Residues of Ethion in Milk after Intravenous, Oral, and Dermal Administration to Goats

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Mammary excretion and residues in milk of the organophosphorus insecticide ethion were studied in lactating goats after intravenous and oral administration of [^{14}C]ethion (2 and 10 mg/kg, respectively) and after dermal application of 100 mg/kg ethion. Intravenous injection resulted in residues of ethion in milk above the recommended maximum residue level (MRL) for less than a week but for more than 2 weeks if based on ^{14}C. After oral administration, ethion is almost completely metabolized before absorption, leading to very low levels of ethion but high levels of ^{14}C in milk (above the MRL) and plasma for more than 2 weeks. Dermal application of ethion results in a limited but very prolonged absorption and levels of ethion above the MRL in milk for up to 5 weeks.

INTRODUCTION

In areas where tick-borne diseases like East Coast fever, anaplasmosis, and babesiosis are serious problems as in eastern Africa, dipping of livestock is practiced routinely to control ticks and hence the diseases caused by them. For many years organochlorines (OCs), e.g., technical BHC, lindane, and toxaphene, have been the main acaricides used for dipping or spraying animals to control ticks. However, with OCs there have been problems of tick resistance to the acaricides and also of persistence of the acaricides in the tissues of the dipped animals. In such cases organophosphates (OPs) have been the alternative. Thus, for example, Kenya shifted to dioxathion after banning the use of toxaphene in 1976 (Keating, 1977), and today Tanzania is using dioxathion in areas where resistance to toxaphene is a problem.

After cattle are dipped in OPs like dioxathion and ethion, residues are known to occur in adipose tissue with the disappearance half-life being approximately 15 days in both cases (Palmer et al., 1977). Information on the excretion of most OPs including ethion in milk after dipping is scarce despite the fact that this acaricide has been in use since 1956 (Martin, 1968). To avoid ethion residues in milk, it is important to know the pattern of its excretion in milk, and it was therefore the purpose of the present work to study ethion residues in goat milk after intravenous, oral, and dermal administration of the drug.

MATERIALS AND METHODS

The following terms are used: ethion, the parent compound; [^{14}C]ethion, labeled ethion; ^{14}C, [^{14}C]ethion plus [^{14}C]ethion metabolites calculated as ethion.

(a) Chemicals. Technical (95% pure) ethion [*O,O,O',O'*-tetraethyl *S,S'*-methylenebis(phosphorodithioate)] and ethion emulsifiable concentrate (1010 g/L) were generously supplied by Cheminova A/S. [^{14}C-*methylene*]Ethion (11.9 mCi/mmol) with a radiochemical purity of more than 96% was purchased from Amersham. The [^{14}C]ethion was added to the technical ethion so that the specific activities of the final solutions for oral and intravenous administration were 0.4 and 2 μCi/mg, respectively. Both preparations were dissolved in glycerol formal before administration. The ethion emulsifiable concentrate was diluted 1:4 with water before it was applied to the skin.

(b) Animal Experiments. All goats in the three groups were lactating and weighed between 41 and 56 kg. They were fed hay and nutritive concentrate and given water ad libitum.

(*i*) *Intravenous Experiments.* Four goats were administered [^{14}C]ethion intravenously (IV) at the dose of 2 mg/kg. The injection was done into one jugular vein, and blood samples were collected from the other. The dose of 2 mg/kg was chosen on the basis of previous experiments (Mosha and Gyrd-Hansen, 1990), where 2 mg/kg IV did not produce signs of toxicity but 5 and 10 mg/kg IV did.

(*ii*) *Oral Experiments.* Three goats were orally administered [^{14}C]ethion at the dose of 10 mg/kg. The dose of 10 mg/kg was chosen on the basis of a pilot trial, where a 20 mg/kg oral dose was followed by signs of toxicity in the goat. The administration was done by using a narrow stomach tube.

(*iii*) *Dermal Experiments.* Three goats were administered ethion on the skin at the back at 100 mg/kg. This is about 5 times the normal dose received after dipping in the recommended 0.075% ethion solution if it is assumed that 1–1.5 L of dipping solution is enough for a 50-kg goat. A pilot trial using 20 mg/kg dermal application, which corresponds to the normal dipping dose, was followed by very low concentrations of ethion (<0.01 ppm) in blood plasma. The 600-700-cm^2 areas of application were clipped before the diluted emulsifiable concentrate was administered.

(c) Sampling. Blood sampling was done through a polyethylene catheter (Bard-I-Cath) placed in the jugular vein just before dosing and at 1, 4, 8, 12, and 15 h and thereafter by using an 18G needle at 24, 30, and 48 h and then each morning until day 14 after exposure. Blood was collected in heparinized tubes and plasma separated by centrifugation. All plasma samples were stored at −20 °C until analyzed.

