Fate of the Juvenile Hormone Mimic 1-(4'-Ethylphenoxy)-3,7-dimethyl-6,7-epoxy-*trans*-2-octene (Stauffer R-20458) following Oral and Dermal Exposure to Steers

G. Wayne Ivie,* James E. Wright, and Harry E. Smalley

The juvenile hormone mimic [1-(4'-ethylphenoxy)-3,7-dimethyl-6,7-epoxy-*trans*-2-octene (Stauffer R-20458)] was completely metabolized and the phenyl-labeled materials were essentially quantitatively excreted after oral administration of the radiolabeled compound to a steer. About 85% of the dose was eliminated in the urine and about 15% in the feces. Metabolism of R-20458 by the steer involved epoxide hydration, α-oxidation of the 4'-ethyl moiety, ether cleavage, and undefined biotransformations that resulted in a large number of uncharacterized metabolites. After R-20458-^{14}C was applied to the hair and skin of a second steer, about 30% of the dose was absorbed and subsequently excreted through the urine and feces during the first 7 days after treatment. About 40% of the dose remained at the application site after 7 days, and more than 90% of this was unchanged R-20458. Analysis of tissue samples taken 7 days after both the oral and dermal treatments indicated that in each case radiocarbon retention within the body was minimum.

The juvenile hormone mimic 1-(4'-ethylphenoxy)-3,7-dimethyl-6,7-epoxy-*trans*-2-octene (R-20458 of Stauffer Chemical Co., Mountain View, Calif.) has shown potential as a selective insect control agent (Pallos and Menn, 1972; Pallos et al., 1971). Laboratory and field studies with R-20458 indicate that the compound may be useful in suppressing populations of the stable fly (*Stomoxys calcitrans*) in cattle feedlots (Wright, 1972; Wright et al., 1973). The larval and pupal stages of this insect develop in the top few centimeters of the manure-rich soil, and surface treatment of the soil with R-20458 is effective in preventing emergence of the blood-feeding adults.

Previous studies have shown that R-20458 is highly susceptible to metabolic degradation after oral or intraperitoneal administration to laboratory rats and mice and that the numerous metabolic products generated are rapidly and essentially quantitatively eliminated through the urine and feces (Gill et al., 1972, 1974; Hoffman et al., 1973). The major identified metabolic pathways included ether cleavage, epoxide hydration, and α- and β-oxidation of the 4'-ethyl moiety. Although these studies indicated that R-20458 is highly biodegradable in mammals, the potential use patterns of the compound in cattle feedlots dictate that the dynamics of absorption, metabolism, and excretion of R-20458 be evaluated in cattle. Certainly, low-level consumption of residues or body surface exposure seems inevitable if animals are held within treated areas. In the studies reported here, radiolabeled R-20458 was used to evaluate the metabolic and residual behavior of the compound after oral and dermal administration to steers.

MATERIALS AND METHODS

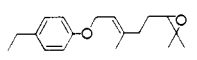
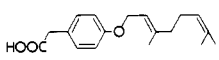
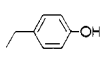
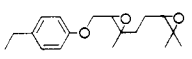
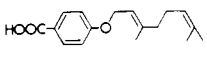
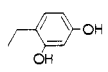
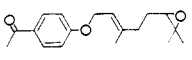
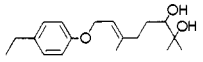
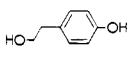
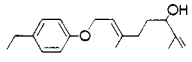
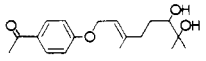
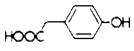
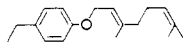
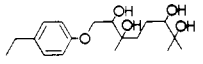
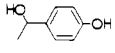
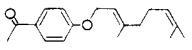
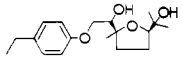
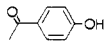
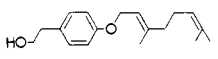
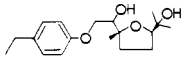
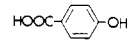
Chemicals. R-20458-phenyl-*U*-^{14}C (17.0 mCi/mmol) was provided by Stauffer Chemical Co., Mountain View, Calif., from material synthesized by Kalbfeld et al. (1973). Because the animal treatments in the current study were separated by a time interval of several months, the radiochemical purity of the R-20458-^{14}C was determined in each case just prior to use. For the oral treatment, the radiochemical composition as determined by thin-layer chromatography (TLC), radioautography, and subsequent liquid scintillation counting (lsc) of the appropriate gel regions was as follows: R-20458 (the ethyl epoxide, see Table I for structures of compounds and their trivial names), 98.0%; ethyldiene, 1.0%; ethyl diepoxide, 0.4%, ethyldiol, 0.5%; other products, 0.1%. For the dermal study, the radiochemical composition was ethyl epoxide, 97.6%; ethyldiene, 1.0%; ethyl diepoxide, 0.2%; ethyldiol 1.0%; other products, 0.2%.

