Absorption, Distribution, and Excretion of the ^{14}C-Labeled Acaricide Hoe 2910 Following Oral Application to Milk-Producing Ruminants and Dermal Application to a Calf

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Orally as well as dermally applied Hoe 2910-^{14}C was absorbed; the percutaneous absorption had only a subordinate significance compared to the enteral. The elimination proceeded rapidly; the half-lives for urine, feces, and milk were on the average 15 ± 2.4 hr for the sheep and 18 ± 4 hr for the cow. The amounts eliminated with milk were small, and for the sheep were less than 0.2% and for the cow less than 1% of the administered dosage. The residue in the organism of the sheep was only 0.5% of the administered radioactivity 4 days after administration and for the cow was only about 1% 5 days after administration. Of those organs and tissues intended for human consumption only the liver and (of the sheep) the retroperitoneal fat contained concentrations of 0.1 μg per gram at those times; the content of the muscles was only 0.01 μg per gram. The concentrations in the calf that was killed 13 days after dermal application were considerably lower.

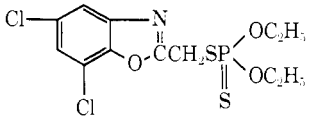
Ectoparasitic ticks are found in many species of animals all over the world. Due to their blood-sucking method of feeding, they are carriers of animal and human diseases. Other consequences of severe tick infestation are blood losses, which have a negative effect on the development of the animals, and skin lesions. The economic losses caused by ticks on domestic animals are considerable. Especially affected by these economic losses are regions in tropical and subtropical zones.

The compound Hoe 2910 (code name for Batestan, registered trademark of the Farbwerke Hoechst A.-G., Frankfurt (Main), Hoechst, West Germany; generic name: Benoxafos) is an ester of dithiophosphoric acid developed in the laboratories of the Farbwerke Hoechst A.-G. which is effective principally against ticks on cattle, horses, and sheep. Moreover, it can be used to fight lice on cattle and sheep, biting lice on cattle, sheep ked on sheep, and red mites of poultry (by treatment of the poultry house).

In practice, essentially two methods of treatment are used: dip bath and spraying. The possibility of incorporation through percutaneous absorption exists for both methods. In the dip bath method, the active component can also enter the body through oral intake of some amounts of the bath liquid. For these reasons, the kinetics of radioactively labeled Hoe 2910 were examined following oral administration to milk-producing ruminants, as well as the dermal administration to a calf.

MATERIALS AND METHODS

Hoe 2910 was labeled with carbon-14 in the benzene ring. Due to the method of labeling, the radioactive carbon was uniformly distributed within the ring. The specific radioactivity of the compound given to sheep and the calf was 23 mCi per gram and that given to the cow was 10 mCi per gram of Hoe 2910 (radiochemically and chemically pure product of the Radiochemical Laboratory of the Farbwerke Hoechst A.-G., Frankfurt (Main), Hoechst).



O,O-diethyl-*S*-(5,7-dichlorbenzoxazol-2-ylmethyl)dithiophosphate

Two milk sheep, of the East Frisian type, weighing 63 kg each, and one Holstein cow, which weighed 494 kg at the beginning of the experiment, were given the single oral dose of 2.5 mg of Hoe 2910-^{14}C per kg of body weight in the form of a 0.1% aqueous emulsion (Hoe 2910-^{14}C 200 mg; Emulsogen EL 23 mg; phenylsulfonate *ca.* 17 mg; Genamin 0-080 6 mg; xylene 44 mg; tap water up to 200 g) through a stomach tube in the morning into the rumen. In order to prevent radioactivity losses by adherence of the suspension to the tube, 500–1000 ml of tap water were used to rinse it down. The dosages of 0.16 g given to sheep contained 3.7 mCi and the dosage of 1.3 g given to the cow contained 13 mCi of carbon-14.

One-hundred milliliters of an 0.08% emulsion of the above-described composition were applied with a brush on the coat of the back (last cervical to last lumbar vertebra) and the lateral chest and abdominal wall of an approximately 6-weeks-old female Holstein calf weighing 38 kg. This dosage of 80 mg contained 1.8 mCi of carbon-14. The liquid immediately penetrated the coat and came into contact with the skin. The treated area measured approximately 0.3 m^2. Because of radiation protection the radioactive skin area had to be covered; therefore the body of the calf was wrapped with polyethylene foil which was sealed air-tight at the neck, forelegs, and just anterior to the hind legs. In order to prevent a heat accumulation which could influence absorption, the covering was ventilated. The air flow was varied so that no water of condensation could form on the inside of the bag.

