Toxicokinetics of Methyl Parathion in Lactating Goats

Ronald E. Baynes† and John M. Bowen*

Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602-7389

Lactating goats were exposed by oral administration to a subtoxic dose of methyl parathion (MPT) (5 mg/kg/day; 3 days). Neither MPT nor its metabolite methylparaoxon (MPO) was detected in plasma, urine, or milk. MPT and MPO were unaffected by incubation in milk for 12 h. Conversion of MPT to aminomethyl parathion by rumen microflora limited bioavailability. Absorption did occur because 7.4% of the total dose of MPT was excreted in urine as dimethyl phosphate (DMP) and dimethyl thiophosphate (DMTP), and serum cholinesterase (ChE) activity was depressed to 58% of control. In lactating goats given an intravenous bolus of MPT at a dose (5 mg/kg) that was slightly toxic (salivation and nervousness), the elimination half-life was 0.81 ± 0.17 (mean ± SE) h. Although MPO, DMP, and DMTP were not detected in plasma, 67.8% of the intravenous dose was excreted in urine as DMP and DMTP. ChE activity was depressed to 52% of control. It was concluded that dosages of MPT not causing overt signs of toxicity are not associated with excretion of MPT or its toxic metabolite MPO in milk.

Keywords: *Methyl parathion; methylparaoxon; lactating goats; toxicokinetics*

INTRODUCTION

The excretion of pesticides in milk represents a potential public health hazard in view of the importance of milk in human nutrition. The degree of hazard depends upon the intrinsic toxicity of the parent compound and/or its metabolite and upon the quantity of toxic compound excreted in the milk. The lack of signs or presence of minimal signs of toxicity as may occur in exposure to low levels of organophosphate (OP) compounds may lead to continued use of the milk from exposed animals for human consumption and could represent a health hazard to people if a significant quantity of the toxic compound was excreted in the milk.

Exposure of dairy animals to OPs such as parathion, chlorpyrifos, or coumaphos at subtoxic doses has resulted in pesticide or pesticide metabolite residues in milk (Konar and Ivie, 1988; Lino and da Silveira, 1992; McKellar et al., 1976; Mosha et al., 1990; Osweiller et al., 1985), although in one report (Lino and da Silveira, 1992) contamination may have occurred during transport or processing. Intramuscular injections of parathion of 4 mg/kg/day for 10–12 days in lactating goats caused a 33–69% depression in erythrocyte cholinesterase activity in kids consuming the milk from these goats (Wilber and Morrison, 1955). The data strongly suggest that parathion and/or paraoxon was present in the dams milk, although the milk was not analyzed for these compounds.

Methyl parathion (MPT) is the second most widely used OP applied per acre in forestry and crop production; this includes many animal forages such as alfalfa and corn (Bennett et al., 1990). Because of its acute toxicity, MPT is not recommended for livestock use. The potential for excretion of MPT in milk after exposure to subtoxic concentrations has not been studied. In addition, no toxicokinetic data or metabolic fate studies appear to be available for ruminants exposed to MPT. Data for human subjects suggest that dimethyl phosphate (DMP) is a major urinary metabolite after oral exposure to MPT (Morgan et al., 1977). The metabolism of MPT in mice has been studied extensively (Hollingworth et al., 1967; Hollingworth, 1969). In these studies the urinary dialkyl phosphates, DMP and dimethyl thiophosphate (DMTP), were recovered as the major metabolites of MPT metabolism. In studies where cattle or goats were orally exposed to OPs such as diazinon, chlorpyrifos, or phosmet, dialkyl phosphates also represented the major excretory products of OP metabolism (Gutenmann et al., 1968; Mount, 1984a,b). Limited data also suggest that the presence of urinary dialkyl phosphates in dairy animals may serve as a more useful and reliable indicator of OP exposure than depressed cholinesterase (ChE) activity (Mount, 1984a,b).

The objectives of this study were to (1) determine whether MPT and its metabolites are eliminated in milk secreted by dairy goats following oral or intravenous exposure to MPT; (2) obtain toxicokinetic data from goats given oral or intravenous doses of MPT; (3) determine the ability of caprine rumen fluid to metabolize MPT and of plasma to metabolize methylparaoxon (MPO); and (4) determine the temporal relationship between the presence of the urinary dialkyl phosphates and depression of serum ChE activity.

MATERIALS AND METHODS

Chemical Standards and Reagents. MPT technical grade (43.3%) was obtained from Helena Chemical Co., Nashville, TN. MPO (99%) was obtained from Chem Services, West Chester, PA. Potassium dimethylphosphoric acid (99.9%) was obtained from Ultrascientific Chemical Co., North Kingstown, RI. Potassium dimethylthiophosphoric acid (90%) was synthesized by Dr. C. Dewitt Blanton, College of Pharmacy, University of Georgia, Athens, GA. The above compounds were analyzed by gas chromatography–mass spectrometry and were found to be free of interfering contaminants. All standard solutions were prepared in acetone (MPT and MPO) or acetonitrile (DMP and DMTP) depending on the chemical

* Author to whom correspondence should be addressed [fax (706) 542-8254; e-mail Bowen.j@calc.vet.uga.edu].

† Present address: Cutaneous Pharmacology and Toxicology Center, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606.

