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Liquid Paraffins in Feed Enhance Fecal Excretion of Mirex and DDE from Body Stores of Lactating Goats and Cows

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Contamination of livestock with halogenated hydrocarbons has caused large economic losses in the past; not so much because of acute toxicity to animals but mainly because their high lipid solubility is frequently accompanied by low biodegradation resulting in prolonged half-lives. These characteristics made natural decontamination economically unfeasible so that often disposal rather than salvage of livestock has been arranged in the past. (NYAS, 1979). Liquid paraffins administered in the feed were shown to promote removal of lipophilic pesticides from body stores in rats and rhesus monkeys by enhancing excretion with feces (Richter et al. 1977; Rozman et al. 1981a, b). Addition of white mineral oil or n-hexadecane to the diet enhanced fecal elimination of hexachlorobenzene from body stores of growing lambs without adverse effects on feed intake or diet digestibility (Rozman et al. 1982a). Studies with rats and monkeys demonstrated that the enhanced elimination of lipophilic halogenated hydrocarbons occurred mainly by direct intestinal transfer, mostly in the cecum and colon, rather than by biliary excretion (Rozman et al. 1981a, 1982a, 1983; Rozman and Rozman 1983).

This study examined effects of liquid paraffins added to the feed on the disposition of ^{14}C-mirex in lactating goats under laboratory conditions and effects of white mineral oil in the feed on levels of DDE in body fat, milk and feces of dairy cattle in a field trial.

MATERIALS AND METHODS

Goat study. Three dairy goats weighing 39.6 (± 1.2) kg were placed in metabolism cages during late lactation and adapted for 2 weeks

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to a commercial pelleted feed (OKAY 60-30-10 cubes, Worley Mills, Inc., Clovis, NM) and experimental conditions. Feed was offered twice daily and intakes were adjusted to 1000 ± 50 g so that consumption was accomplished within 3 hr after feeding. Non-labelled mirex (1.74 g, Supelco Inc., Bellefort, PA) and ^{14}C-mirex [dodecachloro-octahydro-1,3,4-methane-2H-cyclobuta (c,d) pentalene] (7.325×10^8 dpm; spec. act. 9.3 mCi/mmol; California Bionuclear Co., Sun Valley, CA) were dissolved in 390 ml absolute ethanol resulting in spec. act. of 421 dpm $(\mu g)^{-1}$. Immediately prior to dosing of goats, the ethanolic mirex solution was mixed with an equal volume of corn oil (Mazola®) and an emulsion was prepared by ultrasonication. Each goat was given 3.3 ml of the resulting emulsion per kg body weight, administered intraruminally by gavage in two portions (1100 h and 1600 h) on day 1 of the experiment, thereby providing 7 mg mirex/kg twice (= 14 mg/kg) on the day of dosage.

Fourteen days after dosage with mirex, light mineral oil (mineral oil, light, viscosity 60 to 90° saybolt, Sargent Welch Scientific Co., Skokie, IL) was added to the feed (5 g/100 g) of two goats each day for 16 days, after which they received the basal diet (without mineral oil) for 16 days. Thereafter, these same two goats received again the basal diet plus mineral oil (5 g/100 g) for another 14-day period and this was followed by another 14-day period when the basal diet (without mineral oil) was fed. The other goat received the basal diet throughout the study except for one 14-day period (days 32 through 46) when reagent grade n-hexadecane (Sigma Chemical Co., St. Louis, MO; purity, 99%) was added to the feed (5 g/100 g). This period of hexadecane feeding coincided with the interlude when mineral oil was not fed to the other two goats.

Feces were collected quantitatively in plastic bags placed within cloth, fecal collection bags with harnesses custom-fit to each animal. Bags were emptied twice daily. Urine was collected quantitatively into polyethylene jugs and emptied once daily. Each goat was hand-milked twice daily by the same individual. Representative samples of fresh feces, urine and milk were procured daily and kept frozen at -20°C until processed. Blood samples were collected by jugular venipuncture into heparinized, sterile tubes on day 14 and each recurring seventh day thereafter.

Concentrations of mirex in samples of feces, blood and milk were determined by combusting aliquots in a Packard 306 sample oxidizer and counting the radioactivity in a Packard 2425 scintillation spectrophotometer. Radioactivity in urine was counted directly by mixing aliquots of urine with a dioxane-based scintillation fluid. It was assumed that all ^{14}C activity represented mirex because mirex is extremely resistant to biodegradation in animal tissues (Wiener et al. 1976).