The sheep and the calf were kept in metabolism cages according to the method of Herok and Gotte (1963). Blood samples were taken from the jugular vein of the sheep and the cow and from a hind leg vein of the calf. Feces and urine were collected separately and in daily portions.

The milk collection times were different for the sheep and cow (Figure 1). The daily feed ration for the cow consisted of 5 kg of concentrate for dairy cattle, 5 kg of dried brewer's grain and 0.1 kg of mineral supplement for dairy cattle. The

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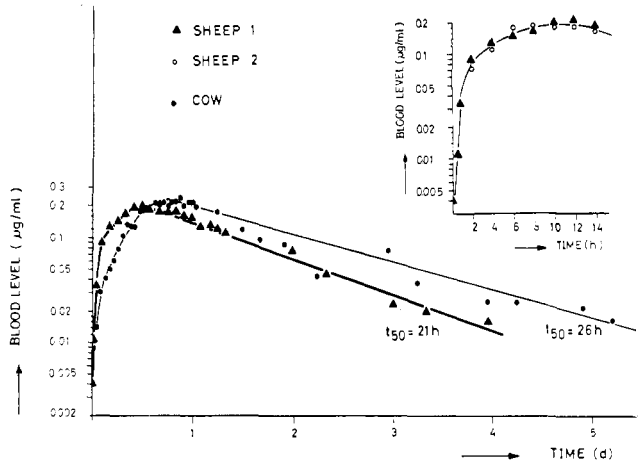

Figure 1. Blood level after single oral administration of 2.5 mg of Hoe 2910-^{14}C per kg of body weight to sheep and a cow

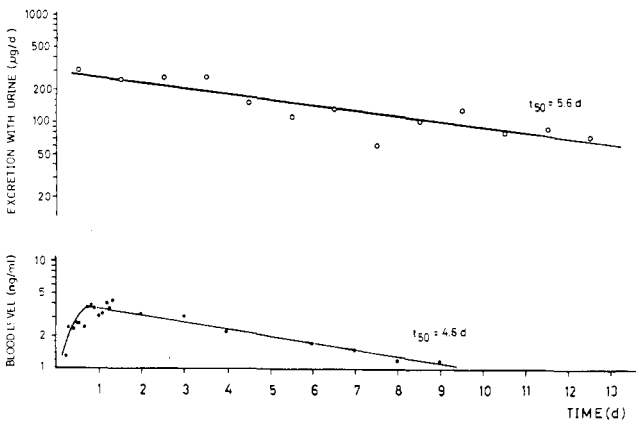

Figure 2. Blood level and excretion with urine after single dermal application of 2.1 mg of Hoe 2910-^{14}C per kg of body weight on a calf

sheep received rolled oats and dried sugar beet pulp according to their weight. Hay and water were available to all animals *ad libitum*. The calf was fed with a commercial milk replacer for calves (Lactina Milk, Schweizerische Lactina A.-G., Kehl/Rhein, Germany).

The sheep, which was killed 14 hr after administration, was perfused with a polymer of partially hydrolyzed gelatin (Haemaccel, Behringwerke A.-G., Marburg) (Kellner *et al.*, 1969). The second sheep was killed 4 days, the cow 5 days, and the calf 13 days after administration by exsanguination under narcosis.

For the determination of radioactivity in blood, 0.1 ml of blood was dissolved in 1 ml 0.1 *N* NaOH and the solution was then discolored with 0.1 ml of hydrogen peroxide (30%). After the addition of 10 ml of dioxane scintillator, the radioactivity was measured.

The radioactivity in urine could also be determined directly after having mixed aliquot portions up to 1 ml with 10 ml of dioxane scintillator.

The feces were dried at 60° after thorough homogenization. Aliquot parts of the residues were burned in an atmosphere of oxygen, as described by Kaiberer and Rutschmann (1961). After absorption of the combustion gases in 10 ml of methanol–butylamine (4:1), aliquot parts were transferred to a toluene scintillator.