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assay that was to be performed. All chemical solvents and reagents used were analytical or pesticide grade. Pentafluorobenzyl bromide (PFB-Br_2) reagent was obtained from Pierce Chemical Co., Rockford, IL. Glycerol formal was obtained from Sigma Chemical Co., St. Louis, MO.

Gas Chromatography Equipment and Operating Conditions. A Hewlett-Packard 5890 gas chromatograph equipped with a flame photometric detector (GC–FPD), phosphorus filter (526-nm), and a 12 meter long, 0.2 mm i.d., HP-1 (100% methyl silicone) 0.33 μm coated narrow-bore Hewlett-Packard capillary column. Flow rates were as follows: carrier gas (helium), 2 mL/min; compressed air, 100 mL/min; hydrogen, 75 mL/min; nitrogen, 10 mL/min. The detector temperature was 200 °C, split injector temperature, 200 °C, and oven temperature, 110 °C. The column was programmed from 110 to 220 °C (10 °C/min) and held for 5 min at 220 °C. Confirmation of the analyte was performed under the same conditions using a Hewlett-Packard 5890 gas chromatograph equipped with a Hewlett-Packard 5970 mass selective detector (GC–MS). In all analyses, 1 μL of the sample was injected into the injection port. Peak areas of standards and spiked sample extracts, run under identical conditions, were compared to determine percentage recoveries at each concentration examined.

The method detection limit (MDL) was calculated using the equation

$$\mathrm{MDL} = t_{(0.99)}\mathrm{SD}$$

where $t_{(0.99)}$ = Student's one-tailed t value appropriate for a 99% confidence level and with $n - 1$ degrees of freedom and SD = standard deviation of the replicate samples.

Animals. Four lactating (two Saanen, two Alpine) dairy goats (1–2 years old) with body weights ranging from 30 to 55 kg were used in this study. The goats were used in experiments at least 2 weeks after the kids were removed. The animals were paired in two stalls and fed a grain/hay diet. After acclimation for 2 weeks, the goats were placed in metabolism stanchions. The stanchions had urine collection pans that were covered by a screen to minimize fecal contamination of urine.

Treatment. Animals were exposed to MPT by oral and intravenous (iv) routes. The oral doses were prepared by adding the calculated concentration of MPT to gelatin capsules immediately prior to administration. The iv doses were prepared by dissolving MPT in glycerol formal. In the first experiment, four lactating goats each received 5 mg/kg MPT orally once each morning for 3 days. After allowing for a minimum 1 month washout period, each goat was given a single dose of MPT (5 mg/kg) by the iv route.

Sample Collection. Urine was collected at 4, 8, 16, and 24 h for the first 24 h and then every 12 h for 4–6 days after final exposure to MPT. Milk was collected every 6 h for the first 12 h and then every 12 h after the final exposure. Blood was collected at 0, 0.25, 0.50, 1.00, 2.00, 4.00, 8.00, 12.00, and 24.00 h for the first 24 h and then every 12 h for 4–6 days after the final exposure. In the oral experiment, samples were collected each morning before the animals were dosed. All samples were stored at −80 °C and analyzed within 1 month of collection.

Analysis of Samples. *Milk.* One milliliter samples were made up to 10 mL with deionized water before being passed through a preconditioned reversed-phase C18 column from Millipore Corp., Milford, MA. The analytes were eluted with 3 mL of chloroform/ethanol (9:1). The organic layer was removed from the eluate, evaporated to almost dryness, and made up to 1 mL with acetone. One microliter of this solution was analyzed by flame photometric gas chromatography. This procedure was performed in duplicate for the determination of MPT and MPO in milk. Additional details of this extraction method have been reported (Baynes and Bowen, 1995).

For the determination of DMP and DMTP in milk, the samples were analyzed according to the urine assay method described below.

Plasma. Venous blood was heparinized and centrifuged (1400*g*, 20 min). One milliliter aliquots of plasma were treated with 1 mL of acetonitrile to precipitate proteins, and the mixture was extracted twice with 3 mL of chloroform/ethanol (9:1). The organic layer was removed, evaporated, and then reconstituted to 1 mL with acetone before being analyzed for MPT and MPO by flame photometric gas chromatography. For the determination of DMP and DMTP in plasma, samples were analyzed according to the urine assay method described below.

Urine. Seven milliliters of acetonitrile was added to 1 mL of goat's urine and vortexed for 20 s. The mixture was centrifuged (1400*g*, 20 min), and the organic layer was removed and evaporated to almost dryness. One milliliter of acetonitrile was added to the residue. Fifty microliters of PFB-Br_2 was added to derivatize the polar metabolites, and 35 mg of potassium carbonate was added as a catalyst for the reaction. This mixture was incubated for 2 h at 90 °C, and then 1 μL was analyzed by flame photometric gas chromatography.

Cholinesterase (ChE) Activity Assay. Serum samples obtained from goat's blood were analyzed for ChE activity according to the method described by Ellman et al. (1961). Two milliliters of a buffer solution (pH 8.0) was mixed with 500 μL of the indicator dithiobis(nitrobenzoic acid) (DTNB) before 20 μL of serum was added. This mixture was incubated at 37 °C for 5 min, and then 200 μL of acetylthiocholine was added. This mixture was analyzed by a Bausch and Lomb Spectronic 2000 spectrophotometer system, and cholinesterase enzyme activity was reported as IU/L.

