Malathion Exposure Studies. Determination of Mono- and Dicarboxylic Acids and Alkyl Phosphates in Urine

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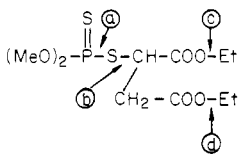
A method for the analysis of the dialkyl phosphate metabolites of organophosphorus pesticides was modified to permit analysis of the monocarboxylic acid (MCA) and dicarboxylic acid (DCA) metabolites of malathion. Recoveries of MCA and DCA from spiked urine are presented, as well as the results of analyses of urine from rats exposed to malathion at five levels. Data from both exposed and unexposed humans are presented.

In support of an epidemiological investigation of pesticide exposure in man, it has become necessary to devise methods for establishing an index of exposure to biodegradable pesticidal compounds. Human exposure to the relatively nonbiodegradable (hard) pesticides usually results in residues of the intact compound in tissues or fluid specimens, by which indices of exposure to these compounds may be established (Thompson, 1972). However, an index of exposure to rapidly degradable pesticides, such as the organophosphorus and carbamate insecticides and the urea herbicides, must be established on the basis of metabolites since the intact compounds may be present in the body for only a brief time.

Shafik et al. (1969, 1971, 1973) and Lores and Bradway (1977) have reported methodology for monitoring organophosphorus pesticide exposure through urinary excretion of the alkyl phosphate metabolites. The methods are based on quantitating alkyl phosphates at levels as low as 0.01 ppm in human urine. A direct relationship between the level of exposure of rats to organophosphorus pesticides and subsequent excretion of alkyl phosphates in the urine was demonstrated (Shafik et al., 1973).

Although the alkyl phosphates constitute the major metabolites of most organophosphorus pesticides, some of these compounds follow other metabolic pathways. In the case of the pesticide malathion, hydrolysis can occur at a



malathion

or b to give the alkyl phosphate metabolites, dimethyl phosphorothionate (DMTP) and dimethyl phosphorodithioate (DMDTP). Hydrolysis at c and/or d produces either malathion α-monocarboxylic acid (MCA) (Chen et al., 1969) or malathion dicarboxylic acid (DCA) which are considered the major metabolites of malathion. Additional metabolites may arise from malaoxon, a metabolite of malathion. It can be assumed that an analogous series of metabolites should be found, differing from malathion metabolites only in that the P=S group has been replaced with P=O.

Kadoum (1969) has developed a method for the analysis of malathion carboxylic acids in grain. The method involves extraction with acetone, methylation with BF_3–methanol, cleanup by silica gel chromatography, and gas chromatographic determination of the derivatized metabolite by electron capture and phosphorus thermionic detection systems.

Table I. Recovery of MCA and DCA from Rat Urine

Spiking level, ppm	% recovery	
	MCA	DCA
1.0	98.8	98.3
0.10	101.0	98.6
0.01	104.0	101.0

The method described in this paper permits broader application of methodology already in general use in monitoring laboratories by providing a specific means for evaluating exposure to malathion.

EXPERIMENTAL SECTION

Materials and Methods. The extraction, alkylation of the urine extracts, and cleanup of the derivatized carboxylic acids were performed according to the procedures reported for the determination of alkyl phosphates in urine (Shafik et al., 1969). Additional cleanup by solvent partitioning and silica gel chromatography was also employed. The gas chromatographic analysis of derivatized MCA and DCA was performed using a flame photometric detector and a 6 ft × 0.25 in. o.d. column packed with 4% SE-30/6% QF-1 on 80/100 mesh Gas-Chrom Q. The column, inlet, transfer lines, and detector were all operated at 200 °C. The flow rates were: nitrogen, 60 mL/min; oxygen, 15 mL/min; air, 50 mL/min; hydrogen, 170 mL/min.

The clean-up procedure reported by Shafik et al. (1971) was modified as follows: an aliquot of urine was extracted twice with equal volumes of diethyl ether, followed by centrifuging to separate the layers. The ether extract was discarded, and the traces of ether remaining on top of the urine were evaporated under a gentle stream of nitrogen. The urine was acidified, extracted, and alkylated as previously described. After methylation, MCA produced the methyl ethyl carboxy ester of malathion and DCA produced the dimethyl carboxy ester of malathion. After ethylation, both carboxylic acids were converted to malathion. MCA and DCA eluted from silica gel with 10 mL of benzene, followed by 10 mL of 10% ethyl acetate in benzene (v/v), collected as one fraction.

