feces, the lowest detectable sensitivity for screening of DPT, DPDT, DCA, and MCA was 0.5 and of DMM was 1.5 μg/g, respectively.

This is a simple and rapid method for the isolation and quantification of the major metabolites of malathion from biological samples. The metabolites are isolated by liquid–solid extraction using anionic trimethylaminopropyl-bonded silica cartridges and quantified by GLC/NPD. The complementary use of capillary GLC–MS unambiguously confirms presumptive peaks identified by capillary GLC–NPD. Compared to older methodologies, the present procedure has the advantages of minimal handling time, clean sample extracts, and no need for concentration of the extract. The method requires only small volumes of sample and organic solvents, and the glassware requirements are also minimal. The analysis time is short, and there is minimal interference from endogenous compounds in both urine and feces. The method has been successfully used for the assay of biological samples following administration of malathion to sheep, as well as for the determination of the metabolites of malathion in different in vitro studies. With minor adjustments, the method should be applicable to most biological sample matrices.

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Registry No. I, 1112-38-5; II, 756-80-9; III, 1190-28-9; IV, 1190-29-0; V, 1116-04-7.

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Elimination of Intravenously Injected Malathion in Sheep

Berit Muan,* Nils E. Søli, and Janneche U. Skåre

The metabolism and excretion of the organophosphorus insecticide malathion were studied after a single intravenous injection of 10 mg/kg body weight of malathion to sheep. A rapid decrease in the plasma concentration and an extensive degradation of malathion to inactive metabolites were observed. Of the administered dose, 81% was eliminated within 24 h, mainly in urine. The major metabolites of malathion were malathion α-monoacid and malathion diacid in both urine and feces.

The organophosphorus compound malathion, *O,O*-dimethyl *S*-(1,2-dicarbethoxyethyl) phosphorodithioate, has a relatively low mammalian toxicity and is widely used both as an agricultural insecticide and as an agent against ectoparasites in various species of livestock and poultry, as well as in humans. Malathion is activated to malaoxon, which is responsible for the toxic effects through inhibition of cholinesterases (Hayes, 1982). The low toxicity of malathion to mammals is due to extensive degradation to inactive metabolites. The most abundant metabolites of malathion, malathion α-monoacid (MCA) and malathion diacid (DCA), are products of the hydrolytic activity of carboxylesterases (Chen et al., 1969; Bradway and Shafik, 1977). Additional metabolites arise from hydrolysis at the P–S and S–C linkages, giving *O,O*-dimethyl phosphorothioate (DPT) and *O,O*-dimethyl phosphorodithioate (DPDT), respectively, and by demethylation, giving desmethylmalathion (DMM) (Bhagwat and Ramachandran, 1975; Nomeir and Dauterman, 1978).

Department of Pharmacology and Toxicology, The Norwegian College of Veterinary Medicine, Post Box 8146 Dep., 0033 Oslo 1, Norway.

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Previous investigators have been concerned with the fate of malathion in different species of animals. In ruminants, however, in which malathion may be used, only limited data are available concerning its pharmacokinetic properties. Besides, in most of the reported studies the metabolites have only been divided into chloroform- and water-soluble components. The present study was undertaken to add to the available information on the biological disposition of the compound. The metabolism and excretion of malathion were studied after a single intravenous injection of malathion to sheep.

EXPERIMENTAL SECTION

Chemicals. Malathion monoacid (MCA), malathion diacid (DCA), *O,O*-dimethyl phosphorothioate (DPT), *O,O*-dimethyl phosphorodithioate (DPDT), and desmethylmalathion (DMM) were donated by American Cyanamid Co (Princeton, NJ). A 10% w/v solution of malathion was prepared by dissolving 5 g of malathion in 25 g of 96% ethanol and diluted with 0.9% sodium chloride to 50 mL.