Twenty derivatives and analogs of R-20458 were used for TLC comparisons with ^{14}C-labeled metabolites that occurred in urine and feces and with degradation products extracted from treated skin. The compounds, their structures, and chemical or trivial names, and the sources from which they were obtained are shown in Table I. Most of the available compounds were those synthesized (Hammock et al., 1974) for use in metabolic and environmental degradation studies with R-20458 and related compounds (Gill et al., 1974). The following compounds were synthesized as described by Hammock et al. (1974). The *cis*- and *trans*-ethyltetrahydrofurandiols were prepared by *m*-chloroperoxybenzoic acid oxidation of the ethyldiol, which resulted in an unstable 2,3-epoxy-6,7-diol intermediate that subsequently cyclized to give approximately equal amounts of the *cis*- and *trans*-tetrahydrofurandiol isomers. The α-hydroxyethyl phenol was prepared by lithium aluminum hydride reduction of the acetophenol, and β-hydroxyethyl phenol was synthesized by lithium aluminum hydride reduction of the methyl ester of hydroxyphenyl acetic acid. The trivial names for R-20458 and its analogs used in Table I and throughout this report are generally the same as those used earlier (Gill et al., 1974; Hammock et al., 1974).

Chromatography. TLC studies with R-20458 metabolites, degradation products, and unlabeled analogs were made by using silica gel F_{254} precoated chromatoplates (0.25 mm gel thickness, EM Laboratories, Elmsford, N.Y.). Resolution of the ^{14}C-labeled products was accomplished by applying the mixtures as a spot or short band to one corner of a 20 × 20 cm chromatoplate and then developing the plate in sequence in the following three solvent systems: (1) benzene–1-propanol (5:1) in the first direction; (2) chloroform–methylene chloride–ethyl acetate–propanol (10:10:1:1) in the second direction; and then (3) carbon tetrachloride–ether (2:1) as a second development in the first direction. This TLC procedure has been used successfully in the resolution of a large number of metabolites and photoproducts of the ethyl epoxide, ethyldiene, ethyl diepoxide, and ethyldiol (Gill et al., 1974). In studies aimed

Veterinary Toxicology and Entomology Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, College Station, Texas 77840.

Table I. Structures, Trivial Names, and Sources of R-20458 and Certain Analogs Considered as Potential Metabolites or Degradation Products

	Ethyl epoxide (R-20458)[a]		β-Carboxymethyl diene[b]		Ethyl phenol[b,d]
	Ethyl diepoxide[b]		Carboxy diene[b]		Ethyl resorcinol[b,d]
	Aceto epoxide[b]		Ethyl diol[b]		β-Hydroxyethyl phenol[c]
	Ethyl-6-hydroxy-2,7-diene[b]		Aceto diol[b]		Hydroxyphenylacetic acid[b,d]
	Ethyl diene[b]		Ethyl tetrool[b]		α-Hydroxyethyl phenol[c]
	Aceto diene[b]		cis-Ethyl-thf-diol[c]		Aceto phenol[e]
	β-Hydroxyethyl diene[b]		trans-Ethyl-thf-diol[c]		Hydroxybenzoic acid[b,d]