In Vitro Stability Studies. *Plasma.* Plasma samples (30 mL) obtained from goat's blood were spiked with MPO at 2.5, 5, and 10 μg/mL, respectively. The mixtures were held at 39 °C (normal goat's body temperature), and four 1 mL samples were obtained at the following times from each of the mixtures: 0, 4, 8, and 16 min. The plasma was analyzed according to the methods described above. For each of the three spiked plasma mixtures, the first-order elimination rate constants (K_e) for the destruction of MPO by plasma in vitro were determined by regression analyses of semilogarithmic plots of the concentration of unchanged MPO vs incubation time. Half-lives were calculated from the relationship $T_{1/2} = 0.693/K_e$.

Milk. The stability of MPT, MPO, DMP, and DMTP in raw fresh goat's milk was studied. MPT (150 μg/0.3 mL of acetone) and MPO (150 μg/0.3 mL of acetone) were added to separate 30 mL aliquots of goat's milk that were then held at 39 °C. At 0, 4, 8, 12, and 24 h, 1 mL was analyzed from each mixture by solid-phase extraction and gas chromatography for MPT and MPO as described above. This procedure was repeated for milk from each animal. DMP (50 μg/0.1 mL of acetone) and DMTP (50 μg/0.1 mL of acetone) were added to separate 10 mL aliquots of goat's milk and held at 39 °C. At 0, 2, 4, and 6 h, 1 mL was analyzed according to the urine assay method described above. The pH of the milk was also monitored during this study with an Orion Research Model SA 250 portable pH meter.

Rumen Fluid. The stability of MPT in the presence of fresh rumen fluid was studied. MPT (500 μg/mL of acetone) was thoroughly mixed with 100 mL of filtered, fresh, goat rumen fluid to provide a concentration of 5 μg/mL, and the mixture was held at the normal goat's body temperature (39 °C). At 0, 0.5, 1.0, 2.0, 4.0, and 8.0 h, the fluid was again mixed and 5 mL of fluid was removed and diluted to 25 mL with acetone. One milliliter of the solution was transferred to a 50-mL volumetric flask containing 1 mL of benzene, and the contents were made to volume with a 2% sodium sulfate solution. The upper benzene layer was removed and evaporated and the residue reconstituted with 1 mL of acetone before being analyzed by flame photometric gas chromatography for MPT and MPO. The pH of the rumen fluid during incubation was monitored.

Kinetic Analyses. Semilogarithmic plots of plasma concentrations (MPT in μg/mL) vs time (min) were constructed for each goat in the study. Visual inspection of these plots allowed estimates as to whether drug disappearance was mono- or biexponential.

Initial estimates of toxicokinetic parameters were obtained via a curve-stripping computer program (RSTRIP, MicroMath Inc., Salt Lake City, UT). This program utilized iterative,

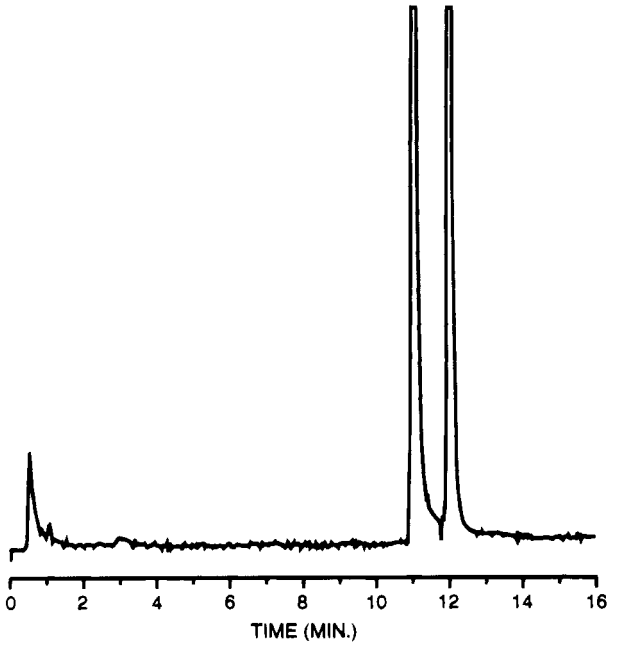

Figure 1. Capillary gas chromatogram of an acetone extract of plasma containing 5 μg/mL of MPO and 5 μg/mL of MPT. Retention time for MPO was 10.99 min, and that for MPT, 11.96 min.

nonlinear regression to give a line of best fit. The final estimates of the parameters were determined by an iterative procedure of fitting the data until the minimum sum of squares was reached.

The toxicokinetic parameters determined were the half-lives (half-life[1], half-life[2]), the y-axis intercepts (A[1], A[2]), the area under the curve (AUC), first-order rate constants (k[1], k[2]), systemic clearance (Cl), and apparent volume of distribution (V_d).

Akaike's information criterion (Yamaoka et al., 1978), based on the mean values of the final estimates of the associated toxicokinetic parameters, was used to determine the number of exponential terms that best described the data.

Statistical Analyses. Variations were expressed using the standard error (SE) of the mean and coefficient of variation (CV) where possible. Daily mean values for milk production, urine concentration, and cholinesterase activity data were compared by two-way analysis of variance (ANOVA). Significant differences between daily means within each treatment group were determined by Duncan's multiple range test. Differences were considered significant if $p < 0.05$.