Cattle study. A commercial dairy herd in Arizona exhibited butterfat p,p'-DDE (2,2-bis(p-chlorophenyl)-1,1-dichloroethylene) levels in the range 0.7 to 2.5 ppm. The source of contamination was assumed to be their feed that was grown on fields heavily sprayed with p,p'-DDT (2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane) in the 1960's. Ten cows in late gestation were selected as representative of the herd, stratified by age and condition scores and allocated into two equal groups. Both groups were treated similarly and fed the same (contaminated) feed except that one group ("treated") received white mineral oil sprayed daily on the concentrate mix whereas the concentrate mix of the other group ("controls") was sprayed with the same amount of water. Mineral oil or water added amounted to 3% of total diet. Dietary treatments with mineral oil or water were continued for 30 days.

Specimens of adipose tissue (tailhead) and feces (rectal grab sample) were collected the day before the onset and the day before cessation of mineral oil or water administration. Milk samples were procured on day 70 after commencement of treatments, which corresponded to 25 ± 14 days post-parturition. Concentration of p,p'-DDE in feces was determined by Wilson Laboratories (Salina, KS) according to EPA Manual (1980). Concentration of p,p'-DDE in fat and milk was measured in our laboratory according to the Report by the Panel on Determination of Organochlorine Pesticides in Foodstuffs of Animal Origin (1979).

RESULTS AND DISCUSSION

Shown in Table 1 is the disposition of mirex (expressed as percentage of dose) in three goats during the first 14 days after dosage. Mirex was detected in urine on the day of dosage, although only trace amounts were detected in feces. Urine levels remained very low throughout the study. Peak values for mirex in feces occurred on the second day after dosage and seemed to decline according to a biphasic pattern. Milk levels peaked on the second and third day after dosing and appeared also to follow a biphasic trend with values one hundredfold greater than in urine at the peak (days 2 and 3) and about tenfold greater from day 11 onward. Cumulative excretion of mirex during 14 days after dosage, expressed as percentage ($\bar{x}$ ± SD) of dose, was 44.5 ± 6.72 in feces, .41 ± .02 in urine and 14.6 ± 1.32 in milk.

At 2 weeks after dosage, approximately 40% of total mirex administered remained as "body burden." At that time, daily excretions into feces and into milk were about equal in magnitude and together amounted to about 1% of body burden. Levels of mirex in whole blood at 2 weeks after dosage were in the range .10 to .18 µg/ml.

Table 1. Excretion of mirex into feces, urine and milk by lactating goats during 14 days after single, intraruminal dosage at 14 mg mirex per kg body weight.[a]

	Percentage of dose excreted daily		
Days	Feces	Urine	Milk
1	(traces)	.034 ± .012[b]	.60 ± .38
2	13.89 ± 6.89	.027 ± .005	2.96 ± 1.47
3	6.41 ± 2.96	.027 ± .006	3.27 ± .80
4	4.87 ± 1.75	.027 ± .010	1.77 ± .18
5	6.42 ± 4.10	.035 ± .036	1.86 ± .74
6	5.91 ± 4.41	.033 ± .079	.78 ± .38
7	2.24 ± 1.64	.034 ± .011	.84 ± .50
8	1.24 ± .86	.037 ± .005	.64 ± .32
9	1.16 ± .76	.031 ± .008	.44 ± .17
10	.83 ± .42	.018 ± .006	.32 ± .07
11	.49 ± .14	.024 ± .007	.29 ± .06
12	.35 ± .08	.029 ± .002	.31 ± .06
13	.37 ± .11	.024 ± .006	.27 ± .06
14	.28 ± .04	.029 ± .008	.21 ± .04
	Cumulative excretion, % of dose		
1 through 14	44.5 ± 6.72	.41 ± .02	14.6 ± 1.32

[a] Dosed : 7 mg/kg at 1100 h + 7 mg/kg at 1600 h.

[b] Mean ± SE (n = 3).

Addition of mineral oil to the diet caused no change in intake of feed. (The goats consumed all of the feed provided.) The levels of liquid paraffins administered caused no diarrhea or laxative effect. Excretion of fresh feces was 695 ± 43.6 g per day during the week before treatment was started and 685 ± 76.2 g during the first week of mineral oil administration. The effect of mineral oil or hexadecane on excretion of mirex in feces is shown in Figure 1. Whether expressed as concentration of mirex in feces (as in Figure 1) or as cumulative percentage of mirex excreted into feces during periods of treatment (data not shown), liquid paraffins increased mirex excretion into feces by about twofold.

