Bull. Environ. Contam. Toxicol. (2005) 74:809–816

DOI: 10.1007/s00128-005-0653-8

Bulletin of Environmental Contamination and Toxicology

Determination of Urinary Metabolites of Phosalone, Methidathion, and IBP after Oral Administration and Dermal Application to Rats

K. J. Min,[1] C. G. Cha,[2] W. Popendorf[3]

[1] Department of Public Health, Keimyung University, Daegu 704-701, Korea
[2] The Center for Traditional Microorganism Resources, Keimyung University, Daegu 704-701, Korea
[3] Department of Biology, Utah State University, Logan UT 84322, USA

Received: 9 December 2004/Accepted: 14 February 2005

The use of organochlorine pesticides has largely been banned or restricted after evidence of their toxicity, persistence and bioconcentration in the aquatic environment became apparent. For this reason, organophosphorus pesticides are widely used in agriculture (Serrano *et al.*, 1997). In Korea, phosalone (*S*-5-chloro-2,3-dihydro-2-oxobenzoxazol-3-ylmethyl *O,O*-diethyl phosphorodithioate), methidathion [*S*-(2,3-dihydro-5-methoxy-2-oxo-1,3,4-thiadiazol-3-ylmethyl) *O,O*-dimethyl phosphorodithioate], and IBP or iprobenfos (*S*-benzyl *O,O*-diisopropyl phosphorodithioate) are widely used for the control of pests such as white fly, rocket, caterpillar, beetle, scale, lygus, and aphid (Montgomery, 1996).

The occupational hazards of organophosphorus pesticides are high because toxic effects may occur following either inhalation, ingestion, or skin absorption. The majority of organophosphorus pesticides are lipophilic and not ionized; they are absorbed rapidly following inhalation or ingestion. Dermal absorption is slower, but severe poisoning may still ensue if exposure is low but prolonged (Spear *et al.*, 1977; Vale, 1998). Organophosphorus pesticide exposure can be assessed by biological monitoring methods such as blood cholinesterase inhibition or urinary metabolite excretion (Richardson and Seiber, 1993). The inhibition of blood cholinesterase activity has often been used as a measure of acute exposure to these pesticides, but the method is not sensitive to the small inhibitions caused by the chronic exposure levels commonly encountered in occupational settings (Aprea *et al.*, 1996).

Most organophosphorus pesticides are metabolized to dialkyl phosphates in humans and other animals. Thus, the measurement of dialkyl phosphate metabolites has also been used to determine exposure to organophosphorus pesticides (Spear *et al.*, 1977; Churchill *et al.*, 1978; Miles and Dale, 1978; Moody *et al.*, 1985; He, 1993). The six dialkyl phosphates (*O,O*-dimethyl phosphate (DMP), *O,O*-diethyl phosphate (DEP), *O,O*-dimethyl phosphorothioate (DMTP), *O,O*-diethyl phosphorothioate (DETP), *O,O*-dimethyl phosphorodithioate (DMDTP), and *O,O*-diethyl phosphorodithioate dimethyl phosphate (DEDTP) constitute the major metabolites of most organophosphorus pesticides (Lauwerys and Hoe, 1993).

Correspondence to: C. G. Cha

This study was aimed to determine the elimination kinetics of oral and dermal doses of phosalone, methidathion and IBP as measured by urinary dialkyl phosphate metabolites.

MATERIALS AND METHODS

Phosalone (98% purity), methidathion (99% purity) and IBP (95.5% purity) were obtained from Kyung Nong corporation in Korea and used without further purification. Dialkylphosphates (95% purity) were purchased from Ultra Scientific Inc. (N. Kingstown, RI, USA). The butylating agent, tetrabutyl-ammonium hydroxide (TBAH), was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). All solvents used were pesticide residue grade with no further treatment.