The emptying of the udder of milk followed the same sequence as blood sampling. For the dermal experiments milking and blood sampling were further done in the morning on days 32, 38, and 46 postexposure. All milk samples were stored at −20 °C until analyzed.

(d) Analytical Methods. (*i*) *Distribution of Ethion in Milk Components.* Blank milk samples were spiked with [^{14}C]ethion (2 μCi/mg) at the rate of 2 μg/mL and then mixed for 1 h. The milk components, i.e., cream, skim milk, casein, and whey, were separated according to the method of Ziv and Rasmussen (1975). The quantification of ethion in the milk components was performed by liquid scintillation counting.

(*ii*) *Protein Binding.* The binding of ethion to milk proteins was determined in vitro by equilibrium dialysis (Ehrnebo et al., 1971). Skim milk (Ziv and Rasmussen, 1975) was spiked with [^{14}C]ethion (2 μCi/mg) to obtain concentrations between 0.1 and 4 μg/mL. By use of a Dianorm dialyzer with Teflon cells each milk sample was dialyzed against an equal volume of phosphate buffer, pH 6.83, at 37 °C for 5 h as equilibrium was obtained within that time. The membrane used was Visking cellophane tubing, average pore size 2.4 nm with a molecular weight cutoff of 12 000–14 000. After dialysis, aliquots from both sides of the

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Table I. Distribution of Ethion (2 μg/mL) in Milk Components

component	ethion, %	component	ethion, %
cream	97.1	whey	1.2
skim milk	2.9	casein	1.8

Table II. Protein Binding of Ethion in Milk

milk concn, ng/mL	protein binding, %	milk concn, ng/ml	protein binding %
100	98.2	2000	99.9
200	99.2	4000	99.9
500	99.6		
1000	99.3	mean ± SD	99.4 ± 0.6

membrane were assayed for ethion by using gas chromatography and liquid scintillation counting.

(iii) Gas Chromatography. The quantification of ethion in plasma, milk, and buffer was done by gas chromatography. Ethion was extracted from plasma and buffer by an equal volume of hexane containing parathion as an internal standard. Some hexane extracts were concentrated so that low levels of ethion could be quantified. The hexane extracts were analyzed on a Hewlett-Packard 5890A gas chromatograph with a nitrogen phosphorus detector (NPD). Chromatographic conditions were as follows: column, glass column 6 ft × 2 mm i.d. packed with Chromosorb W-HP 100/120 coated with 3% SE-30; carrier gas, helium, flow rate 25 mL/min; temperatures, injector 240 °C, column 220 °C, and detector 290 °C; injection volume, 4 μL; retention times, parathion 2.2 min, ethion 4.0 min. Recovery rate for ethion in plasma was 94 ± 5%. Detection limit for ethion was 3 ppb.

Ethion was extracted from milk according to the method of Muan and Skaare (1986) for extraction of malathion from milk. Instead of 15 mL of milk, 1.5 mL was used with the corresponding reduction in volume of the chemicals. The final 1-mL extract containing parathion as internal standard was chromatographed. The recovery rate of ethion from milk was 101 ± 4%.

(iv) Liquid Scintillation Counting. The concentration of ^{14}C in plasma, buffer, and milk was determined by liquid scintillation counting using an LKB Wallac 1217 Rackbeta scintillation counter with automatic quench correction by the external standards method. Each sample (100 μL) was counted in duplicate in 3 mL of OptiPhase HiSafe (FSA Laboratory Supplies) scintillation cocktail.

RESULTS

The distribution of ethion in the different components of milk is given in Table I, which shows that about 97% of the acaricide was retained in cream and only 3% in skim milk. About two-thirds of the ethion in skim milk was bound to the casein fraction. Protein binding in milk was found to be as high as 99.4 ± 0.6% for ethion concentrations between 0.1 and 4 μg/mL (Table II).

Figure 1 shows the levels of ^{14}C and the much lower levels of ethion in milk and plasma after intravenous application of 2 mg/kg [^{14}C]ethion. The maximum concentration of ^{14}C in milk was 3.1 ± 1.4 ppm obtained after 4 h. The ^{14}C level in milk was higher than in plasma for the first 4 days after which time both fluids contained approximately 0.4 ppm, falling to 0.1 ppm during the rest of the 14-day experiment. The peak level of ethion in milk was 1.2 ± 0.5 ppm, obtained 4 h after administration. The levels of ethion in milk were more than 10 times higher than in plasma up to 24 h and detectable for 5–7 days.

