Pharmacokinetics of Mirex in Goats. 1. Effect on Reproduction and Lactation

Ann L. Smrek,* Samuel R. Adams, John A. Liddle, and Renate D. Kimbrough

Groups of five male and five female goats were given 0 and 1 mg of mirex/kg of bodyweight daily for 61 weeks. An additional group of female goats was given 1 mg of mirex/kg of bodyweight daily for 18 weeks. The female goats were given daily doses of 10 mg of mirex/kg of bodyweight for an additional 4 weeks after the exposures to 1 mg of mirex/kg of bodyweight for 61 and 18 weeks. Goats exposed 61 weeks were bred twice and those exposed 18 weeks were bred once. Plasma, milk, and adipose tissue were analyzed for mirex at intervals. Adipose tissue levels of mirex were lower in female than in male goats and were not noticeably affected by pregnancy. A steady mirex concentration was not reached in adipose tissue, whereas the plasma levels became stationary after about 5 months of exposure.

Mirex [dodecachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[*cd*]pentalene] (Figure 1) has been used as an insecticide, particularly to combat fire ants in the southeastern United States. It is manufactured by Hooker Chemical Co. and has also been marketed as a flame retardant under the tradename Dechlorane (Thiery, 1971). Mirex has been used on a limited scale for the control of yellow jackets and seems effective in controlling termites (Markin et al., 1972; Alley, 1973). It is a very stable compound similar to such halogenated aromatic substances as hexabromobiphenyl, octabromobiphenyl, hexa-, hepta-, and decachlorobiphenyl, and halogenated terphenyls. Some of these compounds have industrial uses, or have been suggested as flame retardants. Because of their stability, these compounds will accumulate in the environment if used extensively and will eventually end up in the food chain. Since mammals seem to be unable to metabolize chemicals of this type to any great extent, mirex was selected as a representative of this group of very stable compounds to be used in gaining additional information on the pharmacokinetics of these chemicals in mammals.

The goat was selected as the experimental animal because it is inexpensive, reproduces easily, is easily maintained, and is large enough for sequential surgery. In addition, milk collection is easy. The goat is also very easy to handle and only a minimum amount of sedation is necessary for minor surgery. Goats take the stress of surgery well and heal rapidly. Since mirex is a very stable compound, the chemical was not expected to be affected in the rumen. However, it is not known how efficient the absorption of mirex from the gastrointestinal tract is in ruminants.

It was the purpose of this study to determine the accumulation of mirex in adipose tissue, its concomitant concentration in plasma, and how these parameters were affected by pregnancy, parturition, and lactation. The results are reported in this paper.

MATERIALS AND METHODS

A total of 15 female and 10 male adult common goats of unknown genetic background were divided into groups of five. For 61 weeks one group of males and one group of females were given daily doses of 1 mg of mirex/kg of bodyweight mixed in sugar; then the five females were given 10 mg of mirex/kg of bodyweight for 4 weeks. The mirex was given orally in capsules. Control groups of five male and five female goats were given capsules of the same size filled with sugar. An additional group of female goats were started on the regimen of 1 mg of mirex/kg of bodyweight when the kids were born after the first breeding cycle. After 18 weeks of dosing this group of goats was given 10 mg of mirex/kg of bodyweight orally in capsules daily for 4 weeks, after which exposure was discontinued. The dose was increased to determine what immediate affect this would have on plasma and adipose tissue levels. The goats were initially weighed weekly and later biweekly. The mirex dose was adjusted to the bodyweight. The goats were kept in an open pasture. They were bred at the beginning of the study and again 32 weeks after onset of exposure. The goats were also given a concentrated ration [0.5–2.0 lbs $(head)^{-1}$ $(day)^{-1}$] to maintain normal body flesh during days and seasons when pastures were not lush.

During lactation, the mirex-treated female goats were kept separate from the controls. Endoparasite control was maintained with Trianthen (Ft. Dodge), Thiabendazole (Merck), and Ripercol-L (American Cyanamid) which were administered alternately at 3–5-week intervals in the dosages recommended by the respective manufacturers. Foot rot therapy consisted of periodic formaldehyde application by footbath and routine hoof trimmings. Seriously affected goats were placed on antibiotic therapy. Blood, milk, and adipose tissue samples were obtained at intervals as indicated in Figures 2–7 and Figure 9.

