Mendoza, C. E., Vijay, H. M., Shields, J. B., Laver, G. W., *Toxicol. Appl. Pharmacol.* **41**, 127 (1977b).
Mischutin, V., Ger. Offen. Patent No. 2352923, Oct 25, 1972 (1974) (CA08114079326Q).
Negishi, H., Tokai, Y., Matsunaka, S., Azuma, M., Japan, Patent No. 72-41425, 1972 (CA08004015866N).
Pashin, A. S., Nesmerchuk, N. S., Shed'ko, G. E., *Plast. Massy* 1, 40 (1974).
Raley, Jr., C., U.S. Patent No. 3668155, 1972 (CA07712076111X).
Strik, J. J. T. W. A., *Meded. Rijksfac. Landbouwwet. Gent* **38**, 709 (1973).
Villeneuve, D. C., Khera, K. S., *Environ. Physiol. Biochem.* **5**, 328 (1975).
Vos, J. G., Botterweg, P. F., Strik, J. J. T. W. A., Koeman, J. H., *TNO Nieuws* **27**, 599 (1972).

Recieved for review March 3, 1977. Accepted March 1, 1978.

Pharmacokinetics of Mirex in Goats. 2. Residue Tissue Levels, Transplacental Passage during Recovery

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

Male and female goats were given 1 mg of mirex/kg of bodyweight for 61 weeks, followed by 10 mg of mirex/kg of bodyweight to the females for 4 weeks. Thereafter, mirex levels in adipose tissue and blood were determined at intervals over a 52-week recovery period for the males and a 34-week recovery period for the females. An additional group of female goats was given 1 mg of mirex/kg of bodyweight for 18 weeks and 10 mg of mirex/kg of bodyweight for 4 weeks thereafter. Mirex levels in adipose tissue and blood were determined in these goats at intervals over a 52-week recovery period. During the 34- and 52-week recovery periods, the mirex levels in adipose tissue decreased to half their original value in female goats and in male goats after a 52-week recovery period to about a third of their original value. The proportional decrease in mirex plasma concentrations was much greater than in adipose tissue. At the end of the recovery period on autopsy, mirex was also present in brains and livers of the adult goats and in fetuses of early gestational age.

Mirex [dodecachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[*cd*]pentalene] is a very stable chemical that is used as an insecticide particularly in the control of fire ants. At one time it was also used as a flame retardant. The accumulation of this chemical in goats has previously been reported (Smrek et al., 1977). In that study male and female goats were dosed with 0 or 1 mg of mirex/kg of body weight. During the dosing period the goats were bred twice. A steady state in adipose tissue was not reached during a 61-week dosing period. At the end of the study the adipose tissue levels were lower in female than in male goats but reproduction did not noticeably affect mirex adipose tissue levels. This paper reports the results of a 34–52-week recovery period after dosing of the goats was discontinued.

METHODS

The methods of dosing and maintaining the goats as well as the chemical analyses of the biological specimens have been outlined in detail in a previous paper (Smrek et al., 1977). Briefly, 15 female and 10 male goats were divided into groups of five. All goats were weighed at biweekly intervals throughout the experiment. One group of five males and five females were kept as controls. One group of five males and five females were dosed with 1 mg of mirex/kg of bodyweight per day for 61 weeks. Following this the five females were given 10 mg of mirex/kg of body weight for 4 weeks, and then dosing was stopped. The group of dosed female goats and the controls were bred twice during the dosing period. Dosing was started at the onset of the first pregnancy. Blood and adipose tissue biopsies were obtained from these goats at intervals during the recovery period as indicated in Tables I and II. The female goats were killed 34 weeks after dosing was stopped, and autopsies were performed. At the time of the autopsies the goats were in early pregnancy and fetal material for chemical analysis was also collected. The male goats were killed 52 weeks after dosing was stopped.

An additional group of five female goats was given 1 mg of mirex/kg of bodyweight for 18 weeks and then 10 mg of mirex/kg of bodyweight for 4 weeks. In this group, dosing was started on the first postpartum day after the first breeding cycle. The schedule for adipose tissue biopsies and collection of blood for this group of goats is given in Table III. The goats were killed after a 52-week recovery period. The male and female control goats were killed with the experimental goats. Since dosing was discontinued earlier in the female goats which were allowed to recover for 52 weeks, it was possible to kill all female goats within a 1-week period.