Figure 2 shows the ^{14}C and the very low ethion levels in milk and plasma after oral administration of 10 mg/kg [^{14}C]ethion. ^{14}C in plasma had a peak value of 5.5 ppm at 24–30 h, which was higher than the peak concentration in milk of 3.9 ppm reached after 72 h. From this point on the ^{14}C levels were almost equal in milk and plasma.

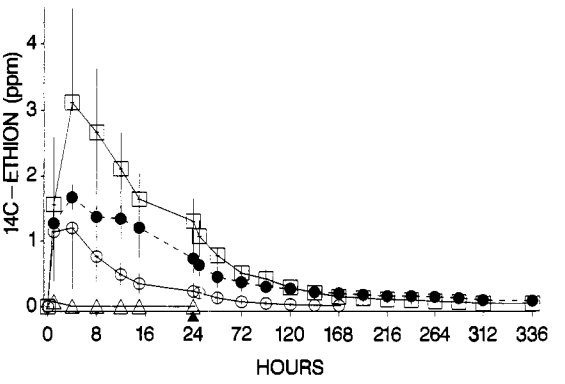

Figure 1. Concentrations of ethion and ^{14}C in plasma and milk after IV administration of 2 mg/kg of body weight of [^{14}C]ethion (n = 4). Plasma: △, ethion; ●, ^{14}C. Milk: ○, ethion; □, ^{14}C. Mean ± SD. ▲ indicates a change in the scale.

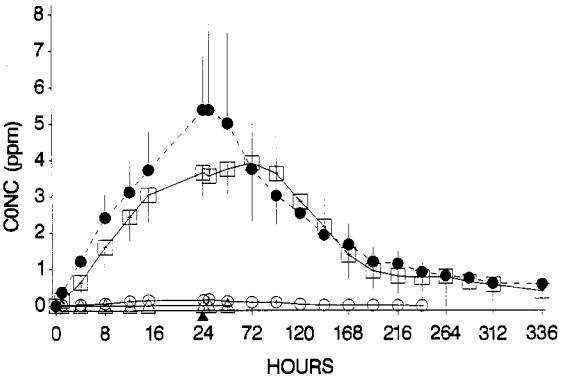

Figure 2. Concentrations of ethion and ^{14}C in plasma and milk after oral administration of 10 mg/kg of body weight of [^{14}C]ethion (n = 3). Plasma: △, ethion; ●, ^{14}C. Milk: ○, ethion; □, ^{14}C. Mean ± SD. ▲ indicates a change in the scale.

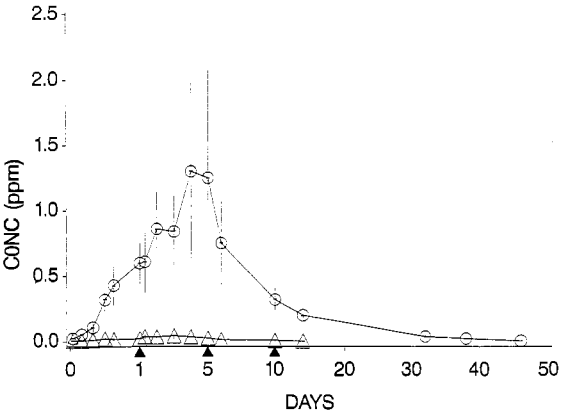

Figure 3. Concentrations of ethion in plasma (△) and milk (○) after dermal application of 100 mg/kg of body weight of ethion (n = 3). Mean ± SD. ▲ indicates a change in the scale.

The concentrations of ethion in milk were higher than in plasma but had a peak value of only 0.2 ppm after 24 h. The ethion levels in milk were thus far lower than the corresponding levels after IV administration of a 5 times smaller dose (Figures 1 and 2) but detectable for 8–10 days.

Figure 3 shows the levels of ethion in milk and plasma after dermal application of 100 mg of ethion/kg. The average peak level in milk was 1.3 ppm, obtained after 4 days. The concentrations in plasma were less than 5% of the milk concentrations at corresponding times. Moreover, plasma levels were hardly detectable after day 14, whereas milk levels persisted much longer and were still detectable

Table III. Cumulative Excretion of Ethion and ^{14}C (Percent of Dose) in Goat Milk after IV, Oral, and Dermal Administration (Values in Parentheses Are for ^{14}C)

days after exposure	route of administration		
	IV	oral	dermal
1	1.0 ± 0.4 (2.5 ± 0.7)	0.01 ± 0.00 (0.4 ± 0.2)	0.01 ± 0.00
2	1.1 ± 0.4 (2.9 ± 1.0)	0.03 ± 0.01 (0.8 ± 0.1)	0.01 ± 0.00
4	1.1 ± 0.4 (3.3 ± 1.2)	0.03 ± 0.01 (1.1 ± 0.2)	0.02 ± 0.02
7	1.2 ± 0.4 (3.7 ± 1.1)	0.04 ± 0.01 (1.5 ± 0.2)	0.03 ± 0.02
9	1.2 ± 0.4 (3.9 ± 1.2)	0.04 ± 0.01 (1.6 ± 0.2)	0.04 ± 0.02
14	1.2 ± 0.4 (4.3 ± 1.3)	0.04 ± 0.01 (1.7 ± 0.3)	0.05 ± 0.02

on day 38 to a level of 0.02 ppm. Table III shows the amounts of ethion and ^{14}C excreted in milk for 2 weeks as percentage of dose after the three routes of administration.