Adipose tissue samples were obtained surgically from an area immediately under the skin of the rump. The goats were sedated for surgery with an intravenous dose of 4 mg of xylazine/goat. Blood samples were drawn from the jugular vein into vacutainer tubes containing EDTA at the time the biopsies were performed. During lactation milk samples were collected at intervals (Figure 9).

Plasma, milk, and subcutaneous fat samples were analyzed for mirex content using the method of Markin et al. (1972). One milliliter of plasma or milk, or 100 milligrams of fat, was pipetted or weighed into a glass centrifuge tube. The sample was extracted with isopropanol and *n*-hexane. The isopropanol was removed by washing the extracts with saturated NaCl solution and with deionized water. The extract was filtered through anhydrous sodium sulfate and was concentrated to 5 mL under a stream of nitrogen. The concentrated extract was eluted through a column packed with an anhydrous sodium sulfate layer, a 1-g column of Whoelm silica gel, and another layer of anhydrous sodium sulfate. The eluent was concentrated to an appropriate volume with a stream of nitrogen. An internal standard was added to each sample, and the mirex was quantitated by electron-capture gas chromatography. The gas chromatograph column consisted of a U-shaped glass tube, 6 ft × 0.25 in., packed with 1.5% OV-17–1.95% QF-1 on 80/100 mesh Chromosorb WHP. Operating conditions were as follows: column, 206

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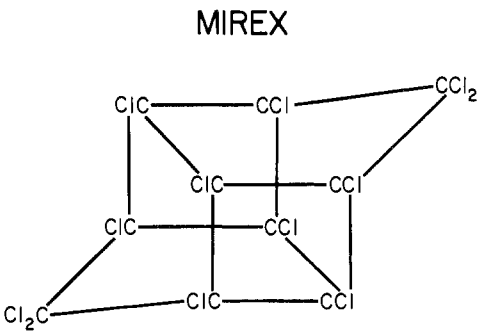

Figure 1. Mirex (dodecachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[*cd*]pentalene).

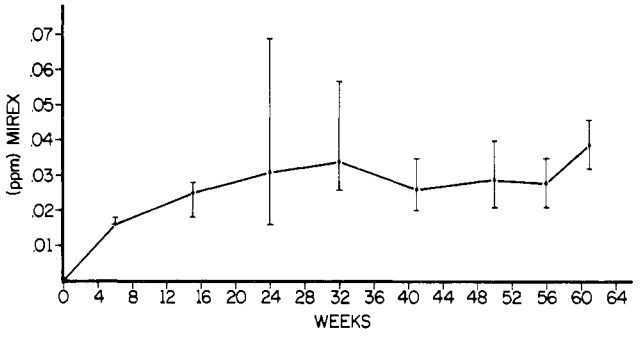

Figure 2. Range and mean concentration of mirex (ppm) in plasma of group V male goats dosed with 1 mg of mirex kg^{-1} day^{-1} for 61 weeks.

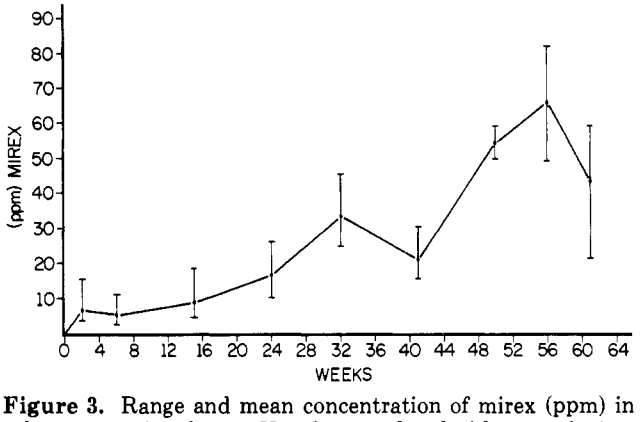

Figure 3. Range and mean concentration of mirex (ppm) in subcutaneous fat of group V male goats dosed with 1 mg of mirex kg^{-1} day^{-1} for 61 weeks.