The serial adipose tissue samples were obtained by open biopsy from the rump as described previously (Smrek et al., 1977). At autopsy, all tissues from major organs were fixed in 4% formaldehyde for microscopic examination. All tissue sections were stained with hematoxylin and eosin. Tissues were also collected for chemical analysis from brain, liver, superficial and deep sucutaneous adipose, and omental fat. These tissues were stored frozen until chemical analysis was done.

RESULTS AND DISCUSSION

The bodyweight of the female goats fluctuated but showed no particular trend. The male goats continued to gain weight throughout the experiment (Figure 1).

The results of the chemical analysis of the adipose tissues, brain, liver, and plasma are given in Tables I–IV. In the female goats (Tables I and III), the mirex plasma and adipose tissue levels declined very slowly during the

Toxicology Branch and Animal Resources Branch, Bureau of Laboratories, Center for Disease Control, Public Health Service, Department of Health, Education, and Welfare, Atlanta, Georgia 30333.

Table I. Ranges and Means (Arithmetic) of Mirex (ppm) in Plasma and Subcutaneous Fat of Female Goats Dosed with 1 mg of Mirex kg^{-1} Day^{-1} for 61 Weeks followed by 10 mg of Mirex kg^{-1} Day^{-1} for 4 Weeks during a 34-Week Recovery Period

Weeks after dosing	Body wt, kg	No. of samples	Plasma range	Plasma mean	Fat range	Fat mean
0	41	4	0.036-0.059	0.050	32.6-47.0	38.6
5	43	4	0.020-0.046	0.036	14.7-31.9	25.6
14	47	4	0.016-0.039	0.029	10.5-34.2	26.7
23	46	4	0.015-0.028	0.022	9.1-31.6	24.1
34	39	4	0.008-0.023	0.017	6.6-24.2	18.9

Table II. Ranges and Means (Arithmetic) of Mirex (ppm) in Plasma and Subcutaneous Fat of Male Goats Dosed with 1 mg of Mirex kg^{-1} Day^{-1} for 61 Weeks during a 52-Week Recovery Period

Weeks after dosing	Body wt, kg	No. of samples	Plasma range	Plasma mean	Fat range	Fat mean
0	51	4	0.032-0.046	0.039	21.7-59.5	44.0
5	54	5	0.021-0.036	0.028	14.8-49.0	29.9
28	69	4	0.004-0.013	0.011	2.4-9.7	7.4
53	70	4	0.004-0.012	0.008	6.1-27.6	15.8

Table III. Ranges and Means (Arithmetic) of Mirex (ppm) in Plasma and Subcutaneous Fat of Female Goats Dosed with 1 mg of Mirex kg^{-1} Day^{-1} for 18 Weeks followed by 10 mg of Mirex kg^{-1} Day^{-1} for 4 Weeks during a 52-Week Recovery Period

Weeks after dosing	Body wt, kg	No. of samples	Plasma range	Plasma mean	Fat range	Fat mean
0	41	4	0.031-0.042	0.037	11.5-21.2	16.4
7	41	4	0.009-0.022	0.015	11.0-18.0	13.7
25	41	4	0.007-0.022	0.015	9.0-23.5	16.2
37	48	4	0.003-0.016	0.010	4.4-18.6	10.0
52	41	4	0.004-0.012	0.008	3.6-12.4	8.0

Table IV. Ranges and Mean (Arithmetic) of Mirex (ppm) in Autopsy Samples

Type of tissue	No. of samples	Female goats dosed 65 weeks, recov. 34 weeks		Female goats dosed 22 weeks, recov. 52 weeks		Male goats dosed 61 weeks, recov. 52 weeks	
		Range	Mean	Range	Mean	Range	Mean
Subcutaneous fat	4	6.6-24.2	18.9	3.6-12.4	8.0	6.1-27.6	15.8
Deep subcutaneous fat	4	22.8-30.7	28.3	4.3-13.5	8.9	9.9-28.0	18.5
Omental fat	4	21.9-34.4	28.4	3.9-11.3	7.8	10.6-27.3	18.7
Brain	4	0.17-0.29	0.25	0.02-0.17	0.10	0.09-0.33	0.19
Liver	4	0.91-1.47	1.34	0.23-0.52	0.36	0.29-0.76	0.46

Table V. Concentration of Mirex in Fetuses from Goats Dosed with 1 mg of Mirex kg^{-1} day^{-1} for 18 Weeks followed by 10 mg of Mirex kg^{-1} day^{-1} for 4 Weeks after a 34-Week Recovery Period

Fetus from goat number	Ppm mirex in whole fetus on a wet weight basis	Ppm mirex in plasma collected at time fetuses were obtained
250 (control)	0	0
15	0.032	0.019
131	0.039	0.023
252	0.038	0.018

recovery period. After a 52-week recovery period (Table III), the plasma level was about one-fourth while the adipose tissue level was one-half of the original value. In the male goats (Table II), the plasma levels over a 52-week recovery period declined to almost one-fifth and the adipose tissue levels to about one-third of the original value. This greater decrease of the mirex levels in males was partly due to the continued weight gain of the males who weighed almost one-third more at the end of the recovery period (Figure 1).