Animal Experiment. Three healthy adult ewes, 1 year of age, weighing 61–71 kg, were used in the experiment. The animals were tied up in metabolism stalls and fed hay and concentrates twice daily at 8 a.m. and 3 p.m. Water was available ad libitum. The urine excreted was collected through permanent bladder catheters (Folaflate balloon catheter), and feces were gathered in plastic cases placed behind the animals. The ewes were given 10 mg/kg body weight (bw) of malathion as a single intravenous injection in the jugular vein.

Blood samples (about 10 mL) were drawn from the jugular vein in heparinized vacutainers immediately before dosing, 1, 2, 3, 4, 6, 9, 12, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 min after administration, thereafter at 10-min intervals up to 2 h, at 20-min intervals up to 5 h, at 30-min intervals up to 7 h, and at 8, 12, 24, 36, 48, 72, and 96 h after the administration. Of the blood samples 2.5 mL was prepared for measurement of the cholinesterase activities in erythrocytes and plasma.

Urine and feces samples were collected immediately before treatment. Then urine and feces were gathered continuously and samples withdrawn at 2, 4, 6, 8, 12, 24, 36, 48, 60, 72, and 96 h after administration. The urine volume was determined at each time interval, and the samples were filtrated through filter paper. The feces was weighed and thoroughly mixed. All biological samples were stored at −20 °C until analysis.

Determination of Malathion and Malaoxon in Plasma, Urine, and Feces. For determination of malathion and malaoxon in the plasma, urine, and feces samples, a method described by Muan and Skåre (1985), based on liquid extraction, gas chromatographic determination, and mass spectrometric confirmation, was used. The detection limits for malathion and malaoxon in plasma and urine were 0.004 and 0.04 μg/mL, respectively. In feces, the detection limits were 0.01 and 0.1 μg/g for malathion and malaoxon, respectively.

Determination of the Metabolites of Malathion in Urine and Feces. MCA, DCA, DMM, DPDT, and DPT in urine and feces were determined by a method combining sorbent extraction, methylation, gas chromatographic quantification, and mass spectrometric identification. Details of the method are described in the preceding paper in this issue. Recoveries of the metabolites were checked by fortification of sheep urine and feces. The average recoveries for all compounds in urine and feces varied from 21 to 125% and 18 to 66%, respectively, with variation coefficients (CVs) within 20 and 30, respectively. The detection limits in urine were 1 μg/mL for DPT, DPDT, and DMM and 0.2 μg/mL for DCA and MCA. In feces the detection limits were 0.5 μg/g for DPT, DPDT, DCA, and MCA and 1.5 μg/g for DMM.

Determination of Cholinesterase Activities. The cholinesterase activities in plasma and erythrocytes were determined according to the radiochemical method of Sterri and Fonnum (1978), as described previously (Muan and Skåre, 1985).

RESULTS

Plasma Concentration of Malathion and Malaoxon. A semilogarithmic plot of the concentration of malathion in plasma as a function of time after intravenous administration of 10 mg/kg bw of malathion to three sheep is shown in Figure 1. The concentration of malathion in plasma decreased rapidly, and there were no detectable amounts of malathion in the plasma beyond 1 h after administration. Malaoxon was not detected in any of the plasma samples.