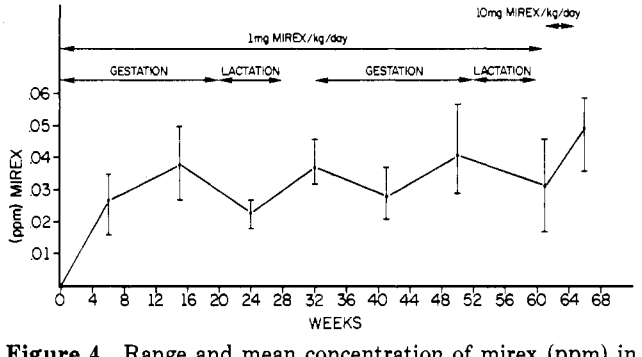

Figure 4. Range and mean concentration of mirex (ppm) in plasma of group II female goats dosed with 1 mg of mirex kg^{-1} day^{-1} for 61 weeks and then with 10 mg of mirex kg^{-1} day^{-1} for 4 weeks.

°C; detector, 250 °C; carrier nitrogen flow, 40 mL/min.

The content of fat in milk samples was determined by the Roese–Gottlieb method (AOAC, 1970). One milliliter of milk was mixed with 1 mL of isopropanol and 2.5 mL

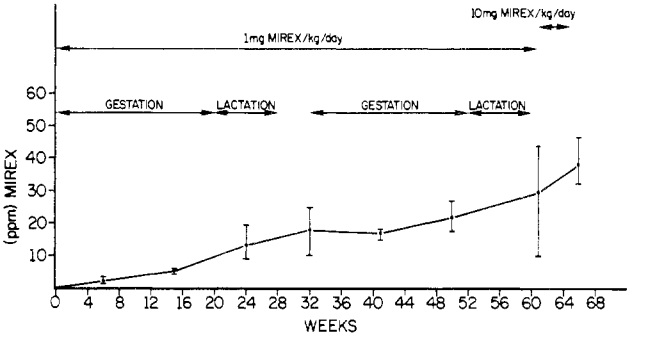

Figure 5. Range and mean concentration of mirex (ppm) in subcutaneous fat of group II female goats dosed with 1 mg of mirex kg^{-1} day^{-1} for 61 weeks and then with 10 mg of mirex kg^{-1} day^{-1} for 4 weeks. The mirex concentration was expressed on a lipid basis.

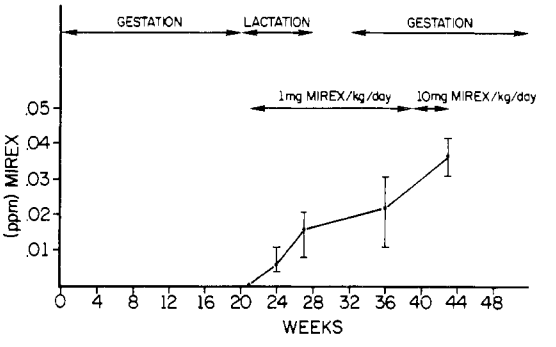

Figure 6. Range and mean concentration of mirex (ppm) in plasma of group III female goats dosed with 1 mg of mirex kg^{-1} day^{-1} for 18 weeks and then with 10 mg of mirex kg^{-1} day^{-1} for 4 weeks.

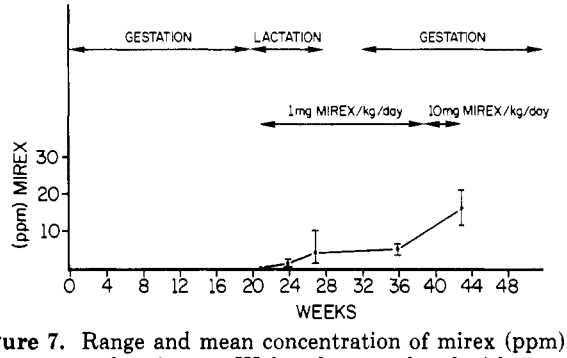

Figure 7. Range and mean concentration of mirex (ppm) in subcutaneous fat of group III female goats dosed with 1 mg of mirex kg^{-1} day^{-1} for 18 weeks and then with 10 mg of mirex kg^{-1} day^{-1} for 4 weeks. The mirex concentration was expressed on a lipid basis.