The concentration of mirex in whole fetuses from three female goats and the corresponding blood levels of the nannies are given in Table V. The concentration of mirex in the whole fetus on a wet weight basis was about twice that of the plasma level of the nanny.

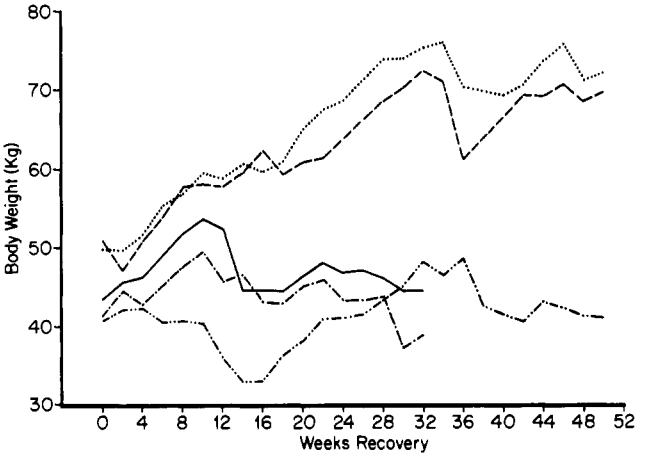

Figure 1. Bodyweights of the five groups of goats weighed at biweekly intervals: (—) female controls, (—·—) female goats dosed with 1 mg of mirex kg^{-1} day^{-1} for 61 weeks, followed by 10 mg of mirex kg^{-1} day^{-1} for 4 weeks; (—··—) female goats dosed with 1 mg of mirex kg^{-1} day^{-1} for 18 weeks, followed by 10 mg of mirex kg^{-1} day^{-1} for 4 weeks; (.....) male controls, (- - - - -) male goats dosed with 1 mg of mirex kg^{-1} day^{-1} for 61 weeks.

At the end of the study the analyses of adipose tissue from different sites (Table IV) of the female goats with

the shorter recovery period showed that the deep subcutaneous fat and the omental fat had higher mirex levels than the superficial adipose tissue. In the males the superficial adipose tissue levels were slightly lower than the mirex concentrations in the remainder of the adipose tissue samples, while a group of females with generally lower adipose tissue levels of mirex showed no real difference in the concentration of mirex in adipose tissues from different sites.

All adipose tissue levels were expressed on a lipid basis, and brain, liver, and plasma levels were expressed on a wet weight basis. The concentration of fat in the goat livers was about 5% and in goat brain 12.5%. In spite of the lower fat content of the liver, the mirex levels were higher by a factor of 3 or more in comparison to brain levels and between 10 and 20 times higher than plasma levels, while the difference between plasma and adipose tissue was ~500- to 1000-fold if the concentrations were compared on a wet weight basis.

Because of a severe generalized skin infection, one male goat that had received mirex was killed 3 months after dosing was stopped. Two male control goats died during the course of the experiment. None of the female control goats died; however, one female goat died after it had been given mirex for only 2 months and one female goat receiving mirex died during the delivery of a kid. In all but the female goat that died during delivery, the cause of death was definitely unrelated to mirex exposure.

All but one goat that died suffered from severe lung disease secondary to infestation with a nematode, most likely *Muellerius capillaris*, which is very difficult to control in goats kept in a pasture. Most of the goats at autopsy showed a few nodules in the lungs measuring about 2 cm in diameter which contained nematodes or amorphous eosinophil material, surrounded by fibrosis, mononuclear, and multinucleated cells as well as proliferated epithelium. The goats that died suffered also from acute bronchopneumonia and pulmonary edema. Occasionally sections of abdominal lymph nodes showed nematodes surrounded by a chronic inflammatory reaction. Microscopic examination of the other organs did not reveal any abnormal morphological findings except that the hepatocytes of liver sections from goats which had received mirex appeared slightly enlarged around the central veins and occasionally showed inclusions. One goat that died had, in addition to the lung disease, acute glomerulitis and another had focal interstitial nephritis. Furthermore, the one male goat that was killed 3 months after dosing was stopped had a generalized skin infection. Sections of skin from this goat showed hyperplasia and papillary proliferation of the epithelium with ballooning of epithelial cells, infiltration of the epidermis by inflammatory cells, and small basophil microorganisms.

