Use of Aliphatic Hydrocarbons in Feed To Decrease Body Burdens of Lipophilic Toxicants in Livestock

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Sheep were contaminated with hexachlorobenzene and then fed a conventional diet with or without 5% mineral oil or hexadecane. Similar to nonruminant species, both treatments enhanced fecal excretion of hexachlorobenzene about 3-fold and reduced levels of hexachlorobenzene stored in adipose tissue. Normal digestive functions of the animals, including fiber digestion by rumen microbes, were not affected by the administration of the aliphatic hydrocarbons. Dietary administration of mineral oil could be an inexpensive way to save livestock contaminated with toxic lipophilic compounds, such as many pesticides and industrial chemicals.

Accidental or environmental contamination of humans and livestock with lipophilic compounds is a common occurrence. Some of the compounds with prolonged tissue retention pose a long-term hazard or risk to exposed humans. Some have caused economic damage to the food-producing industries (Knöppler, 1976; Beall, 1976). Studies with rats and rhesus monkeys contaminated with halogenated hydrocarbons such as hexachlorobenzene (HCB), mirex, Kepone, hexabromobiphenyl, and pentachlorobiphenyl have revealed that the addition of 5–8% light liquid paraffin, mineral oil, or hexadecane to the diet enhances fecal elimination of these compounds 3–13-fold (Richter et al., 1977, 1979a,b; Rozman, K., et al., 1981a,b; Rozman, T., et al., 1981). The present experiment was conducted by using ruminant animals to determine whether similar responses to aliphatic hydrocarbons occur in these animals and to investigate whether dietary mineral oil impairs rumen function.

MATERIALS AND METHODS

Six wether lambs of uniform breeding weighing 22.8 ± 2.3 kg were housed in regular sheep metabolism cages with access to a controlled amount of pelleted, commerical sheep feed and water. After an adaptation period of 2 weeks, the animals were dosed 3 times with 14 mg/kg ^{14}C-labeled HCB (0.0425 mCi/mmol). The doses were administered on 3 consecutive days in a 1% methyl cellulose suspension by a stomach tube. [^{14}C]HCB was obtained from the Gesellschaft für Strahlen- und Umweltforschung mbH, München, West Germany, with a specific activity of 5 mCi/mmol and a radiochemical purity of 99%. Labeled HCB was diluted with repeatedly recrystallized commercially available unlabeled HCB (Aldrich Chemical Co., Inc., Milwaukee, WI), the chemical purity of which was determined to be >99.5% by gas chromatography. Urine and feces were collected daily from each animal. One week after the last dose of HCB and every other week thereafter about 1 g of subcutaneous fat was taken from the ventral part of the abdomen of each animal by fat biopsy. Concurrently, 10 mL of blood was drawn from the vena jugularis externa of each animal. Starting on day 10 after the first dose, three animals received a diet fortified with 5% mineral oil for 4 weeks (Sargent & Co., Denver, CO), whereas the other three animals served as controls. On day 38 the sheep treated with mineral oil were sacrificed, and the other lambs used as controls were placed on a diet containing 5% hexadecane (Sigma Chemical Co., St. Louis, MO) for 2 weeks. Bile present in the gall bladder of the sacrificed animals was also collected.

Concentration of HCB and/or metabolites was determined in all samples except urine by combusting aliquots in a Packard 306 Tri-carb sample oxidizer and counting the radioactivity in a Packard 2425 Tri-carb scintillation spectrometer. Samples of urine (0.5 mL) were mixed with a dioxane cocktail and counted directly. Samples of diet and feces were analyzed for dry matter, crude protein, and acid–detergent fiber (Association of Official Analytical Chemists, 1980), and digestibility coefficients were calculated conventionally.

RESULTS AND DISCUSSION

The average fecal excretion of HCB and/or its metabolites during the first 10 days of the experiment was 73% of the dose administered (Table I). Thus, it can be estimated that about 25% of the HCB administered was absorbed.