of ether. Then 2.5 mL of petroleum ether was added, and the ether solution was transferred to a preweighed aluminum dish. The remaining liquid was extracted two more times with 2.5 mL of ether and 2.5 mL of petroleum ether. These extracts were added to the aluminum dish. The extracts containing the fat were evaporated to dryness and then dried to constant weight in an oven at 102 °C. The dish containing the fat was cooled in a desiccator and weighed.

Fat was separated from adipose tissue by digesting the samples in warm 18 N H_2SO_4, followed by rinsing the separated fat with water to remove any traces of H_2SO_4. The separated fat was transferred to a desiccator to dry before weighing appropriate samples for the determination of mirex. The recovery data for in vitro fortified pool samples were as follows: plasma 90.9 ± 1.9, milk 91.4 ± 2.6, and fat 89.7 ± 4.6%.

Table I. Number and Birth Weights of Kids of Control and Mirex Treated Nannies

Exposure	Number of nannies	Number of kids	Kid weights range	Kid weights mean
0 (controls)	4	9	1.4-2.4	2.1
1 mg of mirex $kg^{-1}day^{-1}$ for 21 weeks	5	10	2.3-2.6	2.4
0 (controls)	4	9	1.4-2.3	1.8
1 mg of mirex kg^{-1} day^{-1} for 51 weeks	4	10	1.4-2.3	1.7
1 mg of mirex kg^{-1} day for 18 weeks followed by 10 mg of mirex kg^{-1} day^{-1} for 4 weeks (dosing stopped 9 weeks before delivery)	4	7	1.4-2.4	1.8

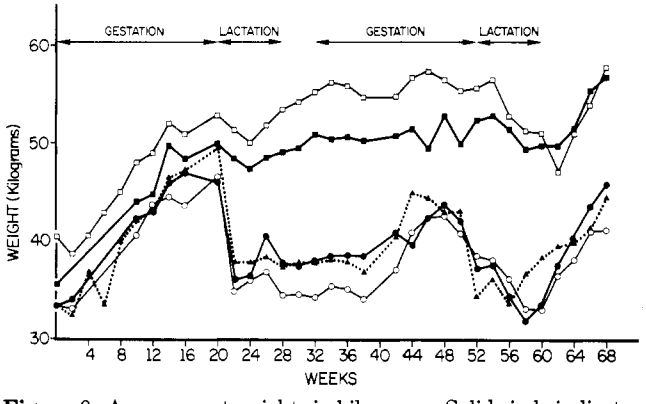

Figure 8. Average goat weights in kilograms. Solid circle indicates female controls; open triangle, females dosed 65 weeks; open circle, females dosed 22 weeks; solid square, male controls; open square, males dosed 61 weeks.

RESULTS

The ranges and means of the bodyweight and the levels of mirex measured in subcutaneous fat tissue, plasma, and milk are given in Figures 2–9. The goats did not show any signs of toxicity. The bodyweights of the male goats showed only minor variations, while the weight of the female goats fluctuated during pregnancy. No difference in bodyweight between treated and control goats was noted.

The number of kids and the weight of the kids in the three groups at birth are given in Table I. No abnormalities were observed in any of the kids. They were killed 2 months after birth. Gross inspection at autopsy and microscopic examination of the tissues were normal.

One female goat of the group given mirex for 61 weeks died while giving birth, and one in the group given mirex for only 2 months died, but none of the female controls died. In contrast, two of the control males but none of the experimental male goats died during the experiment. Results of the findings at autopsy will be reported in a later paper.

The mirex plasma levels varied between the individual male goats, but had stabilized after about 15 weeks of exposure (Figure 2). The subcutaneous adipose tissue levels, on the other hand, continued to increase gradually and independently of the plasma levels (Figure 3). At 32 weeks the adipose tissue levels of mirex were 33.6 ppm, at 41 weeks 20.9 ppm, and at 50 weeks 54.8 ppm (Figure 3). The reason for this fluctuation is not known. None of these goats suffered any weight loss or illness during this time.