Animal Treatments. Male Sprague-Dawley rats weighing 400–450 g were used for the animal feeding experiments. Two rats were fed at each dosage level and were housed together. They were dosed by gavage with peanut oil solutions of malathion at 69 mg (0.1 LD_{50}), 6.9 mg (0.01 LD_{50}), 0.69 mg (0.001 LD_{50}), 0.069 (0.0001 LD_{50}), and 0.0069 mg (0.000 01 LD_{50}) per rat per day for 3 days. The urine was collected in glass bottles over 24-h intervals before, during and for 2 days following the last dose. The

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Table II. Excretion of Malathion Metabolites at Five Levels of Exposure

Day	μM excreted				
	DMTP	DMDTP	DMP	MCA	DCA
			0.1 LD_{50} (69 mg/day)		
1	7.6	3.5	0.80	47	71
2	8.8	4.3	1.2	65	86
3	20	19	0.88	83	140
4	0.41	1.1	0.13	1.9	5.0
5	0.16	0.37	0.80	0.96	2.4
			0.01 LD_{50} (6.9 mg/day)		
1	1.8	0.44	0.63	3.9	16
2	1.4	0.56	0.37	2.8	13
3	2.6	0.92	0.72	3.0	17
4	ND[a]	0.13	ND	ND	0.10
			0.001 LD_{50} (0.69 mg/day)		
1	0.092	0.077	0.15	0.20	0.91
2	0.11	0.051	0.12	0.13	0.85
3	0.23	0.078	0.15	0.074	0.91
4	0.015	0.028	ND	ND	0.13
			0.0001 LD_{50} (0.069 mg/day)		
1	0.026	<0.005	0.032	0.0091	0.090
2	0.017	<0.005	0.016	0.013	0.077
3	0.041	0.0057	0.020	<0.005	0.068
4	<0.005	<0.005	ND	<0.005	<0.005
			0.00001 LD_{50} (0.0069 mg/day)		
1	ND	ND	ND	<0.005	0.014
2	ND	ND	ND	<0.005	0.012
3	ND	ND	ND	<0.005	0.014
4	ND	ND	ND	ND	ND

[a] ND = not detected.

Table III. Excretion of Mono- and Dicarboxylic Acids

Level of exposure	Total μM malathion fed	Total μm MCA excreted	Total μm DCA excreted	MCA/DCA
0.069 mg/day	0.627	0.028	0.24	0.08
0.69 mg/day	6.27	0.40	2.80	0.14
6.9 mg/day	62.7	9.7	46	0.21
69 mg/day	627	200	300	0.66

animals were housed in stainless steel metabolism cages which were cleaned twice daily to minimize fecal contamination.

RESULTS AND DISCUSSION

The recoveries of MCA and DCA from spiked control urine samples at the 0.01, 0.1, and 1.0 ppm levels are shown in Table I. The lowest limit of detectability in both rat and human urine was 0.002 ppm for DCA and 0.005 ppm for MCA. These values are dependent on both detector sensitivity and the presence of interfering peaks. Figure 1 shows a gas chromatogram of rat urine extract for an injection equivalent to 0.8 mL of urine from the first day of the lowest level of exposure (0.000 01 LD_{50}). The peaks from the methyl esters of the two carboxylic acids were easily quantitated. Confirmation of identity was carried out by ethylating the urine extract to convert both the DCA and MCA to malathion.

Urine from rats exposed to malathion at various levels was analyzed for the two carboxylic acids and the alkyl phosphates. The results of the analyses are shown in Table II. The urine from the rats exposed to 0.1, 0.01, 0.001, 0.0001 LD_{50} malathion all contained MCA, DCA, DMTP, DMDTP, and dimethyl phosphoric acid (DMP). Only DCA could be quantitated at the lowest level of exposure (0.000 01 LD_{50}).

In all cases, the major metabolite resulting from exposure of rats to malathion was DCA, although with increasing exposure the relative amount of MCA increased steadily, as shown in Table III. Several investigators (Knaak and O'Brien, 1960; Krueger and O'Brien, 1959; O'Brien, 1960) have reported that the major metabolite of malathion in mammals is MCA. Apparently this is true at higher levels of exposure or when urine is collected a short time after exposure (0.5 h). However, our investigation indicates that at very low levels of exposure and in urine collected over a 24-h interval, DCA is the major metabolite in the rat.