RESULTS

Assay Validation. A typical gas chromatogram of MPT and MPO is shown in Figure 1, and gas chromatograms of derivatized dimethyl phosphate (DMP) and dimethyl thiophosphate (DMTP) are shown in Figure 2. There was good resolution and minimal peak broadening and tailing. In addition, there were no interfering peaks with similar retention times in blank plasma, milk, and urine samples.

The MDL ranged from 0.011 to 0.024 μg/mL for MPT and MPO in milk and plasma samples (Table 1). The MDL ranged from 0.074 to 0.204 μg/mL for DMP and DMTP in urine and milk (Table 1). Extraction efficiency was determined by comparing the peak areas from spiked samples with those obtained from direct injection of standards onto the column. The recovery of MPT was approximately 95.9–109% from plasma and 90.0–108% from milk, over a concentration range of 0.05–5.0 ppm (Table 2). The recovery of the active metabolite, MPO,

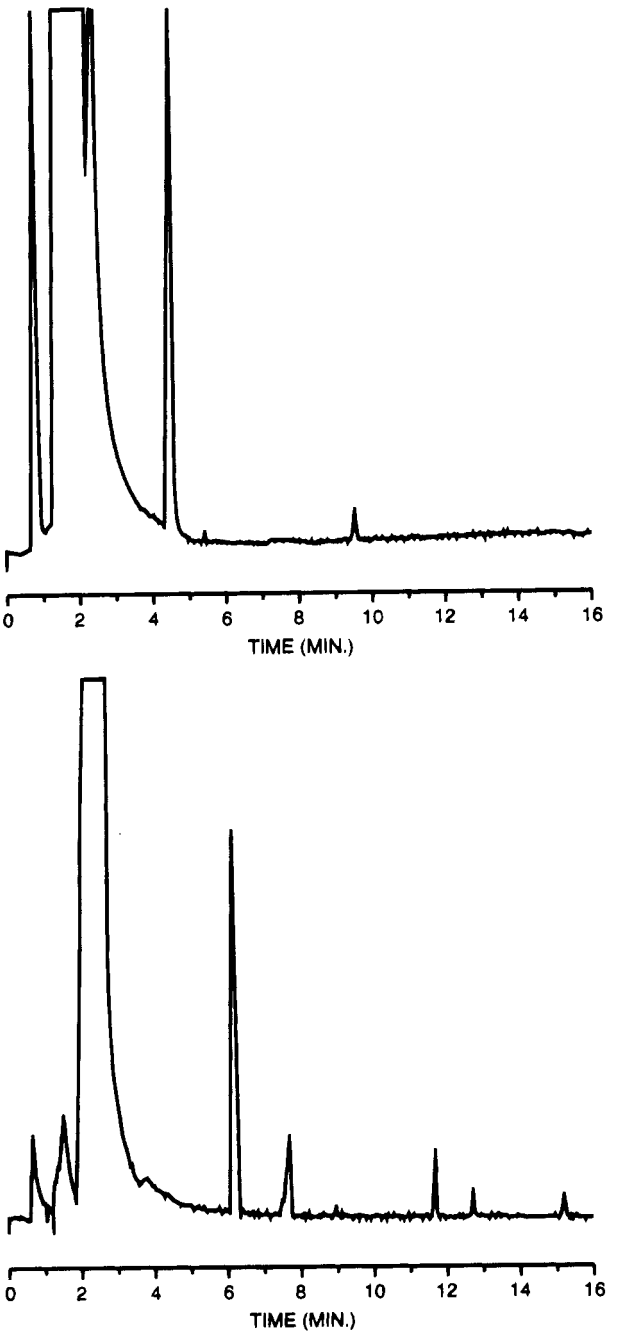

Figure 2. Capillary gas chromatogram of an acetonitrile extract of urine containing 5 μg/mL of dimethyl phosphate (DMP) (top) and 5 μg/mL of dimethyl thiophosphate (DMTP) (bottom). Retention time for the PFB-derivatized DMP was 4.42 min, and that for the PFB-derivatized DMTP, 6.14 min.

Table 1. Method Detection Limits (MDL) for MPT, MPO, DMP, and DMTP in Plasma, Milk, and Urine

matrix	MPT[a] (μg/mL)	MPO[a] (μg/mL)	DMP[b] (μg/mL)	DMTP[b] (μg/mL)
plasma	0.015	0.024		
milk	0.011[c]	0.015[c]	0.074	0.204
urine			0.200	0.155

[a] Concentration added, 0.05 μg/mL. [b] Concentration added, 0.5 μg/mL. [c] Data from Baynes and Bowen (1995).

from plasma and milk was 88.6–96.4% and 92.0–102%, respectively (Table 2). The recovery of the polar metabolite, DMP, from urine and milk was 101–112% and 86.4–95.1%, respectively, over a concentration range of 0.5–5.0 ppm (Table 3). The recovery of the other polar metabolite, DMTP, from urine and milk was 80–110%

Table 2. Analysis of Goat's Plasma[a] and Milk[b] Spiked with Methyl Parathion (MPT) and Methylparaoxon (MPO)

matrix	fortification (μg/mL)	MPT % recovery[c]	MPT CV[d]	MPO % recovery[c]	MPO CV[d]
plasma	5.0	95.9	10.5	96.4	9.44
	0.5	102	12.9	96.2	5.89
	0.05	109	7.49	88.6	14.9
milk[e]	5.0	93.6	8.07	92.0	9.90
	0.5	90.0	3.62	92.0	7.01
	0.05	108	5.46	102	7.79

[a] Plasma analyzed by liquid–liquid extraction, followed by GC–FPD. [b] Milk analyzed by solid-phase extraction, followed by GC–FPD. [c] Average of five replicates for each concentration analyzed. [d] Coefficient of variation. [e] Data from Baynes and Bowen (1995).