The plasma levels of mirex in the female goats were similar to those in the male goats (Figure 4). Throughout the experiment, they varied within the same range. They decreased slightly during lactation and seemed to increase during the latter half of gestation.

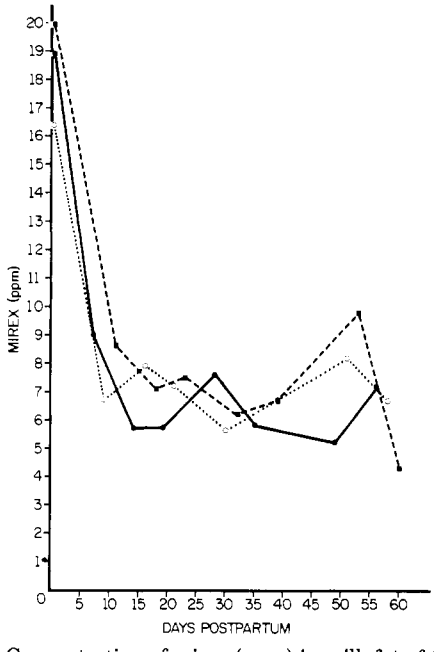

Figure 9. Concentration of mirex (ppm) in milk fat of three goats at different intervals postpartum (open circle, solid circle, open square). Goats had been dosed with 1 mg of mirex/kg of bodyweight per day for 21 weeks at the onset of milk excretion. The mirex concentration was expressed on a lipid basis.

An increase in the dosage at the end of the study produced an increase in the plasma level of mirex (Figure 4). The concentration of mirex in the subcutaneous adipose tissue gradually increased over the 61 weeks of exposure. This gradual buildup of mirex did not seem to be affected by pregnancy or lactation (Figure 5).

The adipose tissue levels of mirex in the female goats were generally lower than in the males. At the end of the study, when the daily dose of mirex was increased in the females from 1 to 10 mg/kg of bodyweight, no dramatic increase in the adipose tissue levels was observed (Figures 5 and 6).

An additional group of goats was started on mirex on the first postpartum day. This group showed plasma mirex levels which were similar to those of female goats which were started on mirex in early pregnancy. At 6 and 15 weeks after onset of exposure the plasma levels of mirex were lower in this group than in the goats exposed during early pregnancy. This is consistent with the finding that plasma levels tend to drop during lactation (Figure 4).

An increase in the dosage at the end of the study again resulted in an increase in the mirex plasma level (Figure 6).

The adipose tissue levels of mirex in the group of goats started in early pregnancy and in the ones started on the first postpartum day also showed no marked discrepancies.

A tenfold increase in the dose over a 4-week period again did not produce a very remarkable increase in the adipose tissue concentration (Figure 7).

Figure 9 gives the results of the analysis of milk obtained from goats at the end of the first pregnancy. Mirex levels in the adipose tissue and the plasma of the goats are given in Figures 4 and 5. The lactation period in these goats began 21 weeks after onset of exposure. At that time, the subcutaneous adipose tissue contained a mean of 13.5 ppm mirex, and the concentration of mirex in colostrum fat was 16.4 to 20 ppm (Figure 9). While the subcutaneous adipose tissue levels slightly increased over the next 8 weeks, the levels of mirex in milk fat decreased to less than half the amount excreted in colostrum immediately after the birth of kids. The mirex excretion in the milk fluctuated but showed no particular trend (Figure 9) after an initial drop of the mirex concentration when colostrum was formed. Although the mirex concentrations were calculated on a fat basis, the goat seems to eliminate more mirex in colostrum than in regular milk.

At no time during the study was mirex found in blood, adipose tissue, or milk of the control goats. This finding shows that the goats did not obtain mirex from the pasture through fecal excretion of mirex by the treated goats. All goats were kept in the same pasture except during lactation when the treated females were separated from the rest of the goats.

DISCUSSION

Mirex is a very stable compound. It has been reported in the literature that it can be broken down by ultraviolet light (Alley et al., 1974) and anaerobic bacteria (Andrade and Wheeler, 1975), but it does not seem to be metabolized in the mammalian system (Gibson et al., 1972; Ivie et al., 1974; Kutz et al., 1974).