Milk production of the goats declined from 1074 ± 179 ml/d during the first week after dosage to 485 ± 120 ml/d during the last week of the experiment. The decline was due in part to the late stage of lactation and also, perhaps, to the conditions imposed by experimentation. Concentrations of mirex in milk overlapped during day 15 through 74 without regard whether liquid paraffins were added to the feed or not (Figure 2). Likewise, there were no detectable differences in levels of mirex in whole blood (Figure 3) during periods when diets contained liquid paraffins or when the animals received basal diet.

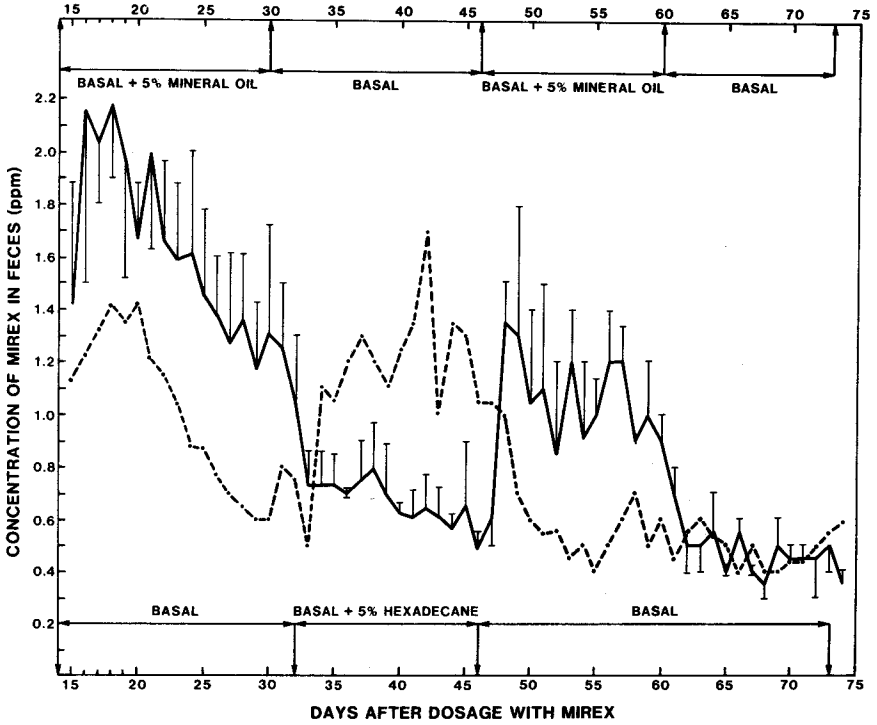

FIGURE 1. Concentrations of mirex in feces of goats fed 1,000 ± 50 g of feed daily, as affected by liquid paraffins added to the feed (50 g daily) during interludes after dosage with 14 mg mirex/kg body weight. Solid line represents the average of values for two goats and interrupted line represents values for a single goat.

Prior to treatment, fecal excretion and fat levels of DDE were similar in both groups of cows (Table 2). Addition of mineral oil to the diet increased fecal concentration of DDE by threefold ($P < .001$). Levels of DDE in adipose tissue and milk were not significantly affected (Table 2).

Both studies described herein confirm previous reports that addition of liquid paraffins to the feed enhances fecal excretion of lipophilic chemicals. Fecal excretion of mirex was increased by about twofold (Figure 1) which is similar to results obtained in rhesus monkeys during the first month after mirex dosage (Rozman et al. 1981b). A much larger effect (fivefold increase) was observed in monkeys half a year after dosing, which was attributed to redistribution of mirex (Pittman et al. 1976) into the "very deep" compartment (fatty tissues) and to the fact that mineral oil treatment appears to affect primarily this compartment and only

Table 2. Effect of mineral oil on the concentration of p,p-DDE in feces, fat and milk of dairy cows.[a]

	Concentration of p,p-DDE (ppm)					
	Controls			Treated[b]		
	Feces	Fat	Milk	Feces	Fat	Milk
0	.036 ± .010[c]	2.02 ± .30	--	.032 ± .012	2.16 ± .27	--
30	.034 ± .010	2.04 ± .29	--	.103 ± .007*	2.06 ± .22	--
70	--	--	2.68 ± .14	--	--	2.38 ± .22

[a]Exposed to unknown amounts of p,p-DDE in the daily feed.

[b]Their daily feed was amended with 3% mineral oil for 30 consecutive days.

[c]Mean ± SEM (n = 5).

*Statistically different from control, $P < .001$.