Table 3. Analysis[a] of Goat's Urine and Milk Spiked with Dimethyl Phosphate (DMP) and Dimethyl Thiophosphate (DMTP)

matrix	fortification (μg/mL)	DMP % recovery[b]	DMP CV[c]	DMTP % recovery[b]	DMTP CV[c]
urine	5.0	101	8.20	80.00	6.43
	0.5	112	9.31	110	9.90
milk	5.0	86.4	8.92	90.0	6.42
	0.5	95.1	4.15	102	8.06

[a] Urine and milk analysis by derivatization with PFB-Br_2 (n = 4). [b] Average of four replicates for each concentration analyzed. [c] Coefficient of variation.

and 90–102%, respectively, for concentrations of 0.5 and 5.0 ppm (Table 3). Identification of these metabolites was confirmed based on GC–MS spectra and fragmentation patterns for the compound peak of interest (Figure 3A,B).

The precision of the assay system was assessed by calculating the coefficient of variation (CV). These CV values were usually less than 10% (Tables 2 and 3).

In Vitro Studies. *Plasma.* Incubation of MPO with fresh goat's plasma over a 16 min period resulted in the rapid destruction of MPO. Biotransformation of MPO by plasma followed apparent first-order kinetics, with a mean MPO half-life of 5.99 min (Figure 4).

Milk. When goat's milk was spiked to 5.0 μg/mL of MPT and MPO and incubated at 39 °C, there was no change in concentration of these compounds in milk after 12 h. However, after 24 h of incubation, the concentrations of MPT and MPO were reduced to 3.98 and 1.67 μg/mL, respectively. Milk pH ranged from 5.97 to 6.27 during the first 12 h but decreased to 4.25 after 24 h of incubation. No changes in DMP concentrations in spiked milk were observed after 6 h of incubation. The DMTP spiked concentration declined by 25.7% after 6 h of incubation. DMP was not detected after DMTP was incubated in milk.

Rumen Fluid. After fresh rumen fluid was spiked to 5 ppm MPT, neither MPT nor MPO was recovered at any time during the 8 h study. Two unidentifiable peaks (based on retention time) that were not detected in the control samples were observed following GC–FPD analysis. GC–MS analysis confirmed that one of these peaks was the metabolite aminomethyl parathion (Figure 3C). (Aminomethyl)paraoxon was not confirmed by GC–MS analysis.

Clinical Effects. No clinical evidence of toxicoses was observed in goats exposed to MPT by the oral route. However, goats exposed to MPT by the intravenous route displayed mild signs of anxiety and increased salivation during the first 30 min after exposure. The more classical signs that are usually associated with OP

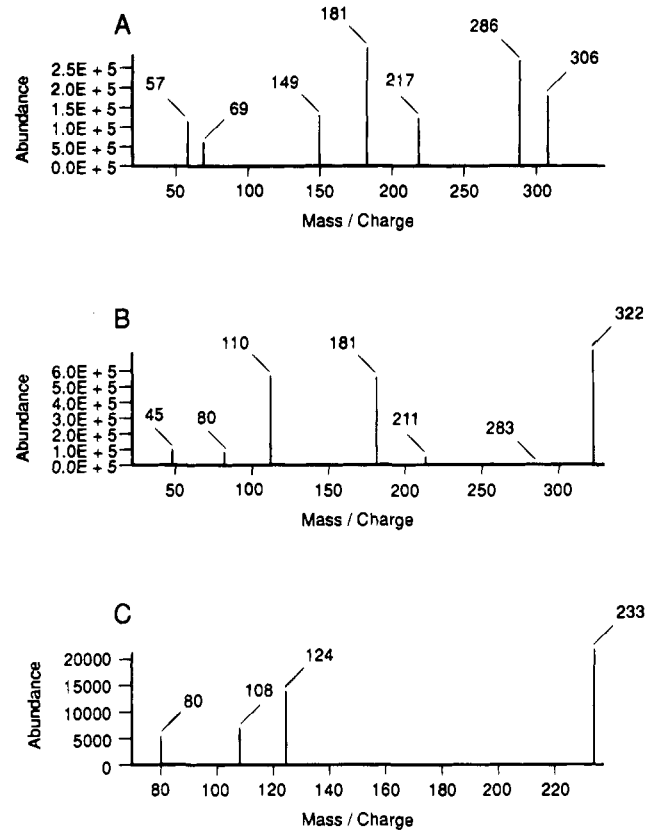

Figure 3. GC–MS analysis of a urine extract containing PFB-derivatized dimethyl phosphate (A) and dimethyl thiophosphate (B) and a rumen fluid extract containing aminomethyl parathion (C).