Mehendale et al. (1972) estimated that the half-life for mirex following final distribution in rats was more than 100 days. (After a single dose of mirex, blood levels will first be high and drop fairly rapidly, because mirex is deposited in the organs and in adipose tissue. Following this final distribution, mirex is only very slowly excreted.) Pittman et al. (1975) developed a mathematical model and predicted that the biological half-life of mirex in rhesus monkeys was extremely long, with only a 2% decline in adipose tissue over a 10-year period. The extremely long biological half-life and the lack of metabolism of mirex in these animals suggests poor excretion of the compound. This agrees with our finding in the goats, which shows that mirex deposits in adipose tissue increased throughout a 61-week exposure period. It has always been assumed that lipid-soluble, chlorinated hydrocarbons, such as dieldrin or aroclor 1016 and 1242, if administered at a constant rate, will reach a steady state in adipose tissue within a period ranging from a few weeks to a few months, depending on the dose administered and that even though dosage is continued at the same rate, adipose tissue levels will remain stationary (Burse et al., 1974; Deichmann et al., 1968). This is not the case for mirex.

Most storage distribution studies with chlorinated hydrocarbons have been conducted in rats. Exposure has been achieved by mixing the material in the diet. The food consumption in rats decreases on a bodyweight basis as rats become older, and therefore, the amount of the chemical that is consumed also decreases. This may affect long-term pharmacokinetic studies in rats. However, Ivie et al. (1974) found that no tissue plateau occurred in rats or male quail which were given daily doses of mirex-^{14}C for a 16-month period. The results reported in the literature and our findings with goats indicate that, if people were exposed to this material in the diet, they would conceivably accumulate increasing amounts of mirex throughout their life span without being able to eliminate much of it. Trace amounts of mirex have been found in a few human adipose samples (Kutz et al., 1974). All of these samples originated in Georgia; however, no definite link to the imported fire ant control program was established, and exposure could have been in connection with the use of mirex as a flame retardant.

During the study in goats no obvious signs of toxicity were noted at this low dose. The effect of sudden stress or loss of weight on high concentrations of mirex stored in adipose tissue needs further study.

The different biological compartments in the goat (plasma, milk, and adipose tissue) seem to be independent of each other. While plasma levels stabilize, adipose tissue levels continue to increase, colostrum levels are higher than milk levels. Milk levels fluctuate but show no particular trend. The plasma levels, after having stabilized, will increase when the dosage of mirex is increased so that the capacity of plasma to transport mirex is not a limiting factor in the plasma level at these low doses. After oral exposure the liver may play an important role in controlling mirex plasma levels. If plasma levels do not increase in proportion to the concentration in adipose tissue, then the determination of plasma levels in chronic exposure is of little predictive value for adipose tissue levels. As long as dosing was continued, neither pregnancy nor lactation caused any decline in adipose tissue levels. Further studies are in progress to determine how pregnancy affects tissue levels after dosing has been stopped.

Although the female goats showed a steady increase in mirex levels in adipose tissue (Figures 3 and 5), the adipose tissue levels of the male goats fluctuated a great deal. Similar fluctuations in adipose tissue levels of mirex have been reported in roosters (Medley et al., 1974).

Numerous chemicals have been suggested as flame retardants for building materials, clothing, and many other products (Pearce and Liepins, 1975). The toxicological properties for many of these products have not been established. Many of them are extremely stable chemicals and will, if used in great quantities, create environmental pollution problems in the future. An additional problem is that toxic combustion products may form (Petajan et al., 1975).

Because of the extent that these chemicals are being used, it is extremely important to evaluate their potential hazards and to select those chemicals from the congeners that are the least dangerous for both acute and long-term human exposure.