Male Sprague-Dawley rats (180~200 g), five per dose group, were used in this study. Rats were housed 1/cage in Nalgene metabolic cages (Rochester, NY, USA) that were cleaned daily to minimize fecal contamination. Standard laboratory chow and water were furnished *ad libitum*. The lights of the animal room were kept on from 6 am to 6 pm; temperature, at 22±1°C; and relative humidity, at 55±5%. Rat urine samples were collected in metabolic cages for increasing time intervals up to 168 hours after oral administration and dermal application of phosalone, IBP, and methidathion as shown in Figs. 1 and 2. Blank urine samples were collected for 24 hr prior to beginning the exposure of the rats to the pesticides. Urine samples were frozen and stored at -30°C until analyzed.

Phosalone, methidathion, and IBP were dissolved in propylene glycol and given orally at selected doses of 10 mg (0.2 LD_{50}) for phosalone, 2.16 mg (0.1 LD_{50}) for methidathion, and 9.8 mg (0.1 LD_{50}) for IBP (Montgomery, 1996). All oral solutions were prepared so that 5 mL of solution was given per kg of body weight. Phosalone, methidathion, and IBP were dissolved in propylene glycol and applied dermally at selected doses of 7.8 mg (0.1 LD_{50}) phosalone, 66.5 mg (0.1 LD_{50}) methidathion, and 80 mg (0.1 LD_{50}) IBP (Montgomery, 1996). All of the dermal solutions were prepared so that 5 mL of solution was applied per kg of body weight. Pesticides were applied directly to a clipped area of 6.25 cm^2 (2.5 cm × 2.5 cm) on the back and sides of each rat, uniformly spread, and covered with a semi-occlusive dressing for 96 hours.

The analytical method employed was that of Richardson and Seiber (1993). A 1 mL aliquot of urine was transferred to a 15 mL screw-cap centrifuge tube, followed by 1 mL 6N-HCl. After vortexing for 1 min, 1 mL of 5% ethanol/ethyl acetate was added. The tubes were capped, vortexed again for 2 min, and centrifuged (2000 rpm) for 15 min. A 1 mL aliquot of the organic phase was transferred to a second tube and held in an ice bath. After discarding the residual solvent, the extraction was repeated and the second 1 mL organic extract combined with the first. The combined filtrate was then dried in a rotary evaporator under vacuum at 35°C. The residue was redissolved in 1 mL of ethyl acetate. Forty microliters of TBAH (tetrabutylammonium hydroxide, 1.0 M in

methanol, Aldrich Chemical Co.) was added for each 1 mL portion of extract dissolved in ethyl acetate.

Identification of the derivatized urinary metabolites was determined by GC/MS using a Hewlett-Packard model 5890 Series II Plus gas chromatograph directly interfaced to an HP 5972 mass selective detector. For the data were analyzed using an HFG 1034C MS Chemstation. An HP5-MS column (length 30 m, id 0.25 mm, film thickness 1 μm) was coupled to the ion source. Helium at a flow rate 0.7 mL/min was employed as a carrier gas. Samples were split injected at a split ratio of 10:1. The injector and transfer line in the GC/MS were at 280°C and 300°C, respectively. The oven temperature was set at 80°C initially, subsequently increased by 5°C per min to 150°C, held there for 1 min, subsequently increased by 10°C per min to 300°C, and held there for 1 min. A 2 μL aliquot of the derivatized sample was injected. The mass spectrometer was operated at 70 eV in the electron impact (EI) mode using SCAN.

The derivatized urinary metabolites were quantified by GC/NPD. A Thermo-Finnigan TRACE GC equipped with nitrogen phosphorus detector (NPD) was used. A 2 μL volume was injected in all GC analyses. The analytical column used for the analysis of the parent organophosphorus pesticides and their dialkyl phosphate metabolites was a 30m×0.53mm×1.0μm DB-17 (J&W Scientific). The nitrogen carrier gas flow rate was 1 mL/min. Operating temperatures were as follows: detector, 300°C; column programs, 80°C for 0 min, 5°C/min to 150°C, 10°C/min to 270°C, 270°C for 5 min; split ratio (10:1); and injector, 280°C. However, none of the three parent compounds was detected in any urine sample.