Various recent publications have shown that addition of aliphatic hydrocarbons to the diet of rats and rhesus monkeys enhances fecal excretion of halogenated compounds such as HCB, mirex, chlorodecone (Kepone), 2,4,5,2′,4′,5′-hexabromobiphenyl, and 2,4,6,2′,4′-pentachlorobiphenyl (Richter et al., 1977, 1979a,b; Rozman, K., et al., 1981a,b; Rozman, T., et al., 1981). This treatment also results in an enhanced removal of HCB from adipose tissue of rhesus monkeys (Rozman, K., et al., 1981a). Thus, it was of interest to examine the possible usefulness of the treatment in an animal species of economic importance with a digestive system different than the previously investigated ones.

Similar to other species contaminated with various lipophilic compounds, sheep also responded to the aliphatic hydrocarbon treatment with enhanced fecal excretion of HCB (Table I). During the first week of mineral oil treatment, fecal excretion of HCB and/or metabolites was 2.6 times higher in treated sheep than in controls. The control lambs excreted during the 4-week period (days 11–38), 3.7% of the administered HCB into the feces, whereas the mineral oil treated ones excreted 9.1%. Urinary excretion of HCB and/or metabolites during this same period was 0.45 and 0.36% in untreated and treated animals, respectively (Table I). The 20% lower excretion of HCB into urine of mineral oil treated sheep may be treatment related as similar results have been reported in rats (Richter et al., 1977). However, the contribution of urine to the total excretion and thus to body burden reduction of HCB and/or metabolites is only 11 and 3.7%

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Table I. Effect of Mineral Oil and Hexadecane on the Excretion[a] of HCB and/or Metabolites in Sheep[b]

	dietary treatment					
	basal		basal + 5% mineral oil		basal + 5% hexadecane	
days	urine	feces	urine	feces	urine	feces
1-10	14.4 ± 2.7[c]	2895 ± 83.7	13.9 ± 1.5	3224 ± 223		
11-17[d]	9.1 ± 1.4	74.6 ± 17.7	7.5 ± 0.2	197 ± 45.6		
18-24	7.3 ± 1.5	57.0 ± 12.6	5.7 ± 0.5	149 ± 32.9		
25-31	5.6 ± 1.0	48.2 ± 10.1	4.5 ± 0.2	114 ± 22.8		
32-38	5.0 ± 0.9	39.5 ± 10.1	3.7 ± 0.4	88.0 ± 15.2		
39-45[e]					5.1 ± 0.7	114 ± 22.8
46-52					2.3 ± 0.2	105 ± 14.2

[a] Data for excretion are given as micrograms per kilogram per day. [b] Dosed on days 1, 2, and 3 with 14 mg/kg [^{14}C]HCB. [c] Data are expressed as mean ± SE (n = 3). [d] Sheep were placed on the mineral oil treatment on day 10. [e] Sheep receiving basal diet until day 38 were used for the hexadecane treatment.

Table II. Effect of Mineral Oil and Hexadecane on the Concentration[a] of HCB and/or Metabolites in Fat and Blood of Sheep[b]

	dietary treatment					
	basal		basal + 5% mineral oil		basal + 5% hexadecane	
day	fat	blood	fat	blood	fat	blood
10[c]	88.6 ± 24.7[d]	0.2 ± 0.1	81.7 ± 17.6	0.1 ± 0.1[d]		
24	64.6 ± 13.9	0.3 ± 0.1	50.5 ± 7.9	0.2 ± 0.1		
38	50.3 ± 8.1	0.1 ± 0.1	30.3 ± 5.5	0.1 ± 0.1		
52[e]					42.2 ± 13.3	0.1 ± 0.0

[a] Data for concentration are given in micrograms per gram (ppm). [b] Dosed on days 1, 2, and 3 with 14 mg/kg [^{14}C]hexachlorobenzene. [c] Prior to any treatment. [d] Data are expressed as mean ± SE (n = 3). [e] Sheep receiving basal diet until day 38 were used for the hexadecane treatment.

of that in the feces for control and treated lambs, respectively (Table I).

Hexadecane in the diet caused a 2.9-fold increase of fecal excretion of HCB and/or metabolites during the first week of treatment over that excreted the week before treatment. Total fecal excretion of HCB over a 2-week period prior to the hexadecane treatment (days 25–38) was 1.5% of the dose administered, whereas the same animals excreted in feces 3.7% of the dose when fed a diet containing hexadecane (5%) for 2 weeks (days 39–52). Similar to that observed with mineral oil treatment, urinary excretion during the 2-week control period (0.18% of the dose) was about 30% lower than during the hexadecane treatment (0.12% of the dose) (Table I).