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Determination of Residues of Chlorpyrifos, Its Oxygen Analogue, and 3,5,6-Trichloro-2-pyridinol in Tissues of Cattle Fed Chlorpyrifos

Henry J. Dishburger,* Richard L. McKellar, Joan Y. Pennington, and James R. Rice

Cattle were fed chlorpyrifos daily for 30 days at levels of 3, 10, 30, and 100 ppm. Muscle, liver, kidney, omental fat, renal fat, and subcutaneous fat were collected at the end of this period. In addition, omental fat was collected by biopsy at weekly intervals for 5 weeks following withdrawal of the highest level of 100 ppm chlorpyrifos. Residues of chlorpyrifos and its oxygen analogue were determined by thermionic or flame photometric gas chromatography. The 3,5,6-trichloro-2-pyridinol moiety as the trimethylsilyl derivative was determined by electron-capture gas chromatography. The procedures were used to quantitate chlorpyrifos and its oxygen analogue down to 0.01 ppm and 3,5,6-trichloro-2-pyridinol to 0.05 ppm. Residues of chlorpyrifos were mainly in the fat tissues and averaged 0.02 ppm (<0.01–0.05 ppm) with 3 ppm in the diet and 3.28 ppm (2.28–4.70 ppm) in fat of cattle fed 100 ppm. The 3,5,6-trichloro-2-pyridinol was predominantly in the liver and kidney and averaged 0.20 ppm (0.16–0.23 ppm) in liver and 0.11 ppm (0.09–0.15 ppm) in kidney at 3 ppm; 2.41 ppm (2.16–2.61 ppm) in liver and 1.75 ppm (1.46–1.95 ppm) in kidney at the 100 ppm feeding level. No chlorpyrifos oxygen analogue was detected in any tissue at any feeding level.

DURSBAN, trademark of The Dow Chemical Company for insecticide products containing chlorpyrifos [*O,O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate], as the active ingredient is effective for control of numerous soil and foliar pests of crops. The many potential uses of chlorpyrifos, certain ones of which could conceivably cause residues of chlorpyrifos in cattle feeds, prompted this study. Information relating oral intake to residues in animal tissues is essential for safe utilization of the insecticide on field crops.

McKellar et al. (1972) found residues of chlorpyrifos predominantly in fat tissue and 3,5,6-trichloro-2-pyridinol in liver and kidney tissues of swine when the animals were fed chlorpyrifos in their diet for 30 days. The levels of residues were small, <0.05 ppm, even at the highest dietary concentration of 10 ppm. The residues declined rapidly to undetectable or very low levels within 7 days after withdrawal of the insecticide from the feed. Similar results were obtained when chickens were fed chloropyrifos (Dishburger et al., 1972). To define more critically the potential residue hazards that could be encountered from such uses, a feeding study was conducted with cattle. Reported herein are the results of a study to determine the level of residues of chlorpyrifos, its oxygen analogue, and the 3,5,6-trichloro-2-pyridinol moiety in tissues of cattle fed known amounts of chlorpyrifos.

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EXPERIMENTAL SECTION

Eighteen Hereford crossbred heifers were fed chlorpyrifos for a 30-day period at levels of 0, 3, 10, 30, or 100 ppm on a daily, dry matter intake basis. The cattle were subdivided into six groups of three heifers each, one for each level through 30 ppm and two groups at 100 ppm. The subdivision was based on body weight in an attempt to minimize differences between groups. The experimental animals varied in body weight from 347 to 524 lb. The cattle in each group were penned together and allowed to share a conditioning ration composed of 50% concentrate and 50% roughage for 36 days prior to the study. At the end of the acclimation period, the feed was changed to a 75% concentrate and 25% roughage ration which was fed for the duration of the study. Chlorpyrifos (commercial production lot) was administered in gelatin capsules each morning with a balling gun and the amount given was derived from the average, daily, dry matter intake of the concentrate–roughage ration. Capsules were formulated within 7 days of use by pipetting the insecticide in acetone onto a few grams of ground concentrate in the capsule, closing the capsule, and holding them in a sealed glass container at −10 °C until fed.

All cattle fed 0, 3, 10, and 30 ppm and one group fed 100 ppm chlorpyrifos were sacrificed at the end of the 30-day period. Samples of muscle, liver, kidney, omental fat, renal fat, and subcutaneous fat were taken for residue analysis. At this time, chlorpyrifos was withdrawn from the second group on the 100 ppm level and the animals were allowed to continue on the concentrate–roughage ration. Omental