RESULTS AND DISCUSSION

Fortification/recovery studies were conducted by introducing known quantities of dialkyl phosphate standards into the urine of one unexposed rat. Average recoveries (n = 6) were more than 87% for all dialkyl phosphates (Table 1).

Table 1. Average recoveries of dialkyl phosphates from fortified urine samples

spiked level	% recovery (mean ±S.D.) (n=6)					
(μg/L)	DMP	DMTP	DMDTP	DEP	DETP	DEDTP
10	93±4.2	90±2.3	87±4.6	91±2.2	89±6.1	87±4.4

The same urinary metabolites were detected following both oral and dermal exposures of each insecticide. DEP, DETP, and DEDTP were detected in rat urine after both oral administration and dermal application of phosalone. The identity of the molecular ions of these diethyl phosphate metabolites was confirmed by GC/MS at m/z ratios of 181, 226, and 242, respectively.

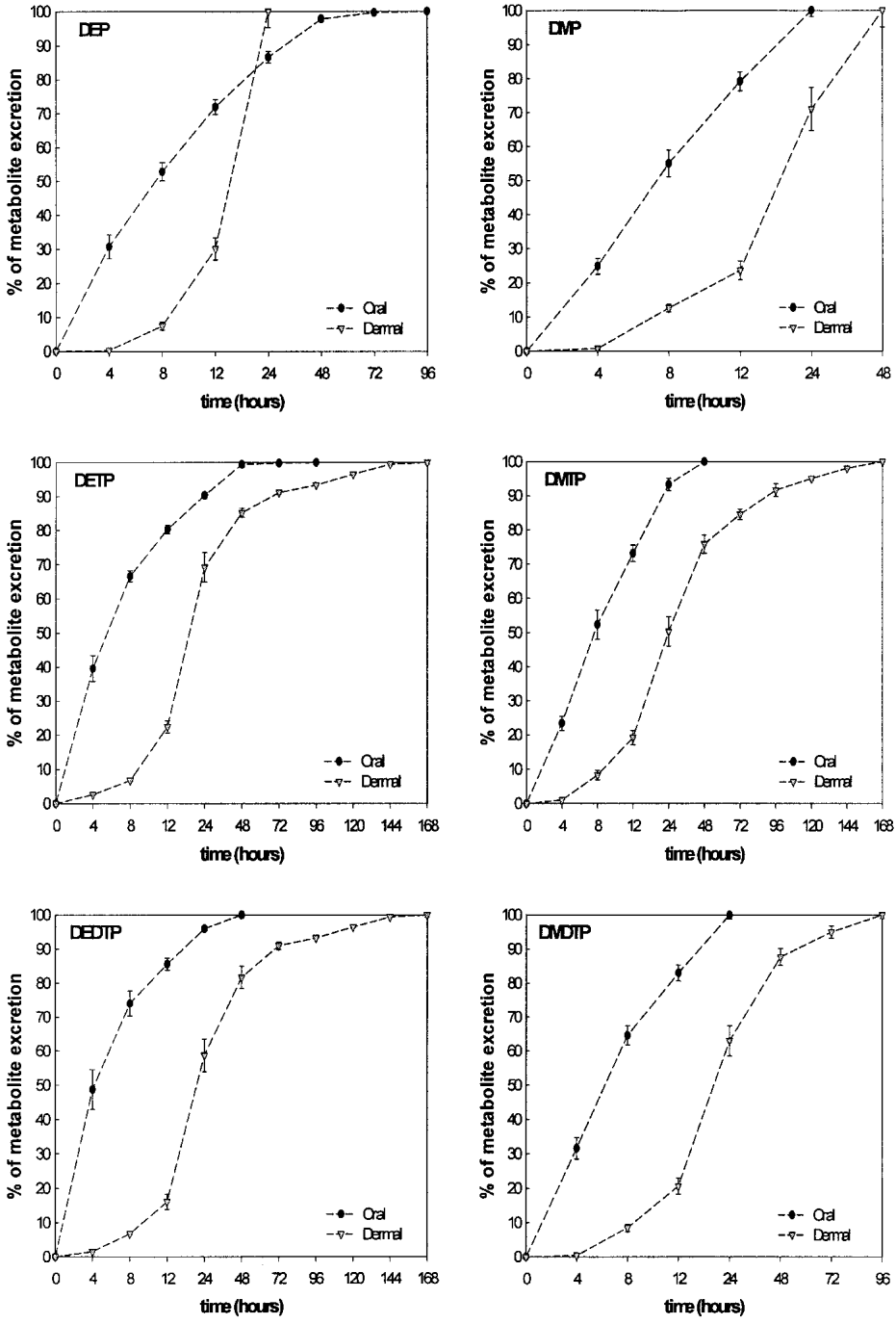

Figure 1. Comparison of the cumulative excretion of: DEP, DETP, and DEDTP from oral and dermal exposures of phosalone to rats: DMP, DMTP, and DMDTP from oral and dermal exposures of methidathion to rats. Values are mean ± S.D., n = 5.

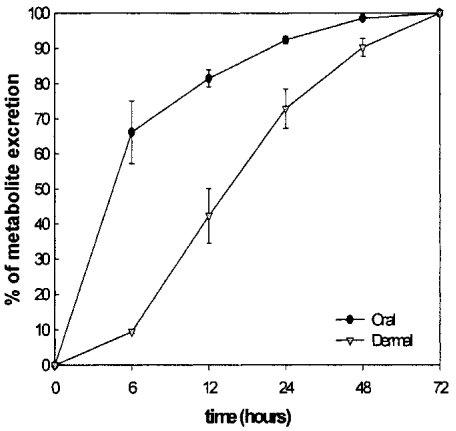

Figure 2. Comparison of the cumulative excretion of urinary diisopropyl phosphorothioate from oral and dermal doses of IBP to rats. Values are mean ± S.D., n = 5.

DMP, DMTP, and DMDTP were detected in rat urine after both oral administration and dermal application of methidathion. The identity of the molecular ions of these dimethyl phosphate metabolites were confirmed by GC/MS at m/z ratios of 153, 198, and 158, respectively. For IBP, only the single urinary metabolite of diisopropyl phosphorothioate was detected in rat urine after both oral administration and dermal application of IBP. The mass spectrum of its ion was confirmed by GC/MS at an m/z ratio of 254.