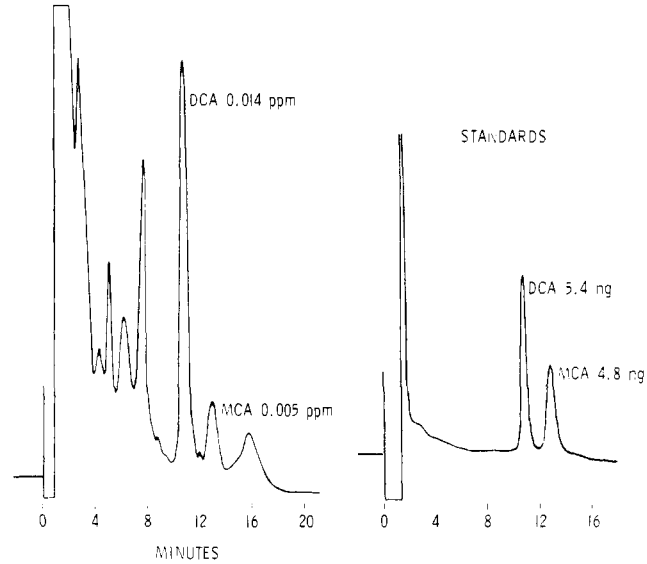

Figure 1. Gas chromatograms of methylated rat urine extract and methylated standard MCA and DCA using the phosphorus mode of a flame photometric detector.

The method for the determination of malathion MCA and DCA was applied to the analysis of urine samples collected from ten people with no history of exposure to

Table IV. Human Urinary Metabolites from a Malathion Poisoning Case

Metabolite	Urine, human control, ppm	Urine, human exposed, ppm
DCA	<0.005	12
MCA	<0.005	223
DMTP	ND	96
DMDTP	ND	20
DMP	ND	50
MMP[a]	ND	8

[a] Monomethyl phosphate.

malathion. Carboxylic acids were found in most of the urines, the highest level being 0.01 ppm of the monoacid. The average of the ten, however, was less than 0.005 ppm of either acid.

Urine was available from a human poisoning case in which a man attempted suicide by drinking about 200 mL of 50% malathion preparation. Urine for the first 24 h was not available, but that from the second 24 h following exposure was analyzed. Table IV shows the results of the analysis for phosphorus-containing metabolites. Malathion monoacid is the most significant metabolite, followed by DMTP and DMP.

The method for the determination of DCA and MCA in urine has proven its value in monitoring animal and human exposure to malathion. In addition to the quantitative data, the analytical procedure provides confirmation of identity of the metabolites through the phosphorus-specific flame photometric detector and elution in the proper fraction from silica gel. Ethylation of MCA and DCA to form malathion provides additional confirmation.

This method provides a specific and sensitive procedure for the determination of malathion carboxylic acids in urine. The determination of such urinary metabolites helps provide an index of human exposure to organophosphorus pesticides which is essential to the human monitoring programs.

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Tissue Residue and Comparative Metabolism Studies on Tiazuril in the Chicken, Rat, Dog, and Monkey

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The metabolism of the anticoccidial compound tiazuril (2-[3,5-dimethyl-4-(4-chlorophenylthio)-phenyl]-*as*-triazine-3,5(2*H*,4*H*)-dione) was studied in the chicken and laboratory animals. Following oral administration of tritium-labeled tiazuril (^{3}H-T) to the chicken at 1 mg/kg, ^{3}H-T equivalents of radioactivity was 0.06 ppm or less in edible tissues and plasma 5 days after dosing. Throughout the withdrawal period, radioactivity was higher in plasma, liver, and kidney than muscle, skin, and fat. Tiazuril (T) is metabolized by oxidation of the sulfur atom to T-SO and T-SO_2 and by hydroxylation of the chlorophenyl ring to HO-T, HO-T-SO, and HO-T-SO_2. T-SO_2 and HO-T-SO are the major metabolites in liver and excreta, respectively. Broilers maintained on T in feed at 0.0015% for 8 weeks showed less than 0.01 ppm of T and 0.03 ppm or less of T-SO_2 in plasma and edible tissues 4 days after drug withdrawal. In comparative metabolism studies, the dog is distinguished from the chicken, rat, and monkey by maintaining persistent concentrations of T in plasma and by its inability to hydroxylate the drug or its sulfoxidation products.

Tiazuril (2-[3,5-dimethyl-4-(4-chlorophenylthio)-phenyl]-*as*-triazine-3,5(2*H*,4*H*)-dione) belongs to a class of aryltriazines with structures similar to 6-azauracil that possess potent broad spectrum anticoccidial activity in the chicken (Miller, 1975; Miller et al., 1977; Mylari et al., 1977). It was proposed for treatment of broilers at a projected use level of 0.0010–0.0020% in feed. The anticoccidial activity, site of action, and metabolism of other potentially useful animal health products of this series have been described (Chappel et al., 1974; Ryley et al., 1974; Rash and Lynch, 1976).

The study reported here was part of a safety evaluation process incorporating suggestions of Perez and Weber (1975, 1976, 1977). The distribution and the rate of disappearance of metabolic residues of tiazuril remaining in edible tissues of the chicken under both laboratory and

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