[a] Provided by Stauffer Chemical Co., Mountain View, Calif. [b] Provided by B. D. Hammock (Department of Entomology, University of California, Riverside, Calif.) and J. E. Casida (Department of Entomology and Parasitology, University of California, Berkeley, Calif.). [c] Synthesized as described by Hammock et al. (1974). [d] Also available from Aldrich Chemical Co., Milwaukee, Wis. [e] Aldrich Chemical Co., Milwaukee, Wis.

at characterizing the ^{14}C-labeled products obtained from the steer urine, feces, or treated skin, the isolated metabolites or ^{14}C-labeled mixtures were applied to TLC as described above, except that one of the unlabeled analogs (Table I) was added to the mixture. After development of the plate in the three TLC solvent systems, the plate was examined for coincidence of the unlabeled standard with any of the ^{14}C-labeled products. Radioautography was used to locate the ^{14}C-labeled materials, while the standards were visualized either by their quenching of gel fluorescence when viewed under short-wavelength ultraviolet light or by spraying the plates with molybdophosphoric acid reagent and then heating (Stahl, 1969).

Animal Treatment. *Oral.* A 221-kg steer (mixed breed) was housed in a metabolism stall, and a latex collection device was cemented around the prepuce to allow separation of urine and feces. The animal was provided food and water ad libitum. The radiochemical (0.8 mCi) was dissolved in corn oil, mixed with a small amount of crushed feed, and administered orally as a single dose with a balling gun. The treatment was equivalent to 0.05 mg/kg body weight.

Dermal. A second steer (234 kg, mixed breed) was placed in a metabolism stall as before, and areas of skin 15 × 15 cm square were marked off on each side of the animal near the top of the back just below the withers. These areas were chosen for the dermal treatments because they were so located that rub-off could not occur when the stanchioned animal lay down in the stall. The hair immediately surrounding the treatment area was clipped; however, hair on the treatment area itself was not disturbed. The radiolabeled R-20458 was diluted with a formulation of unlabeled R-20458 emulsifiable concentrate and water such that the final treatment solution was equivalent to the mixture normally used in feedlot applications (1% R-20458 in water) though it contained R-20458-^{14}C in a concentration of 7.1×10^6 dpm/ml (specific activity of 800 dpm/μg). The treatment solution was applied to the hair and skin of the two treatment areas with an artist's brush to the point of saturation but not run-off. The total applied dose contained 52 μCi of radiocarbon and 144 mg of R-20458.

Sample Collection. After treatment of each animal, total urine and feces samples were collected periodically, and 0.2-ml aliquots of urine were assayed for radiocarbon content by lsc. Samples of feces were mixed thoroughly, and about 0.5-g portions were retained for radiocarbon quantitation by combustion analysis. Also, substantial portions of each sample were quickly frozen and held at −70°C for later analysis. Venous blood samples were collected from each animal at frequent intervals after treatment through an intravenous catheter inserted in the juglar vein. The animals were sacrificed 7 days after both the oral and the dermal treatments. Samples of major tissues were collected at this time for determination of radiocarbon content. In the dermal study, the treated areas of skin were carefully removed for analysis, and samples of skin and subcutaneous tissues in close proximity were also collected for study.

Analytical Procedures. LSC measurements were made with a Beckman LS-235 spectrometer equipped with external standardization. A toluene based scintillation cocktail was used in which 2-methoxyethanol was incorporated to improve miscibility with aqueous samples. Combustion analyses were performed by first air drying up to 0.5 g (wet weight) of tissue and feces or 1.0 g of whole blood, and then burning the samples under 1 atm of oxygen. The combustion gases were bubbled through a carbon dioxide trapping solution (equal volumes of 2-aminoethanol and 2-methoxyethanol), and the trapped radiocarbon was then quantitated by lsc. Radioactive areas on developed TLC plates were located by radioautography (Kodak No-Screen Medical X-ray Film) and quantitated by scraping the appropriate gel regions into scintillation vials for lsc.