One milliliter of freshly homogenized milk was dissolved in 1 ml of Digestin (Merck, Darmstadt, West Germany) and a

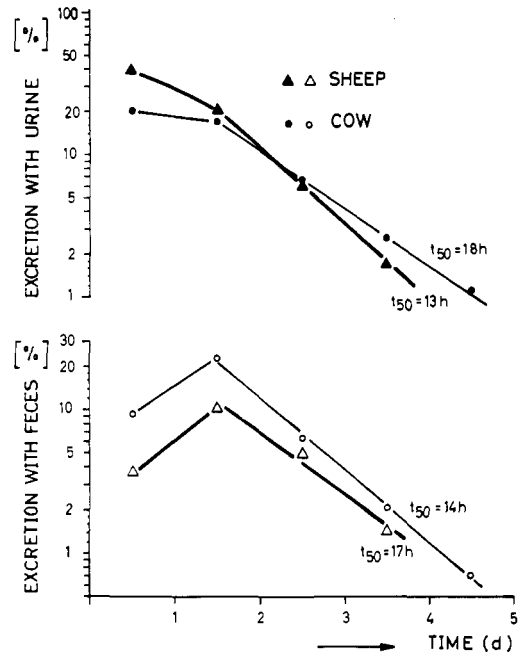

Figure 3. Excretion with urine and feces after single oral administration of 2.5 mg of Hoe 2910-^{14}C per kg of body weight to sheep and a cow. Percent of the administered radioactivity

mixture of 2 ml of toluene scintillator and 8 ml of dioxane scintillator was added.

The dioxane scintillator consisted of 5 g of 2,5-diphenyloxazole (PPO), 100 mg of 1,4-bis-2-(5-phenyloxazolyl)benzene (POPOP), and 100 g of naphthalene per liter of dioxane. The toluene scintillator consisted of almost the same combination, with the exception of POPOP, 150 mg per liter of which was present. The reagents were purchased from Merck A.-G., Darmstadt, West Germany. The overall error of individual values for blood, milk, urine, and feces was up to 10%, including errors in administration, sampling, pipetting, preparation, and measurement of radioactivity.

All samples were counted in a Packard Tri-Carb liquid scintillation spectrometer, series 3000 (Packard Instrument Co., Downers Grove, Ill.). All quenching corrections were made with the aid of automatic external standardization using the channel ratio method.

All data expressed in micrograms were obtained from the percentage of radioactivity content without regard to the probability that metabolization of the compound had occurred in the animals.

BLOOD LEVEL

The blood levels obtained following oral administration of Hoe 2910-^{14}C are shown in Figure 1. Absorption occurred slowly, whereby the invasion proceeded faster in both of the sheep than in the cow. The maximum blood levels were very uniform (0.19 to 0.25 µg per ml). The course of elimination which could be traced for one sheep and the cow proceeded, after reaching the maximum, in both species in only one phase, which means total elimination can be described by a single half-life. The half-lives (t_{50}), 21 and 26 hr, respectively, were only slightly different from each other. At the time the animals were killed, 96 hr (sheep) and 125 hr (cow) after administration, the concentrations in the blood of both species lay under 10% of the maximum blood level.

Following application of the emulsion on the skin, absorption occurred slowly, as it also did following oral administra-

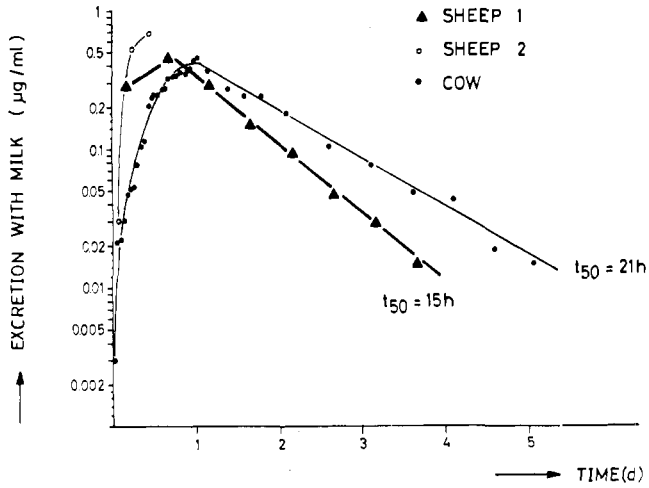

Figure 4. Excretion with milk after single oral administration of 2.5 mg of Hoe 2910-^{14}C per kg of body weight to sheep and a cow

tion. The concentrations detected were extremely low (Figure 2); the highest blood level value was 0.004 μg per ml, less than 2% of the maximum concentration found in the cow. The half-life for the concentration decrease in the blood was 4.6 days, considerably longer than the elimination half-life following oral administration. This can be explained by the continuing percutaneous absorption. As time went on, the amount of acaricide which had been applied to the skin decreased as a result of, for example, dust formation, rubbing off on the protective covering, physiological regeneration processes of the skin, etc., so the half-life of 4.6 days can be considered valid for this decrease, but not for the elimination of Hoe 2910-^{14}C (and/or metabolites containing ^{14}C), which had a half-life of 21 and 26 hr, respectively.