Figure 1 shows the daily fecal excretion of HCB from mineral oil treated and untreated lambs for 4 weeks and for hexadecane-treated animals for a subsequent period of 2 weeks. Fecal concentration of HCB and/or metabolites shows a much faster decline in the treated animals than in the controls.

Table II depicts subcutaneous fat levels of HCB in sheep as a measure of the effect the treatments had on tissue stores of HCB. While Mull et al. (1978) have suggested that omental fat is a more reliable indicator of body burden of HCB than is subcutaneous fat, the latter is more conveniently obtainable and should be an acceptable semiquantitative indicator of the effectiveness of the treatments. The subcutaneous fat level decreased by 43% in the control sheep over a 4-week period, whereas the decrease was 63% in the treated animals, indicating that increased fecal excretion does result in enhanced depletion of HCB from tissues. Blood levels of HCB and/or metabolites in untreated and treated lambs show considerable variations and are inconclusive with respect to the possible involvement of the circulatory system in the transport of HCB and/or metabolites to the site of elimination (Table II).

To determine if the mineral oil effect on HCB excretion decreased with time, we compared the concentration of HCB in feces to that in fat. In order to eliminate the

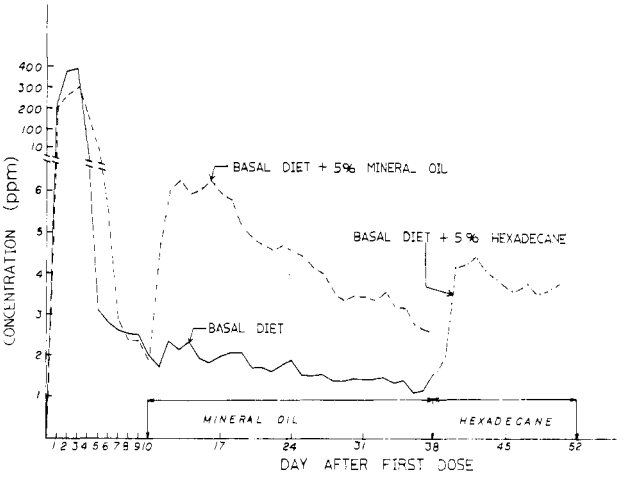

Figure 1. Daily fecal excretion of HCB and/or metabolites in sheep dosed with 14 mg/kg [^{14}C]HCB 3 times. The animals were fed basal diet, basal diet plus 5% mineral oil, or basal diet plus 5% hexadecane. Each curve represents average values of three sheep exhibiting less than a 40% standard deviation from the mean.

Table III. Effect of Mineral Oil and Hexadecane on the Relative Excretion of HCB[a]

days	dietary treatment	rel excretion, f[b]
11-38	basal	27.9 ± 3.1[c]
11-38	basal + 5% mineral oil	88.4 ± 17.6
39-52	basal + 5% hexadecane	87.7 ± 5.1

[a] Dosed on days 1, 2, and 3 with 14 mg/kg [^{14}C]HCB. [b] f = (ppm of hexachlorobenzene and/or metabolites in feces)/(ppm of hexachlorobenzene and/or metabolites in fat) × 10^3. [c] Data are expressed as mean ± SE (n = 3).

variable that HCB is depleted faster from fat in the treated group than in the controls and the individual variations of HCB fat levels, we have correlated (Table III) the concentration of HCB in feces to that in fat (f). The concentration of HCB in the fat for each day was estimated