Urine samples were acidified to pH 2.0 with HCl and

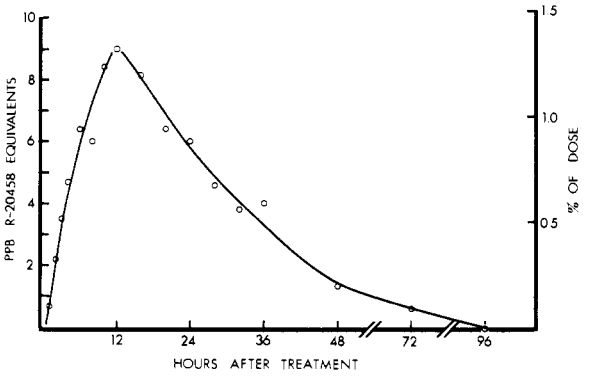


Figure 1. Radiocarbon residues in whole blood of a steer after oral treatment with R-20458-^{14}C at 0.05 mg/kg.

then extracted 4 times with equal volumes of ethyl acetate. Aliquots of the combined ethyl acetate extracts and the aqueous phase were subjected to lsc to determine partitioning characteristics; then the organic phase was dried over anhydrous sodium sulfate, concentrated, and applied to TLC. Radiocarbon remaining in the urine aqueous phase after solvent extraction was further analyzed as follows. The samples were placed on a rotary evaporator under moderate heat (40–45°C) and vacuum to remove all traces of ethyl acetate. Next, 5 ml of 0.2 *M* sodium acetate was added to 5 ml of the aqueous phase, the solution was adjusted to pH 4.5, β-glucuronidase-aryl sulfatase (from *Helix pomatia*, Calbiochem, LaJolla, Calif.) was added, and the samples were incubated for 24 hr at 37–38°C. The levels of enzyme added to each sample were equivalent to about 50000 Fishman units and ≤25000 Whitehead units. After incubation, the samples were adjusted to pH 2.0, extracted as before with ethyl acetate, and subjected to TLC analysis. Samples in which the enzyme was first deactivated at 100°C for 20 min were also tested to determine the extent of nonenzymatic conversion of water-soluble products under the incubation conditions used.

Feces samples (20 g) were homogenized in 40 ml of water with a blade homogenizer (Virtis 45, The Virtis Co., Gardiner, N.Y.); then the samples were adjusted to pH 2.0, 60 ml of ethyl acetate was added, and the samples were homogenized thoroughly with a Polytron homogenizer (Brinkman Instruments, Westbury, N.Y.). After centrifugation to break the resulting emulsion, the organic phase was removed, and the water–residue slurry was extracted three additional times with ethyl acetate. The combined ethyl acetate extracts contained amounts of interfering material sufficient to prevent adequate TLC analysis; thus the samples were subjected to a cleanup procedure as follows. A quantity of the ethyl acetate extract equivalent to 8 g of feces was transferred to 1–2 ml of methylene chloride, and the solution was applied to a column containing 5 g of 60–100 mesh PR Grade Florisil (The Floridin Co., Berkeley Springs, W.Va.). Elution with methylene chloride removed the less polar metabolites with little accompanying pigmented material that interfered with chromogenic visualization of the higher R_f metabolite standards. Further elution with a 1:1 mixture of acetone and methylene chloride removed all but about 10% of the original radioactivity applied to the column. These fractions were analyzed by TLC; however, the chemical nature of the radiocarbon remaining on the column was not further studied because it could only be recovered under conditions which eluted large amounts of interfering compounds. Also, no attempts were made to define the chemical composition of the radioactivity remaining in the water–residue phase after ethyl acetate extraction of the samples of feces.

Radiocarbon residues remaining on the hair and skin 7 days after dermal treatment were extracted by thoroughly washing portions of the skin (hair intact) with methanol. The extract was then concentrated and analyzed by TLC.