EXCRETION

Urine and Feces. The excretion in urine and feces following oral administration is shown in Figure 3. Because of the slow absorption, the renal elimination of the compound and/or metabolites by both species proceeded less rapidly on the first day after administration than in the following examination period, in which the elimination half-lives for the sheep and cow, 13 and 18 hr, respectively, showed good conformity, similar to that of the blood.

Altogether almost $^4/_5$ of the radioactivity orally administered to the sheep and almost one-half of that administered to the cow were eliminated with urine. The elimination of absorbed Hoe 2910-^{14}C and/or metabolites containing ^{14}C was so rapid that 2 days after administration around 90% and 1 day later already about 98% of the total radioactivity found in the urine had been excreted through the kidneys in both species.

The excretion in feces also began somewhat delayed; the maximum occurred on the second day after administration (Figure 3). The subsequent elimination pattern showed good correspondence, again for both species, because the half-lives of 17 hr (sheep) and 14 hr (cow) were also within the range of those determined for renal elimination. Three days after administration, similarly as with urine, over 90% of the total radioactivity excreted with feces had left the body.

In total, 91% of the administered radioactivity was recovered in the urine and feces of the cow in 5 days; for the sheep, 92% was recovered in 4 days.

Following application of Hoe 2910-^{14}C on the skin, only the excretion with urine was measured. The excretion curve is shown in Figure 2. It proceeded evenly with a half-life of 5.6 days (see BLOOD). Within the examination period of 2 weeks, about 2% of the radioactivity applied to the skin was eliminated renally.

Table I. Distribution at Various Times after Oral and Dermal Administration of Hoe 2910-^{14}C

	Concentration (μg/g or μg/ml)			
	14 hr after administration, sheep 1	96 hr after administration, sheep 2	125 hr after administration, cow	13 days after administration, calf
Blood	0.17	0.016	0.016	
Liver	0.96	0.11	0.085	0.002
Bile	1.5	0.053	0.070	0.040
Kidneys	0.85	0.047	0.049	<0.001
Spleen	0.094	0.007	0.070	<0.001
Ovaries	0.57	0.034	0.017	
Rumen	4.4	0.094	0.014	
Omasum	5.2	0.12	0.014	0.002
Abomasum	0.60	0.050	0.017	
Small intestine	0.49	0.013	0.011	
Large intestine	0.32	0.021	0.010	
Mesenteric lymph nodes	0.15	0.009	0.009	
Retroperitoneal fat	0.61	0.10	0.073	0.007
Subcutaneous fat	0.39	0.065	0.052	<0.001[a]
Heart	0.13	0.018	0.032	<0.001
Lungs	0.20	0.013	0.017	<0.001
Brain	0.14	0.004	0.007	<0.001
Spinal cord	0.18	0.008	0.008	
Muscles	0.066	0.009	0.011	<0.001
Peripheral lymph nodes	0.36	0.031	0.014	
Bone marrow	0.34	0.10	0.072	
Skin (without hair)	0.053	0.005	0.013	
Udder			0.019	

[a] From areas where the skin did not come into contact with radioactivity.

Milk. The concentration curves for milk following oral administration of Hoe 2910-^{14}C are shown in Figure 4.

As the blood level rose, the elimination of radioactivity with milk began and reached its maximum in the cow about 1 day and in sheep No. 2 between 8 and 24 hr after administration. The decreases in concentration had half-lives of approximately 15 and 21 hr, respectively, and corresponded to those for renal and fecal elimination. In total, the concentrations in the milk of both species were low. The amounts excreted in 4 or 5 days were very small, and were for the sheep under 0.2% and for the cow under 1% of the administered dose.

Distribution. Table I shows that within the range of maximum blood levels, the organs concerned with absorption and elimination, such as the omasum, rumen, liver, kidneys, and also the bile, showed high concentrations, whereas the heart, lungs, and brain showed low levels.

By reason of the rapid and almost complete elimination, the concentrations measured in the bodies of the sheep and cow 4 and 5 days after the treatment were low. The total residues in the organism of the sheep were only 0.5%, and those in the cow were *ca.* 1% of the administered dose. Of those organs intended for human consumption, only the liver and (in the the sheep) the retroperitoneal fat contained concentrations of around 0.1 μg per gram. Noteworthy is the low content of the skeletal muscles, which was only 0.01 μg per gram.

The concentrations found in the calf which was killed 13 days after dermal application were substantially lower than those found after oral administration (Table I).