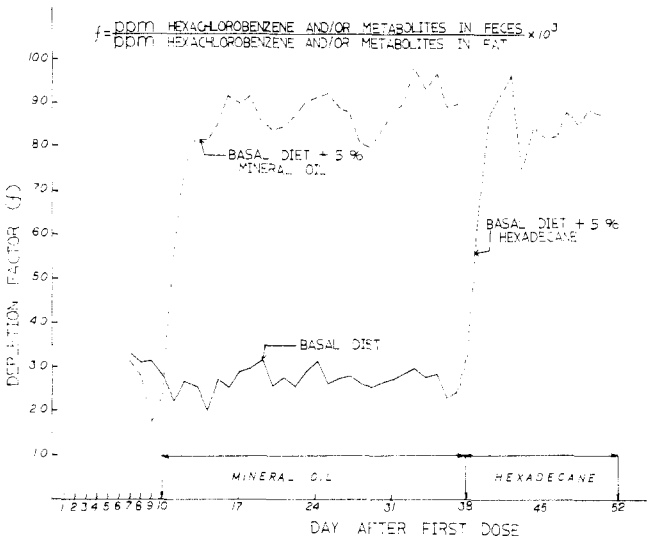

Figure 2. Correlation of daily fecal excretion and adipose tissue depletion of hexachlorobenzene and/or metabolites in sheep dosed with 14 mg/kg [^{14}C]HCB 3 times. The animals were fed basal diet, basal diet plus 5% mineral oil, or basal diet plus 5% hexadecane. Each curve represents average values of three sheep exhibiting less than a 20% standard deviation from the mean.

by extrapolation from the biweekly fat biopsy data (Table II). Figure 2 exhibits the daily pattern of f. It clearly shows that the effect of mineral oil remains virtually constant over extended periods of time. Figure 1, however, could lead to an erroneous conclusion of a diminishing effect. Factor f therefore appears to be a useful measure, especially for laboratory experiments with limitations on animal numbers (i.e., cattle and primates), in standardizing excretion due to variations in exposure or absorption.

The mechanism by which aliphatic hydrocarbons exert their effect upon fecal excretion of lipophilic compounds is not yet fully understood. It was demonstrated in rhesus monkeys and rats that the bile could not account for the effect and that the site where the effect manifests itself is the large intestine (Rozman, K., et al., 1980; Rozman, T., et al., 1980; Rozman, K., et al., 1981a; Richter and Schäfer, 1981). The concentration of HCB and/or metabolites in bile of the sheep was determined to be 0.5 ± 0.1 ppm (n = 3), and assuming the daily bile flow of sheep is 25.4 g/kg of body weight (Dukes, 1955), the total daily biliary output of HCB and/or metabolites is about 13 μg kg^{-1} day^{-1}. The average daily fecal excretion of HCB and/or metabolites during the last 7-day period prior to sacrifice was 87.7 μg kg^{-1} day^{-1} (Table I). Consequently, biliary output of HCB and/or metabolites accounts for only about 15% of the fecal excretion. If reabsorption of biliary HCB and/or metabolites is taken into consideration, then the contribution of bile to fecal excretion of HCB and/or metabolites would be even less. Therefore, the major source of fecal excretion of HCB and/or metabolites in sheep is also likely to be due to intestinal elimination as was shown for HCB in the rhesus monkey and the rat (Rozman, K., et al., 1981a).

Our data indicate no adverse effect of the mineral oil treatment upon normal digestive functions of sheep (Table IV) (Nieman, 1980). This assumption is also supported by the same weight gain in treated and untreated animals over the same time period (0.15 ± 0.03 and 0.16 ± 0.07 kg/day for untreated and treated animals, respectively; n = 3).

Table IV. Effect of Mineral Oil on Digestibility Coefficients in Sheep Contaminated with HCB[a]

dietary component	period of treatment	dietary treatment: basal	dietary treatment: basal + 5% mineral oil
dry matter	2nd week	67.9 ± 1.2[b]	67.8 ± 1.0
	4th week	71.1 ± 0.8	70.0 ± 1.5
protein	2nd week	71.6 ± 2.0	75.4 ± 0.5
	4th week	75.1 ± 0.9	77.7 ± 1.1
acid-detergent fiber	2nd week	38.4 ± 1.6	40.9 ± 2.5
	4th week	44.0 ± 1.4	44.0 ± 2.1

[a] Dosed on days 1, 2, and 3 with 14 mg/kg [^{14}C]HCB. [b] Data are expressed as mean ± SE (n = 3).

Further studies are needed to evaluate the effect of aliphatic hydrocarbons in the diet upon excretion and possible depletion of endogenous lipophilic compounds such as vitamins and hormones. However, the treatment represents a promising start in finding feasible ways to decontaminate livestock.

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