The excretion time courses of the derivatized urinary metabolites of phosalone are shown in Fig. 1. After oral administration, 98% of DEP, 99% of DETP, and 100% of DEDTP were excreted into the urine within 48 hours. However, excretion following dermal application was slower: 100% of DEP, but only 85% of DETP and 82% of DEDTP were excreted within 48 hours, and about 120 hours were required for 95% of the DETP and DEDTP to be excreted into the urine. These latter two metabolites accounted for about 80% of all the urinary metabolites from phosalone. While almost all of the dialkyl phosphates from oral administration were excreted within 48 hours, the metabolites from dermal application were excreted for up to 144 hours.

The excretion time courses of the derivatized urinary metabolites of methidathion are shown in Fig. 1. The metabolites of methidathion were excreted faster than those of phosalone. All of the DMP and DMDTP and 94% of the DMTP were excreted into the urine within 24 hours of its oral administration, and 100% of all three was excreted within 48 hours. Again excretion after dermal application is slower than after oral administration: 100% of DMP, 75% of DMTP, and 87% of DMDTP were excreted into the urine within 48 hours. DMTP was the slowest of these, requiring about 120 hours for 95% of it to be excreted. While almost all of the dialkyl phosphates from oral administration were excreted within 48 hours, metabolites from dermal application were excreted for up to 168 hours.

