimethyl phosphate in urine. MORGAN et al. (1977) compared the urinary alkyl phosphates present in human urine following oral exposure to methyl and ethyl parathion, and found that DMP was associated with the dimethyl form and that DETP appeared consistently in urine following ingestion of the diethyl form. Analysis of urine for specific alkyl phosphates may be useful for identifying the type of organophorphorus insecticide involved in pesticide poisoning. Since numerous OP insecticides produce similar metabolites, the value of alkyl phosphate analyses is limited to determining if either a dimethyl or diethyl OP compound was involved in the incident.

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