RESULTS

Oral Treatment. Radioactivity appeared in venous blood within 1 hr after oral treatment with R-20458-^{14}C, and maximum residues of about 9 ppb R-20458 equivalents were detected 12 hr after dosing (Figure 1). Thereafter, radiocarbon levels in the blood declined steadily to undetectable quantities (<0.25 ppb) by 4-days posttreatment. The maximum residues in blood at 12 hr (after treatment) corresponded to only about 1.4% of the total dose present in the blood at that time, based on the estimated blood content of the animal (Dukes, 1947) and assuming equal distribution of radiocarbon in the blood.

Excretion through the urine was the major mechanism by which radiocarbon was eliminated by the steer after oral R-20458-^{14}C treatment (Figure 2). About 85% of the administered dose appeared in the urine during the 7-day posttreatment period, and the great majority was excreted within 2 days after dosing. Most of the remaining radiocarbon was eliminated in the feces (Figure 2). The total recovery of the administered dose in urine and feces was >98%.

Additional evidence that radiocarbon excretion was essentially complete within 7 days after treatment was obtained by analysis of a variety of tissue samples. Only four tissues contained radiocarbon residues above 0.5 ppb, the level of sensitivity of the combustion method employed. These were fat (8.3 ppb), kidney (1.0 ppb), liver (0.8 ppb), and thyroid (0.8 ppb). The following tissues contained no detectable ^{14}C-labeled residues: brain, heart, lung, lymph node, muscle, spleen, and tongue.

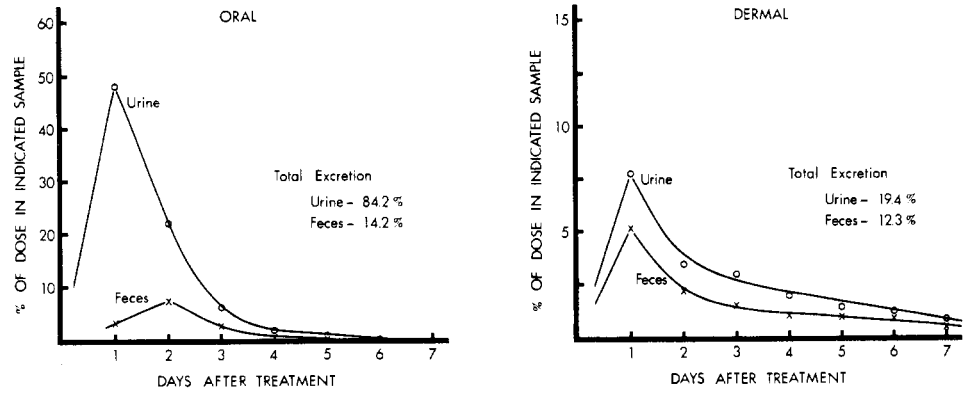


Figure 2. Radiocarbon excretion in urine and feces of steers after oral and dermal treatment with R-20458-^{14}C.

Table II. Partitioning Characteristics of Radiocarbon in Urine and Feces of a Steer Treated Orally with R-20458-^{14}C at 0.05 mg/kg

	% of sample radiocarbon in indicated fraction				
	Urine			Feces	
Hr after treatment	Organic	Enzyme treated[a]	Aq[b]	Organic	Aq residue
0-24	52	44	4	85	15
24-48	48	49	3	69	31
48-72	54	44	2	40	60

[a] Radiocarbon converted to ethyl acetate extractable during incubation of the urine water solubles with β-glucuronidase-aryl sulfatase. [b] Radiocarbon remaining in the aqueous phase after enzyme incubation and solvent extraction.

About half of the radioactivity present in each urine sample analyzed was extractable into ethyl acetate (Table II). TLC analysis of the ethyl acetate extract from the first day's urine revealed the presence of at least 17 metabolites (Figure 3), but only two chromatographed with any of the 21 available standards. These products, the ethylphenol and acetophenol in nonconjugated form, constituted a relatively small portion of the first day's output of urinary radiocarbon. However, TLC analysis of metabolites liberated from the aqueous phase by β-glucuronidase-aryl sulfatase treatment revealed that the ethylphenol and acetophenol were by far the major metabolites in the urine (Figure 3). About 37% of the total radiocarbon in the sample was in the form of these two metabolites as apparent glucuronide or sulfate conjugates.