The excretion time courses of the derivatized urinary metabolite of IBP shown in Fig. 2 follow the same pattern of dermal doses being slower than oral doses. After oral administration, 100% of diisopropyl phosphorothioate was excreted into the urine within 48 hours, while only 87% of diisopropyl phosphorothioate was excreted into the urine within 48 hours; 72 hours was required for 95% excretion after dermal application.

Table 2. Excretion of DEP, DETP, and DEDTP from oral and dermal doses of phosalone to rats.

	Total dose of phosalone (nmol)	Total recovered as urinary metabolites (nmol)			Total % recovered
		DEP	DETP	DEDTP	
Oral administration	27,189	1509 ±422	2917 ±875	5634 ±1405	37 ±10.3
Dermal application	21,207	151 ±33	224 ±54	346 ±73	3.4 ±0.7

Each value represents the mean ±S.D. of five rats.

Table 3. Excretion of DMP, DMTP, and DMDTP from oral and dermal doses of methidathion to rats.

	Total dose of methidathion (nmol)	Total recovered as urinary metabolites (nmol)			Total % recovered
		DMP	DMTP	DMDTP	
Oral administration	7,145	462 ±115	429 ±90	209 ±42	15.4±3.4
Dermal application	219,980	2402 ±793	2217 ±687	1540 ±431	2.8 ±0.9

Each value represents the mean ±S.D. of five rats.

Table 4. Excretion of diisopropyl phosphorothioate from oral and dermal doses of IBP to rats.

	Total dose of IBP (nmol)	diisopropyl phosphorothioate excreted (nmol)	Total % recovered
Oral administration	33,992	3467 ±936	10.2 ±2.8
Dermal application	277,489	5827 ±1690	2.1 ±0.6

Each value represents the mean ±S.D. of five rats.

The total amounts of metabolites recovered from the oral and dermal doses of phosalone are shown in Table 2. Almost 11 times as much of the oral dose of phosalone was recovered as of the dermal dose. DEDTP was the major metabolite from both the oral and dermal doses of phosalone to rats, accounting for 56% and 48% of the three metabolites, respectively. DETP was the second most common metabolite, accounting for another 29% and 31%, respectively.

The total amounts of metabolites recovered from the oral and dermal doses of methidathion are shown in Table 3. About 5 times as much of the oral dose of methidathion was recovered as of the dermal dose. Most of the metabolites from both oral and dermal doses of methidathion were about evenly split between DMP and DMTP; each accounted for about 40% of the three metabolites found.

Table 4 shows that diisopropyl phosphorothioate comprises the sole metabolite recovered from the oral and dermal doses of IBP. Again (as with methidathion), nearly 5 times as much of the oral dose was recovered as was the dermal dose.

In this study, the urinary excretion following dermal applications was lower and more delayed than from oral administration. Despite the low total percent of the insecticide dose that was actually recovered, the standard deviation of these total fractions was a reasonably consistent 26±4% of the total fraction recovered within each route of dosing of each insecticide. These data suggest extrapolations from total excretion back to dose would be possible within a relative standard deviation of about ±25%.

Drevenkar *et al.* (1979), Vasilic *et al.* (1987), and Vasilic *et al.* (1993) have reported finding these urinary metabolites in humans with unquantified exposures. The early study of Drevenkar of chronic occupational exposures showed the slow elimination pattern of the DEDTP, DETP, DEP metabolites in decreasing concentrations as seen here for dermal exposure to rats. The 1993 study by Vasilic *et al.* of five hospital patients who had ingested acute doses phosalone showed highly variable urinary half-lives with higher proportions of DEP than seen here. Vasilic *et al.* (1987) found a higher proportion of DMTP than DMDTP (up to six times more among mixers) and no DMP in the urine from thirty people working with methidathion, in comparison to the roughly equal proportions of DMP and DMTP found here in rats.