DISCUSSION

In milk 97% of the ethion was found in the cream (Table I), which is in accordance with ethion being a lipophilic compound with an octanol/water partition coefficient as high as 10 000 (Cheminova, 1986). This affinity of ethion for lipids explains the high levels of ethion found in milk compared to plasma after IV and dermal administration (Figures 1 and 3).

The binding of ethion to milk proteins was about 99% and not dependent on concentrations within the range tested (Table II). This is very similar to the protein binding of ethion in plasma, which was found to be above 99% (Mosha et al., 1990).

After oral exposure, the levels of ethion in plasma and milk were very low despite the fact that the dose was 5 times higher than the IV one. This can be explained by assuming that ethion, like other OPs, is hydrolyzed by rumen microorganisms before it is absorbed from the gastrointestinal tract (GIT) (O'Brien, 1960) and is subjected to first-pass metabolism by the liver (Braeckman et al., 1983). In goats less than 5% of an oral dose of [^{14}C]ethion is absorbed unchanged, while the absorption of ^{14}C from the GIT is nearly complete (Mosha et al., 1990). This high bioavailability of ^{14}C is reflected by the high plasma levels in the present study (Figure 2). ^{14}C seems to be distributed equally between plasma and milk (Figures 1 and 2), indicating that ethion metabolites are more polar and hence less lipophilic than the parent compound.

After dermal exposure of goats to 100 mg of ethion/kg, a slow and prolonged absorption of approximately 20% of the dose was seen in the first 2 weeks (Mosha et al., 1990). In the present study ethion was detected in plasma for 2 weeks and in milk for at least 5 weeks (Figure 3). Dermal application of another OP, coumaphos, to goats in a dose of 14 mg/kg resulted in coumaphos levels in the milk of only 0.07–0.12 ppm between day 2 and day 7, when the goats were killed, indicating a correspondingly slow absorption for coumaphos (Konar and Ivie, 1988). The concentrations of coumaphos found in milk were 4–5 times the recommended MRL. As cutaneous metabolism of xenobiotics is considered rather limited (Bronaugh et al., 1989), the major part of the ethion is most likely absorbed unchanged and then either metabolized or distributed into tissues and milk fat in accordance with ethion's lipid solubility as reflected in the very high levels in milk compared to plasma (Figure 3). In a similar experiment with dermal application of malathion to cows, administration of 5 g per animal resulted in peak values of only about 0.05 ppm in milk after 4–6 h and levels of less than 0.01 ppm after day 2. Only about 0.01% of the malathion administered was recovered in the milk (Muan et al., 1985). Claborn et al. (1960) reported similar findings after dermal application of a 0.05% malathion emulsion to cows. These findings correspond to the observations in the present study, where only about 0.05% of the dose was recovered in milk after dermal application (Table III).

FAO/WHO (1985) has recommended an MRL of 0.02 ppm for ethion in milk. According to FAO/WHO (1989) the MRL is derived from residues of the parent drug, all metabolites, and drug-based products that result from drug administration to food-producing animals.

In the present study the recommended MRL of 0.02 ppm was reached within 1 week for ethion after 2 mg/kg IV—but not in 2 weeks for ^{14}C (Figure 1).

Oral administration of 10 mg/kg resulted in low levels of ethion in milk, and the 0.02 ppm level was reached in a week; however, even after 2 weeks, the level of ^{14}C was still as high as 0.5 ppm, i.e., 25 times the MRL (Figure 2).

In the experiment with dermal application only ethion itself was measured, but it is clear that due to prolonged absorption the MRL in milk is not reached in less than 5 weeks (Figure 3). However, the dose administered was approximately 5 times higher than the normal dose per dipped goat, but even if adjusted for the higher dose, it appears that ethion levels in milk would not reach the MRL in less than 3–4 weeks and that without taking ethion metabolites into consideration.

ACKNOWLEDGMENT

The study was supported by the Danish International Development Agency (DANIDA). Technical assistance by Miss Kitty Petersen is highly acknowledged.

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Received for review March 5, 1990. Revised manuscript received June 28, 1990. Accepted September 4, 1990.

Registry No. Ethion, 563-12-2.