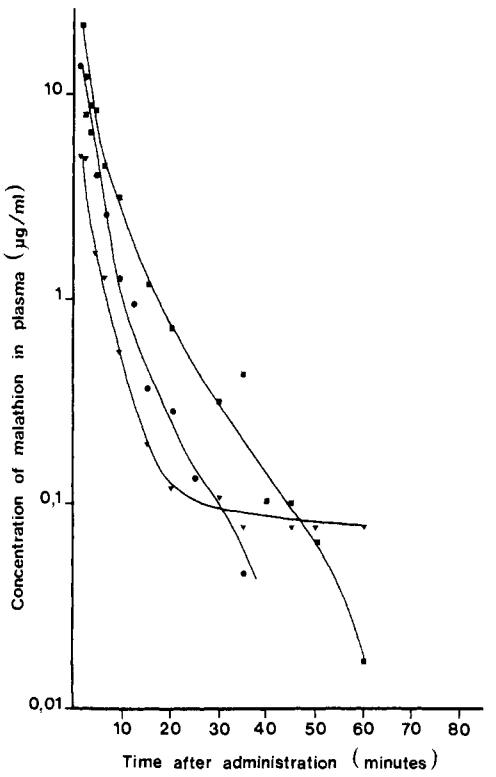

Figure 1. Semilogarithmic plot of the concentration of malathion in plasma as a function of time after intravenous administration of 10 mg/kg of malathion to sheep. Each curve represents an individual sheep.

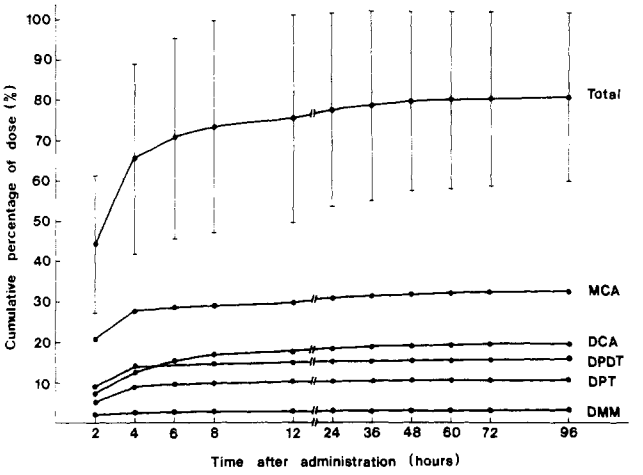

Figure 2. Cumulative excretion of the metabolites of malathion in urine after intravenous administration of 10 mg/kg of malathion to sheep. Each point represents the mean value for three sheep. For the curve depicting total cumulative percentage of dose, standard deviations are also given.

Malathion in Urine and Feces. During 96 h following the administration of malathion, totally only 2×10^{-3}% of the administered dose was excreted unchanged in urine, whereas about 1% of the dose was excreted as malathion in feces.

Metabolites in Urine and Feces. The excretion of malathion and the main metabolites in urine and feces are shown in Figures 2 and 3, respectively, as cumulative

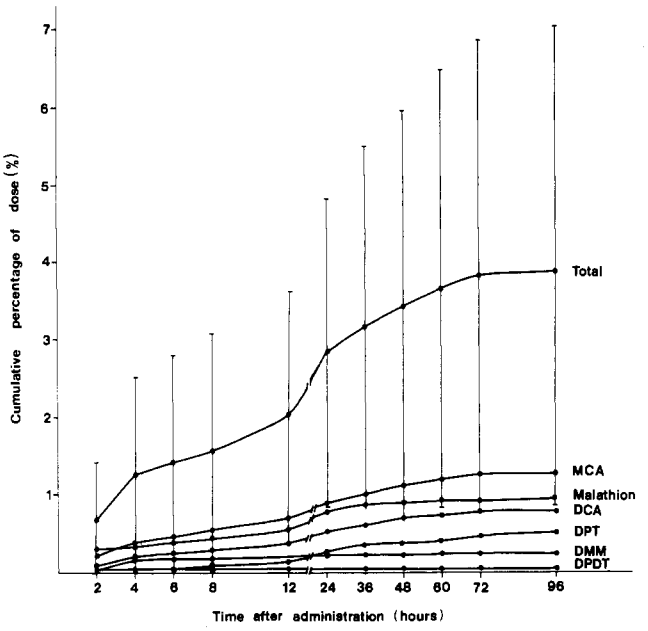

Figure 3. Cumulative excretion of malathion and its metabolites in feces after intravenous administration of 10 mg/kg of malathion to sheep. Each point represents the mean value for three sheep. For the curve depicting total cumulative percentage of dose, standard deviations are also given.