The results reported here should be useful in providing a more quantitative basis between a suspected ingested or dermal dose by people and measured urinary excretion of DEP, DETP, and DEDTP from phosalone, urinary diisopropyl phosphorothioate from IBP, or urinary DMP, DMTP, DMDTP from methidathion. In addition, differences in the ratio among these metabolites between patients or users of these chemicals reported in other studies and the rats reported here suggest that more research is needed. For instance, further work should be done to explore the effect of dose level on urinary excretion, and similar research could be done to explore the excretion of metabolites following exposure to the thiophosphate oxon analogues commonly formed in the environment by some of these compounds.

Acknowledgments. This work supported (in part) by the Ministry of Science & Technology (MOST) and the Korea Science and Engineering Foundation (KOSEF) through the Center for Traditional Microorganism Resources (TMR) at Keimyung University.

REFERENCES

Aprea C, Sciarra G, Lunghini L.(1996) Analytical method for the determination of urinary alkyl phosphates in subjects occupationally exposed to organophosphorus pesticides and in the general population. J Anal Toxicol 20:559-563

Churchill FC, Ku DN, Miles JW (1978) Gas-liquid chromatographic inlet block derivatization of organophosphorus pesticides and related dialkyl phosphorothioates. J Agric Food Chem 26(5): 1108-1112

Drevenkar, V, Frobe Z, Vasilic Z, Tkalcevic B, Stefanac Z (1979) The rate of urinary excretion of phosalone residues in occupationally exposed persons. Sci Total Environ 13(3): 235-244

He F (1993) Biological monitoring of occupational pesticides exposure. Int Arch Occup Environ Heath 65: 69-76

Lauwerys RR, Hoe P (1993) Biological monitoring of exposure to organic substances. In "Industrial chemical exposure; guideline for biological monitoring", CRC Press , pp 256-259

Miles JW, Dale WE (1978) Gas chromatographic determination of organophosphorus pesticides by in-block methylation. J Agric Food Chem 26(2): 480-482

Montgomery JH (1996) Groundwater Chemicals, 2nd edn., Lewis Publishers, Boca Raton, pp 337-344

Moody RP, Franklin CA, Riedel D, Muir NI, Greenhalgh R, Hladka A (1985) A new GC on-column methylation procedure for analysis of DMTP(O,O-dimethyl phosphorothioate) in urine of workers exposed to fenitrothion. J Agric Food Chem 33(3): 464-467

Richardson ER, Seiber JN (1993) Gas chromatographic determination of organophosphorus insecticides and their dialkyl phosphate metabolites in liver and kidney samples. J Agric Food Chem 41(3): 416-422

Serrano R, Lopez F, Roig-Navarro, Hernandez F (1997) Automated sample clean-up and fractionation of chlorpyrifos, chlorpyrifos-methyl and metabolites in mussels using normal-phase liquid chromatography. J Chromatogr A 778: 151-160

Spear RC, Popendorf WJ, Leffingwell JT, Milby TH, Davies JE, Spencer WP (1977) Fieldworkers' Response to Weathered Residues of Parathion. J Occup Med 19(6): 406-410

Vale JA (1998) Toxicokinetic and toxicodynamic aspects of organophosphorus OP insecticide poisoning. Toxicol Lett 102: 649-652

Vasilic Z, Drevenkar V, Frobe Z, Stengl B, Tkalcevic B (1987) The metabolites of organophosphorus pesticides in urine as an indicator of occupational exposure. Toxicol Environ Chem 14:111-127

Vasilic Z, Drevenkar V, Stengl B, Frobe Z, Rumenjak V (1993) Diethylphosphorus metabolites in serum and urine of persons poisoned by phosalone. Chemico-Biol Interact 87:305-313.