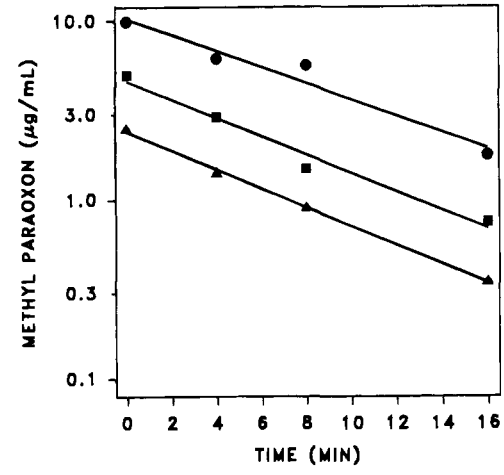

Figure 4. In vitro detoxification of MPO by goat's plasma. Initial MPO concentrations were 10 (●), 5 (■), and 2.5 (▲) μg/mL. Each point represents the mean of quadruplicate samples in which standard deviations were less than 10% of the corresponding means.

toxicosis such as muscle twitching and respiratory distress were not observed.

Oral Administration. *Plasma.* MPT, MPO, DMP, and DMTP were not detected in the plasma of goats after three consecutive daily oral administrations of 5 mg/kg of MPT.

ChE Activity. The mean control value for ChE activity in this experiment was 152 ± 11.6 IU/L. A significant decrease in serum ChE activity was observed within the first 12 h after administration of the first dose (Figure 5). Maximal ChE activity depression to 58% of control occurred on the day after the third and

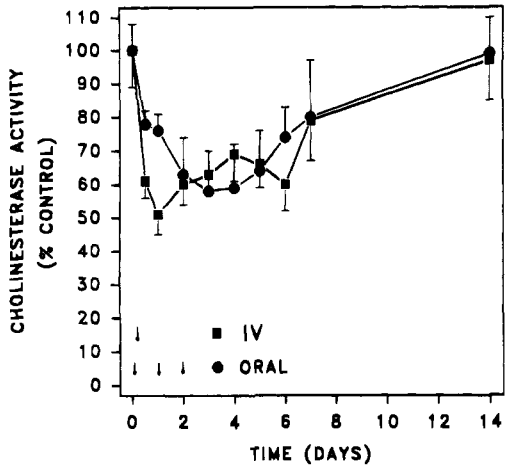

Figure 5. Serum cholinesterase activity in goats exposed to MPT by oral administration (5 mg/kg/day) or intravenous (5 mg/kg) administration. Arrows denote times of MPT administration.

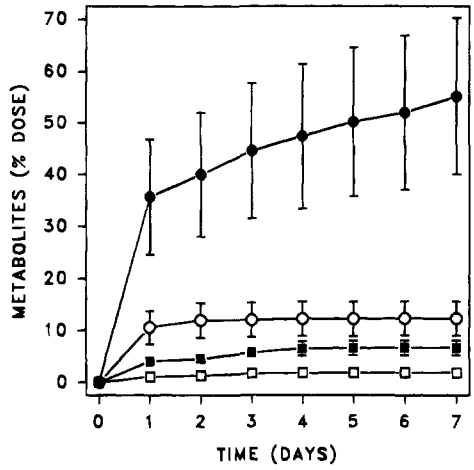

Figure 6. Cumulative urinary excretion of dimethyl phosphate [DMP] (filled symbols) and dimethyl thiophosphate [DMTP] (open symbols) as a percentage of the dose, following administration of a single 5 mg/kg of iv bolus of MPT (circles) and an oral dose of 5 mg/kg/day of MPT (squares) for 3 days.

final oral dose. By day 7 of the study, serum ChE activity had increased to 80% of control, and by day 14, ChE activity had returned to near normal values.

Milk. No effect on milk production was observed after oral administration of MPT ($p > 0.05$). Average milk production was 1792 ± 160 mL/day/goat during the experimental period and was 2075 ± 347 mL/day/goat after this period. The compounds MPT, MPO, DMP, and DMTP were not detected in the milk of goats orally exposed to MPT.

Urine. Traces of MPT and MPO (<0.05 μg/mL) were detected in the urine of two of four animals at 4 or 8 h following oral exposure to MPT. DMP and DMTP were identified and confirmed to be the major metabolites in the urine of goats orally exposed to MPT. On a daily basis, DMP was consistently recovered at higher concentrations than was DMTP. A small percentage (5.5%) of the MPT dose was excreted as DMP and an even smaller percentage (1.9%) of the MPT dose was excreted as DMTP following oral administration (Figure 6). Urine DMP concentrations declined from a mean peak level of 18.6 μg/mL within the first 24 h after the first oral dose to 0.27 μg/mL and nondetectable levels at 4 and 5 days, respectively, after the last oral dose. Urine DMTP concentrations declined from a mean peak level

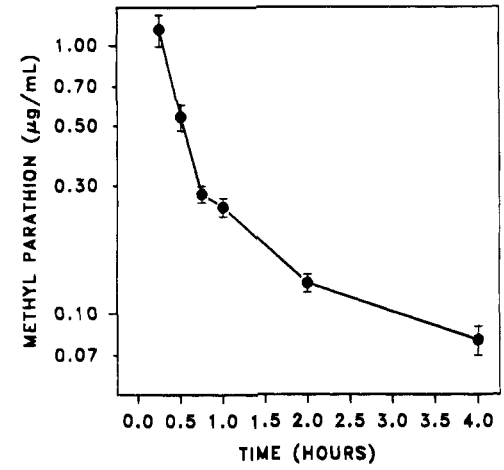

Figure 7. Mean plasma concentrations of MPT following single intravenous administration of 5 mg/kg to four lactating goats.