percentages of the administered dose as a function of time. The cumulative excretion data show that during the 96-h sampling period, 85% of the administered dose was excreted in urine and feces. At 2 and 24 h following administration, 44 and 78%, respectively, of the given dose were excreted in urine. During the 96-h period, 32% of the dose was excreted in urine as MCA and 25% as DCA. The amounts of DPDT, DPT, and DMM excreted in urine accounted for 16%, 10%, and 3%, respectively, of the given dose. Totally, 81% and 4% of the administered dose were excreted in urine and feces, respectively. As in urine, the main metabolite in feces was MCA, which accounted for about 1% of the given dose. Malaoxon was not detected in any of the urine and feces samples.

Cholinesterase Activity. At any time after the administration of malathion, the activities of the cholinesterases were at least 70% of the activities before treatment in both plasma and erythrocytes.

DISCUSSION

The differences in the shapes of the plasma concentration curves seen in Figure 1 may be due to individual variations in the metabolism capacity of the sheep. However, a rapid decrease in the concentration of malathion in plasma is observed for all sheep, probably due to distribution and an extensive enzyme-catalyzed hydrolysis of malathion. A contributing factor may be the possible dilution effect on the blood concentration of malathion as a consequence of an expected replacement of the rather large blood volume sampled during the first day. This makes it difficult to calculate pharmacokinetic parameters. An attempt to fit the data into a nonlinear two-compartment model with bolus input and first-order output gave an elimination half-life of 3–7 min, indicating that distribution equilibrium probably was not reached within 1 h after administration. This is in agreement with results from our previous study on malathion in cattle, where the plasma concentration of malathion decreased rapidly during the first hour following administration (Muan et al., 1985).

Only a limited fraction of the administered dose of malathion was excreted unchanged. However, malathion represents a larger portion (25%) of the amount excreted in feces as compared to the amount excreted in urine. This may be due to biliary excretion, excretion over the intestinal mucosa, or both.

The major metabolite resulting from administration of malathion to sheep was MCA, followed by DCA in both urine and feces. This is in agreement with the findings of O'Brien et al. (1961) who reported that MCA was the principal urinary metabolite after oral administration of malathion to a lactating cow. However, he found that dimethyl phosphate and *O,O*-dimethyl phosphorothioate were the principal degradation products in feces, which may be due to a more extensive degradation of malathion and metabolites by the microorganisms in the rumen when administered orally. Abd-Elroaf et al. (1981) reported that the main urine metabolite of malathion after oral administration to rats was MCA, followed by DMM and DCA. On the contrary, Bradway and Shafik (1977) found that the major metabolite resulting from exposure of rats to malathion was DCA, although with increasing exposure the relative amount of MCA increased steadily.

The cumulative excretion study of malathion showed that the compound was rapidly eliminated, mainly in urine and to a lesser extent in feces. Almost 45% of the administered dose was eliminated within 2 h and 81% within 24 h after injection, and 96 h posttreatment 85% of the dose could be accounted for. The rate of excretion observed for malathion in this study is in agreement with the findings of other authors. O'Brien et al. (1961) found that, after oral administration of malathion for 3 days to a lactating cow, the compound was principally excreted via the urine and that 69% of the dose was excreted within 4 days after the first dose. March et al. (1956b) reported that the greatest amount of activity appeared in the urine within the first 24-h period after dermal application of [^{32}P]malathion to calves. Studies in the hen have shown that about 90% of the dose is eliminated within 24 h after administration, mainly in the urine (March et al., 1956a; Gupta and Paul, 1977; Gupta et al., 1977). In the rat, 93 and 8.5% of the dose were excreted in urine and feces, respectively, after oral administration of malathion (Lechner and Abdel-Rahman, 1986). Bourke et al. (1968) found that 45% of the dose was eliminated in urine within 8 h and 83% within 24 h following oral administration of malathion to rats. The elimination in feces was somewhat slower: Only 0.78% was eliminated within 8 h; however, 5.5% was eliminated within 24 h after administration. The rapid elimination of malathion in rat has also been demonstrated by whole-body autoradiography (Muan and Nafstad, 1989).