Although tissue levels of mirex declined slowly, elimination for goats was much greater than that calculated for rhesus monkeys (Pittman et al., 1975; Pittman et al., 1976). In rats a 40% decline in mirex adipose tissue levels was found over a 10-month period while the reported half-life of mirex in adipose tissue of quail was 20 to 30 days and 4 months in fish (Ivie et al., 1974a). These findings suggests that species differences exist for the accumulation and elimination of mirex. Differences in diet among other factors may affect the elimination of mirex, and mathematical models may not accurately predict the pharmacokinetics of mirex in animals.

Mirex concentrations in adipose tissue obtained from different sites varied in female goats after a 34-week recovery period when mirex residues were still relatively high. Zabik and Schemmel (1973) also found that the levels of other chlorinated hydrocarbons in adipose tissue obtained from different sites varied. Such a difference was not observed in the animals with lower mirex adipose tissue concentrations and longer recovery periods. A reasonable explanation for these differences is not immediately obvious.

Many attempts to demonstrate metabolites of mirex in animals have been unsuccessful (Gibson et al., 1972; Ivie et al., 1974b; Mehendale et al., 1972). From feces of monkeys, Stein et al. (1976) were able to extract a compound with a slightly different retention time than mirex on high-pressure liquid chromatography. They were unable to identify it further, because not enough of the material was available. The authors were unable to extract a metabolite from adipose tissue. The authors concluded that the unidentified material was formed by bacteria in the gastrointestinal tract of the monkeys. Andrade and Wheeler (1975) after exposing mirex to sewage sludge microorganisms under anaerobic conditions were able to demonstrate a metabolite which most likely was the 10-monohydro derivative of mirex. Similar hydro derivatives of mirex can be produced by irradiation with sunlight (Alley et al., 1974). With the methods of analysis used in this study, metabolites were also not demonstrated. It is possible that different analytical parameters are necessary to determine whether mirex is metabolized.

The overall concentration of mirex in fetuses during early gestation is about twice that of the plasma levels of the nannies. Whether bioconcentration occurs in late gestation in specific organs needs to be investigated and at the end of gestation the total fetal body burden may actually be higher than that found in the very young fetus. The present study merely shows that transplacental passage of mirex takes place in goats. Although Bond and Woodham (1975) state that cows exposed to mirex do not accumulate appreciable amounts of mirex in adipose tissue, this does not seem to be the case for goats which are also ruminants. The generalization that ruminants do not accumulate this material can therefore not be made.

LITERATURE CITED

Alley, E. G., Layton, B. R., Minyard, J. P., Jr., *J. Agric. Food Chem.* **22**(14), 727–729 (1974).

Andrade, P., Wheeler, W. B., *Bull. Environ. Contam. Toxicol.* **14**(4), 473–479 (1975).

Bond, C. A., Woodham, D. W., *Bull. Environ. Contam. Toxicol.* **14**(1), 25–31 (1975).

Gibson, J. R., Ivie, G. W., Dorough, H. W., *J. Agric. Food Chem.* **20**(6), 1246–1248 (1972).

Ivie, G. W., Gibson, J. R., Bryant, H. E., Begin, J. J., Barnett, J. R., Dorough, H. W., *J. Agric. Food Chem.* **22**(4), 646–653 (1974a).

Ivie, G. W., Dorough, H. W., Bryant, H. E., *Bull. Environ. Contam. Toxicol.* **11**(2), 129–135 (1974b).

Mehendale, H. M., Fishbein, L., Fields, M., Matthews, H. B., *Bull. Environ. Contam. Toxicol.* **8**(4), 200–207 (1972).

Pittman, K. A., Kennedy, M. W., Treble, D. H., *Toxicol. Appl. Pharmacol.* **33**(1), 196–197 (1975).

Pittman, K. A., Wiener, M., Treble, D. H., *Drug Metab. Dispos.* **4**(3), 288–295 (1976).

Smrek, A., Admans, S., Liddle, J., Kimbrough, R., *J. Agric. Food Chem.* **25**(6), 1321–1325 (1977).

Stein, V. B., Pittman, K. A., Kennedy, M. W., *Bull. Environ. Contam. Toxicol.* **15**(2), 140–146 (1976).

Zabik, M. E., Schemmel, R., *Arch. Environ. Health* **27**, 25–30 (1973).

Received for review December 15, 1977. Accepted March 2, 1978.