Table 4. Toxicokinetic Parameters Descriptive of the Disposition of Methyl Parathion (MPT) in Goats following Intravenous Administration of a Single Dose (5 mg/kg)

	goat no.					
parameter	414	415	435	436	mean	SE
$A[1]$ (μg/mL)		1.56	6.81	2.62	3.66	1.61
$A[2]$ (μg/mL)	1.64	0.42	0.30	0.50	0.72	0.31
$K[1]$ (per h)		4.05	6.41	5.99	5.48	0.63
$K[2]$ (per h)	1.80	0.81	0.58	0.86	1.01	0.27
half-life[1] (h)		0.17	0.11	0.12	0.13	0.01
half-life[2] (h)	0.38	0.85	1.20	0.80	0.81	0.17
AUC (μg·h/mL)	0.92	0.91	1.58	1.02	1.11	0.15
V_d (L/kg)	3.04	6.80	5.44	5.67	5.24	0.79
Cl (L/h·kg)	5.46	5.51	3.14	4.90	4.75	0.56

of 5.28 μg/mL within the first 24 h after the first dose to 0.19 μg/mL and nondetectable levels at 4 and 5 days, respectively, after the last dose.

Intravenous Administration. *Plasma.* Following intravenous (iv) administration of 5 mg/kg of MPT, plasma MPT concentration declined very rapidly over the first hour and was below the MDL after 4 h. Decline of the plasma MPT concentration is illustrated in Figure 7. The active metabolite, MPO, and polar metabolites DMP and DMTP were not detected at any time in plasma. Data from three of the four goats were best described by a biexponential equation, while that of the fourth goat was best described by a monoexponential equation. Mean values for the derived toxicokinetic parameters are summarized in Table 4. MPT distributed very rapidly (half-life[1] = 0.13 h), whereas elimination occurred at a much slower rate (half-life[2] = 0.81 h). The mean volume of distribution was 5.24 L/kg, and a high mean total body clearance of 4.75 L/h·kg was obtained.

ChE Activity. The mean control ChE activity for the goats immediately prior to the start of the single iv dose study was 160 ± 18.0 IU/L. After the goats were exposed to 5 mg/kg of iv bolus, a significant decrease in serum ChE activity ($p < 0.05$) to 50% of control occurred within the first 12 h (Figure 5). Recovery of ChE activity was not evident until the 7th day (78% of control), and by the 14th day, ChE activity was almost at control levels.

Milk. A significant ($p < 0.05$) decrease in milk production was observed after the goats were exposed to an intravenous dose of 5 mg/kg of MPT. Milk production dropped from 2266 ± 328 mL/day/goat before

the animals were dosed with MPT to an average milk production of 1936 ± 288 mL/day/goat after the experimental period. No detectable amounts of MPT, MPO, nor DMTP were observed in milk secreted by goats exposed to the intravenous dose of MPT. However, milk samples from two animals taken at 6 h after receiving the 5 mg/kg of iv bolus contained an average of 0.38 μg/mL of DMP, which represented 0.02% of the dose.

Urine. Traces of MPT and MPO (<0.05 μg/mL) were detected in the urine of two of four animals following iv exposure to the MPT. DMP and DMTP were identified and confirmed to be the major urinary metabolites in the urine of goats given an intravenous dose of MPT, and on a daily basis, DMP was recovered at higher concentrations than was DMTP. DMP represented a larger percentage (55.3%) of the total dose following the 5 mg/kg intravenous dose than did DMTP (12.5%) (Figure 6). Urine DMP concentrations declined from peak levels of 48.7 μg/mL within the first 24 h after the intravenous dose to 8.02 μg/mL at 7 days after the intravenous dose. Urine DMTP concentrations declined from peak levels of 18.1 μg/mL within the first 24 h after the intravenous dose to 0.21 μg/mL at 7 days after the intravenous dose.

DISCUSSION

The oral dose of MPT (5 mg/kg/day; 3 days) used in the present study was selected to be in a range that would not produce easily observable signs of OP toxicity in the goats but would inhibit cholinesterase activity significantly as an indication of absorption of MPT. This exposure was intended to simulate accidental ingestion of a feed having a low level of OP contamination.

Within the detection limits of the milk assay method used in this study (Baynes and Bowen, 1995), neither MPT nor its toxic metabolite MPO was present in milk at concentrations equal to or higher than the MDLs, i.e., 0.011 μg/mL (11 ppb) and 0.015 μg/mL (15 ppb), respectively. Since metabolism of MPT and MPO did not occur in vitro in milk during a 12 h incubation and since DMP and DMTP were not present in milk after oral administration of MPT, the absence of MPT and MPO in milk would not appear to be the result of their biotransformation while in the udder. Rather, it indicates that these compounds were not excreted in milk in measurable concentrations. Even with the much higher plasma concentration of MPT following iv administration, neither MPT nor MPO was detectable in milk.