The measurements of the cholinesterase activities together with clinical observations of the sheep did not reveal any significant toxicity symptoms at any time after the administration of malathion.

This study revealed that in sheep malathion is extensively metabolized to inactive metabolites and that the compound is rapidly eliminated from the body. The main excretion route of malathion in sheep is through urine, but a small portion of the administered dose was excreted in feces. The rapid elimination of malathion by sheep indicates that a drug residue problem should be minimal.

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Registry No. MCA, 1190-29-0; DCA, 1190-28-9; DPT, 1112-38-5; DPDT, 756-80-9; DMM, 1116-04-7; malathion, 121-75-5; cholinesterase, 9001-08-5.

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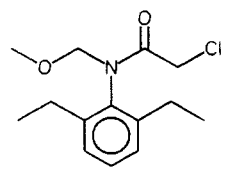
In Vitro Transformation of Chloroacetanilide Herbicides by Rat Liver Enzymes: A Comparative Study of Metolachlor and Alachlor

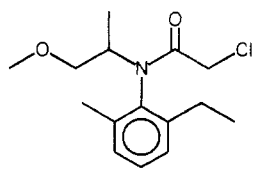
Paul C. C. Feng* and Stephen J. Wratten

The in vitro transformation of metolachlor by rat liver cytosolic and microsomal enzymes was studied. In the presence of glutathione (GSH), the liver cytosolic enzymes catalyzed complete conversion of metolachlor to a more polar metabolite identified as the metolachlor–GSH conjugate. Liver microsomal enzymes, fortified with NADPH, catalyzed O-demethylation, benzylic hydroxylation, and N-dealkylation reactions of metolachlor. Eight oxidized metabolites were identified involving single, double, and triple hydroxylations at four different sites in the metolachlor molecule. The in vitro transformation of metolachlor by rat liver enzymes was similar to that observed with alachlor; both herbicides readily undergo conjugation with GSH and oxidation. The rate of GSH conjugation by rat liver cytosolic enzymes was significantly faster for alachlor than metolachlor. The rate of oxidation by rat liver microsomal oxygenases was similar for alachlor and metolachlor.

Metolachlor [2-chloro-*N*-(2-methoxy-1-methylethyl)-*N*-(2-ethyl-6-methylphenyl)acetamide] and alachlor [2-chloro-*N*-(methoxymethyl)-*N*-(2,6-diethylphenyl)acetamide] are the two most widely used commercial chloroacetanilide herbicides.



Alachlor



Metolachlor

The metabolism of metolachlor in soil, plants, and animals was recently summarized by LeBaron et al. (1988). Although details of the studies were not presented in that report, O-demethylation and hydrolytic dechlorination of metolachlor appeared to be the major pathways of metabolism in rats. O-Demethylation has also been reported as a major pathway of metabolism for metolachlor in a soil fungus (McGahen and Tiedje, 1978) and a soil actinomycete (Krause et al., 1985).

Our laboratory has been conducting metabolism studies of chloroacetanilide herbicides in animals. Based on our experience, in vitro incubations with animal tissue fractions are very useful in predicting the in vivo metabolism and in elucidating the initial and intermediary pathways of metabolism. Alachlor is rapidly degraded in animals through a complex network of several pathways (Sharp, 1988). We have previously examined the in vitro transformation of alachlor by liver and kidney enzymes from rats, mice, and monkeys (Feng and Patanella, 1988, 1989) and have found good qualitative correlation between the results of in vivo and in vitro studies.

Monsanto Agricultural Company, St. Louis, Missouri 63198.

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