DISCUSSION

The examination showed that, following dermal treatment, the active component is absorbed through the skin. However, from the results it can be derived that percutaneous absorption plays only a subordinate role in relation to the amount of Hoe 2910 which enters the body. More important is the oral intake of the active component, for example with the bath liquid, because of the good enteral absorption. But even with the selected high dose of 2.5 mg of Hoe 2910 per kg of body weight, which corresponds to an intake of almost 2.5 l. of the commercial 0.05% bath liquid by a cow, no adverse effects were seen because orally administered Hoe 2910 was eliminated so rapidly and completely that already a short time after the treatment the residues found were very low. This especially applies to the organs intended for human consumption.

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Received for review May 9, 1972. Accepted August 3, 1972.

Metabolism of Bis(chloromethyl) Sulfone in Sheep and Cattle

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Bis(chloromethyl) sulfone is metabolized in sheep and cattle to form carbon dioxide in exhaled air. Chloromethanesulfinic and chloromethanesulfonic acids were identified in urine. Radioactive uric acid and urea in urine and amino acids with carboxyl carbon labeling in liver and kidney tissues were identified as secondary metabolites.

Bis(chloromethyl) sulfone is of interest for its effect on rumen fermentation. Recent work in this field is described by O'Connor *et al.* (1971) and Prins and Seekles (1968). A study of the metabolites present in the urine of sheep and cattle following oral dosing of the compound has been carried out. In addition, the nature of radioactive residues in the liver and kidney of sheep has also been investigated.

METHODS

Radiometric Methods. Bis(chloromethyl) sulfone-^{14}C was prepared by treating trithiane-^{14}C with sulfur dichloride to form bis(chloromethyl) sulfide-^{14}C (Mann and Pope, 1923). The latter was oxidized with hydrogen peroxide. For the animal studies a specific activity of 0.3 to 0.6 μCi/mg was employed. The radioactivity of urine samples and of isolated metabolites was determined by methods described by Tocco *et al.* (1965).

Animal Studies. For the isolation work described in this paper, a wether lamb weighing 31.5 kg was dosed orally with one capsule twice a day for 2 days. Each capsule contained 125 mg of bis(chloromethyl) sulfone-^{14}C at 0.6 μCi/mg, a total of 500 mg and 300 μCi.

A Hereford steer weighing 264 kg was dosed with 1.2 g/day of bis(chloromethyl) sulfone-^{14}C of specific activity 0.3 μCi/mg for 4 days by mixing the compound in the feed. The animal was preconditioned by feeding 0.03% of unlabeled compound in the diet for 5 days prior to dosing. Animals were housed in metabolism cages for separate and total collection of urine and feces.

Urine was collected and frozen until examined. Liver and kidney from sheep were collected and frozen at sacrifice 4 days after dosing.

Collection of Carbon Dioxide from Sheep and Steer. Carbon dioxide was collected from a wether lamb for 4 days after receiving a single dose of 525 mg of bis(chloromethyl) sulfone-^{14}C. The lamb was housed in an air-tight chamber which was ventilated at a rate of 60 l. of air per min. A small side stream of the expired air (2%) was drawn through alkali. A sample of this solution was acidified and the resulting CO_2 was trapped in Hyamine and counted by liquid scintillation procedures. A steer was similarly fitted with a mask and intermittent samples of expired air were obtained over a 4-day period after the animal received 15 g of the compound orally. Carbon dioxide was obtained and analyzed as described above. The total quantity of ^{14}C expired as $^{14}CO_2$ was calculated by multiplying the average rate of expiration over the time increment between sampling periods.

Separation Methods. Urine from steer and sheep was fractionated by the scheme outlined in Figure 1. The pH 8 methylene chloride extract contained the unchanged drug (K_D, [solvent/water] = 14). The amount of intact sulfone present was determined by reverse isotope dilution analysis (Barker *et al.*, 1970). The aqueous effluent from Dowex 1×2 (Cl^-) chromatography was evaporated to a small volume of water from which uric acid crystallized. Alternatively, when the effluent from Dowex 1×2 (Cl^-) was passed through a column of Dowex 1×2 (OH^-), the uric acid was retained and the aqueous effluent yielded crystalline urea upon evaporation.

Elution of the Dowex 1×2 (Cl^-) column was accomplished with either 1 *M* pyridine hydrochloride at pH 2.5 or with methanolic hydrochloric acid prepared by mixing concentrated hydrochloric acid and methanol (1:9). The eluate, consisting of strongly acidic metabolites, was extracted with ether, which removed a small amount of radioactivity (about 10%) which

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