Extraction of urine water-soluble samples that had been incubated with heat-deactivated enzyme also resulted in appreciable partitioning of radiocarbon into the ethyl acetate (up to 40% depending on the sample), and TLC analysis of the extracts of these samples indicated similar metabolite distribution patterns as in nonboiled samples. Thus, heating either did not totally destroy enzyme activity or, more likely, the conjugates themselves underwent cleavage in the pH 2 medium before enzyme treatment or during the incubation procedure itself.

The extraction of feces showed that the proportion of radiocarbon extractable into ethyl acetate decreased substantially as time after treatment progressed (Table II). Thus, 85% of the radiocarbon present in the first day's feces sample partitioned into ethyl acetate, but less than half of the radioactivity in the 3-day sample was organic extractable (Table II). TLC studies with the organic phase from the first day's sample indicated that at least 10 metabolites were present in the mixture. Two were identified as the ethyldiol and the acetodiol (Figure 3). The major component of the feces extract, which comprised 27% of the total radiocarbon in the first day's feces sample (Figure 3), was initially thought to be the unmetabolized parent compound. However, isolation of the product for definitive TLC studies revealed that it included two components (as resolved by TLC solvent system 3) in roughly equal amounts, neither of which cochromatographed with R-20458. One migrated slightly higher than the ethyl epoxide in this system, and the other was chromatographically identical with the ethyldiene. Subsequent treatment of the suspected ethyldiene metabolite with *m*-chloroperoxybenzoic acid gave a product that cochromatographed with the ethyl diepoxide.

Dermal Treatment. Radiocarbon was absorbed and subsequently excreted in both urine and feces after dermal application of R-20458-^{14}C to a steer (Figure 2). During the 7-day posttreatment period before the animal was

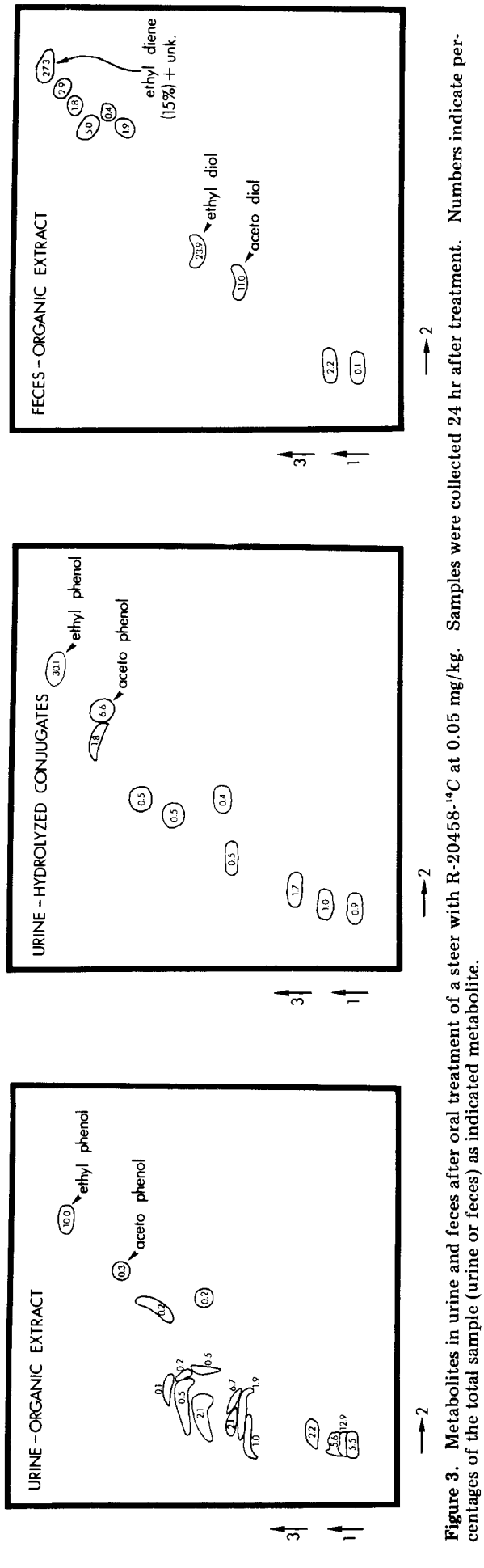


Figure 3. Metabolites in urine and feces after oral treatment of a steer with R-20458-^{14}C at 0.05 mg/kg. Samples were collected 24 hr after treatment. Numbers indicate percentages of the total sample (urine or feces) as indicated metabolite.