The apparent low bioavailability of MPT following oral administration to the goats can, in part, be attributed to reduced absorption as a consequence of MPT's biotransformation by rumen microflora to aminomethyl parathion. This biotransformation was demonstrated through an in vitro study using rumen fluid. Depression of ChE activity and the presence of dialkyl phosphates in urine provided evidence of some MPT absorption even though it could not be detected in plasma. In a toxicokinetic study in which dogs were given an oral dose of 20 mg/kg of MPT, a low bioavailability (1−29%) was also reported (Braeckman et al., 1983), suggesting that factors other than microflora contributed to reduced absorption. Aminomethyl parathion was not detected in either plasma or urine from the goats given oral doses of MPT, suggesting that it was not absorbed. Reduction of parathion to amino parathion in bovine rumen fluid has been reported (Cook, 1957).

The biexponential curve (Figure 7) obtained in most of the iv dose experiments is characteristic of a two-compartment open model which was reported as descriptive of the toxicokinetics of MPT in dogs (Braeckman et al., 1980). The plasma concentration−time data for MPT in goats following iv administration revealed a rapid distribution phase and a slower elimination phase. The distribution phase half-life[1] (0.13 h) and the high V_d (5.24 L/kg) for MPT suggest that this OP compound is rapidly and widely distributed although hepatic first-pass biotransformation of MPT to MPO by cytochrome P450-dependent monooxygenases probably influenced these results. Since MPO was not detectable in plasma, the elimination phase for MPT was probably influenced by the rapid biotransformation of MPO to DMP by hydrolases in goat plasma (in vitro MPO half-life = 5.99 min) and, to a lesser extent, MPT to DMTP. Sultatos et al. (1985) and Zhang and Sultatos (1991) obtained comparable data for in vitro detoxification of paraoxon and MPO in mouse and rat blood. Interaction of MPO and serum and erythrocyte ChE would also contribute to MPO's rapid disappearance.

While plasma protein binding of MPT and MPO in the goat has not been studied, high binding (94%) for MPT has been reported in the dog (Braeckman et al., 1983). Binding could reduce the availability of these compounds for biotransformation which would slow elimination, although the clearance (4.75 L/h · kg) indicated rapid elimination in the goats. The elimination half-life for MPT after iv administration was much longer in dogs (6−8 h) than in the goats (half-life[2] = 0.81 h) which suggests a major species difference.

A small amount of DMP, a nontoxic metabolite of MPO, was detected in milk from two of the four goats 6 h after iv administration of MPT but was not detected in plasma. Incubation of milk with DMP and DMTP for 6 h revealed no change in the DMP concentration but a 25% reduction in the DMTP concentration. This may explain the absence of DMTP in milk after iv administration of MPT. The presence of DMP in milk probably reflects its diffusion into the milk from plasma following its production by metabolism of MPO. No dialkyl phosphates were detected in milk from goats given oral doses of 5 or 10 mg/kg/day of phosmet (Imidan) for 7 days (Mount, 1984b) or from cows fed chlorpyrifos at levels ranging from 0.3 to 30 ppm for up to 2 weeks (Gutenmann et al., 1968; McKellar et al., 1976).

The presence of dialkyl phosphates in urine has been recommended as a more sensitive indicator of OP exposure than inhibition of ChE activity (Mount, 1984a,b). Following oral administration of MPT to the goats in the present study, urinary excretion of DMP and, to a lesser extent, DMTP increased in small increments to day 4, but changes thereafter were not detected (Figure 6). Cumulative excretion of these metabolites was very small (7.4%). Following iv administration, DMTP increased within 24 h, but after day 2, additional excretion was not detected. DMP excretion increased markedly within 24 h, and, although quite variable, the concentration was still increasing at day 7. Cumulative excretion of these metabolites was large (67.8%). The continued excretion of DMP suggests that this DMP was derived from stored DMP and not from MPO biotransformation as release of MPO would have sustained ChE inhibition, which, in contrast, was decreasing at day 7 similar to that associated with oral administration.

ChE activity was significantly depressed to 58% of control following oral administration of 5 mg/kg/day for 3 days and 52% of control following iv bolus administration of 5 mg/kg (Figure 5). Because ChE activity can be used to evaluate the degree of exposure experienced by goats and because ChE activity was not depressed to at least 25% of the control activity (Osweiler et al., 1985), the goats in the present study were exposed to a subtoxic dose of MPT as confirmed by the absence of overt signs of toxicity. The presence of dialkyl phosphates in urine provided an indication of exposure to MPT in goats in the present study as reported for goats given diazinon and phosmet (Mount, 1984a,b). However, on day 7 when ChE activity was still depressed, only DMP in the iv exposed goats was still being excreted. While assessment of dialkyl phosphates in urine allows distinction of OP from non-OP ChE inhibitors, e.g., carbamates, ChE activity still appears to have a better correlation with time course of exposure and progress toward recovery.

Excretion of toxic compounds in milk can be a public health hazard and a hazard to suckling offspring of the lactating goats. However, results of the present study emphasize that milk from lactating goats exposed to subtoxic concentrations of MPT, orally or intravenously, would not be a hazard as measurable concentrations of MPT and MPO were not present. At higher concentrations, excretion might occur, but signs of toxicity would also be present in the lactating goat.

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