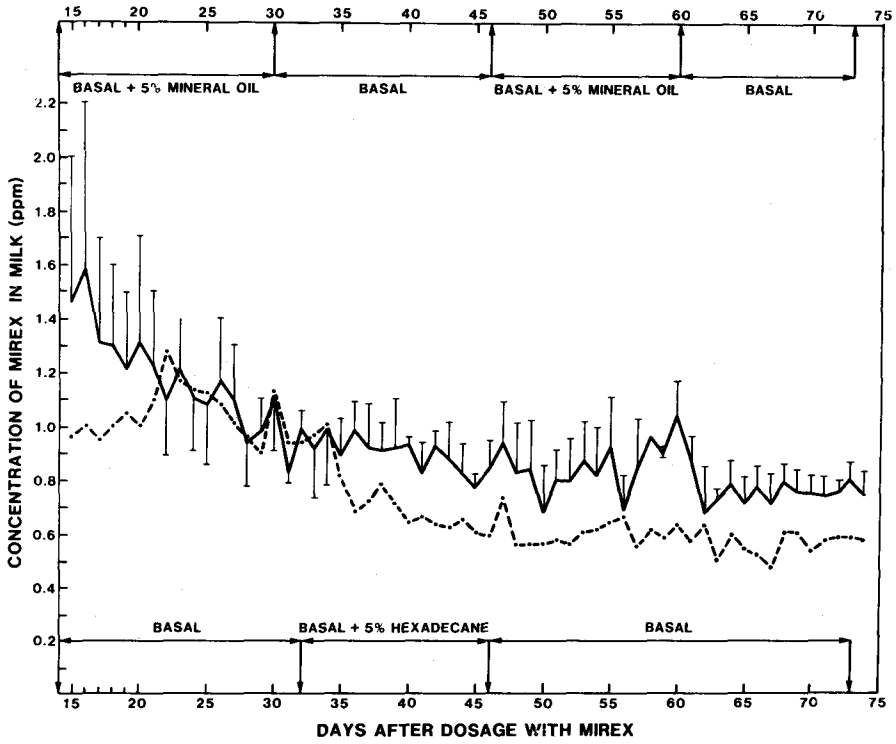

FIGURE 2. Concentration of mirex in milk of goats fed 1,000 ± 50 g of feed daily, as affected by liquid paraffins added to the feed (50 g daily) during interludes after dosage with 14 mg mirex/kg body weight. Solid line represents the average of values for two goats and interrupted line represents values for one goat.

secondarily the peripheral one(s). Redistribution processes may also further complicate the effect of liquid paraffins on mirex kinetics in lactating goats, particularly during the post-lactation period when adipose tissue will be replenished with fats. In contrast to goats that were dosed in late lactation, the cows were apparently exposed to DDE prior to and during treatment. Fecal excretion and fat levels in the control cows on day 0 and day 30 indicate absorption/excretion steady state, which is not surprising since the animals were presumably exposed to the same contaminated feed for many months before, as well as during, the treatment period. Under these conditions 3% mineral oil (ca. 0.5 ml/kg) enhanced fecal excretion of DDE by threefold (Table 2). It could be argued that the effect of mineral oil in this case might be due to malabsorption of DDE. Although it is known that large amounts of mineral oil (5 ml/kg) have a laxative effect and cause malabsorption of lipophilic substances (Forth et al. 1977), it has

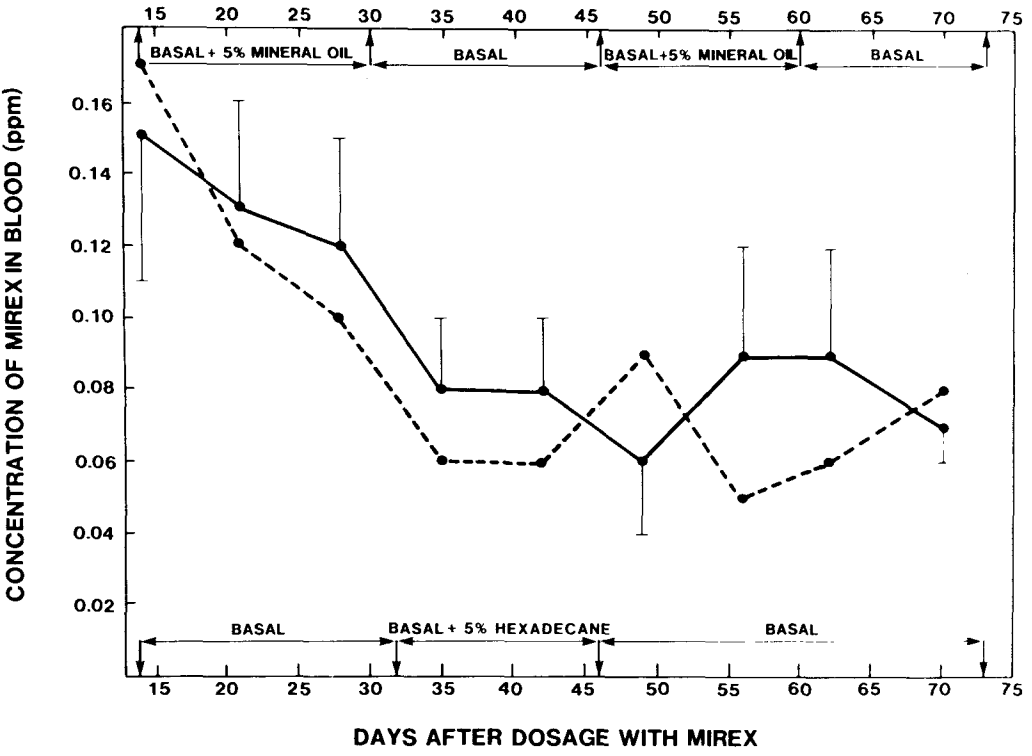

FIGURE 3. Concentrations of mirex in whole blood of goats, as affected by liquid paraffins added to the feed (50 g daily) during interludes after dosage with 14 mg mirex/kg body weight. Solid line represents the average of values for two goats and interrupted line represents values for one goat.

been demonstrated that smaller amounts of mineral oil (1 ml/kg) are nonlaxative and facilitate rather than prevent absorption of lipophilic chemicals (Rozman et al. 1982b). Thus, it is more likely that mineral oil stimulated nonbiliary intestinal excretion of mirex and DDE in these goats and cows as was shown conclusively for chlordecone and hexachlorobenzene in laboratory animals (Boylan et al. 1979; Rozman et al. 1982a, 1983; Rozman and Rozman, 1983).

There are no measured indicators for the body burden of mirex in these goats because we could not obtain clearly identifiable adipose tissue by biopsy at this stage of lactation. However, a very good correlation between enhanced fecal excretion and body burden reduction was established for hexachlorobenzene in rats (Scheufler and Rozman, unpublished). Thus, it is reasonable to assume that continued enhanced fecal excretion would also result in a corresponding reduction of mirex half-life in goats. Under the

steady state conditions of the DDE study with cows the treatment was of insufficient length to reduce body burdens significantly. This finding indicates that utilization of contaminated feed during the process of decontamination is somewhat problematic and would require, at best, a carefully developed schedule between treatment and feeding contaminated and uncontaminated feed.

Similar to previous reports in other species dosed with hexachlorobenzene, blood levels of mirex in goats were not affected by a total of 30 days mineral oil treatment (Rozman et al. 1981a, 1983). This is in contrast to food restriction experiments in rats, in which enhanced fecal excretion of persistent lipophilic compounds was accompanied by a rapid fourfold increase in blood levels (Jondorf et al. 1982; Villeneuve 1975). This is not surprising, since food restriction results in enhanced lipolysis. However, mineral oil does not seem to exert its effect by this mechanism because blood levels of lipophilic chemicals are unaltered at first and decline only slowly in accordance with decreasing body burdens (Rozman et al. 1981a). Thus, it is likely that enhancement of fecal excretion is due to an altered equilibrium between central compartment and intestinal contents, in favor of the latter, as a result of increased lipophilicity of the contents because of the presence of aliphatic hydrocarbons. More importantly, the rate limiting step in the intestinal excretion of lipophilic chemicals may be the adipose tissue/blood equilibrium rather than that between blood and intestinal contents. An unmeasurably small difference between venous and arterial blood could account for the pronounced effect seen in intestinal contents when considering the several thousand circulations per day that maintain the equilibrium.

Similar considerations apply for milk levels which were also not affected significantly by the treatment. This finding is contraindicative of increased lipolysis and favors the hypothesis that the rate determining step between excretion and storage of lipophilic chemicals is the equilibrium between adipose tissue and blood. Since blood levels over a period of one month were not affected by the treatments, milk levels should not be altered either. However, it was clearly demonstrated that blood levels do decline in accordance with body burdens (Rozman et al., 1981a). Thus, it can be expected that milk levels will also decrease correspondingly as body burdens become diminished.

The half-life of mirex might be too long to derive significant benefits from decontamination by mineral oil. Clearly, continued feeding of contaminated feed (as occurred in our study with DDE) tends to defeat somewhat the purpose of decontamination. Yet, many lipophilic compounds have half-lives in the range of months to a few years. Moreover many extensive livestock contaminations are marginal, amounting to a few ppm in adipose tissue, which is usually not more than one to fivefold above the maximal allowed

concentration. In these instances the reduction of a contaminant's half-life in lactating animals by a factor of three to five could make the difference between disposal or salvage of expensive animals.

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