Table III. Radiocarbon Residues in Treated and Peripheral Areas 7 Days after Dermal Application of R-20458-^{14}C to a Steer

Sample location	Radiocarbon residues	
	ppm	% of applied ^{14}C
Treated area		
Hair	4825.0	39.3
Skin	6.9	1.2
Subcutaneous tissue[a]	1.0	
Muscle	0.1	
Peripheral area[b]		
Hair[c]		
Skin	2.5	
Subcutaneous tissue[a]	0.4	
Muscle	0.1	

[a] Connective tissue between skin and muscle. [b] All samples collected 2-3 cm from edge of treatment area. [c] Hair clipped in this area before treatment and thus was not analyzed.

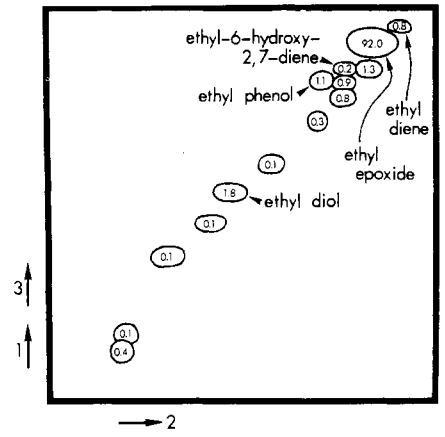


Figure 4. Radiocarbon residues on treated skin and hair of a steer 7 days after dermal exposure to R-20458-^{14}C. Numbers indicate percentages as individual products.

sacrificed, about 19% of the total dose was eliminated in the urine and about 12% in the feces. Low levels of radiocarbon were still being excreted in the urine and feces at the time the animal was sacrificed. Although numerous whole blood samples were analyzed at intervals after the dermal application, none contained detectable radiocarbon residues. Analysis of major tissues after sacrifice indicated residues as follows, in parts per million (ppm): fat, 0.3; kidney, 0.2; all others <0.1, which was the lower limit of sensitivity.

About 40% of the applied radiocarbon remained at the site of application after 7 days (Table III). The great majority was residue on the hair; very little was associated with the skin. Subcutaneous connective tissue below the treatment area contained very low levels of radiocarbon, and muscle tissue beneath the treated skin contained only trace residues (Table III). Likewise, samples collected from areas peripheral to the treatment site contained only very low radiocarbon levels.

Methanol extraction of the treated hair and skin and TLC analysis indicated that the residual R-20458-^{14}C underwent little degradation during the 7-day period after application (Figure 4). More than 90% of the residue was unchanged ethyl epoxide, and no single degradation product was present in appreciable quantity. Small amounts of the ethyldiene, ethylphenol, ethyldiol, and ethyl-6-hydroxy-2,7-diene were identified as components of the extract. No attempts were made to study the chemical nature of radiocarbon residues occurring in urine or feces of the dermally treated steer.

DISCUSSION

R-20458-^{14}C was rapidly absorbed and metabolized, and the phenyl-labeled materials were almost totally excreted after low-level oral exposure to a steer. This rapid metabolism and excretion by the steer was expected, based on earlier studies with laboratory rats and mice that showed R-20458 to be highly biodegradable (Gill et al., 1974; Hoffman et al., 1973).

Although most of the TLC-resolved metabolites occurring in the urine and feces of the treated steer were not characterized, the nature of the products that were identified indicates that the compound is metabolized by similar mechanisms in both laboratory rodents and ruminants. Thus, hydration of the 6,7-epoxide moiety, cleavage of the phenyl geranyl ether bond, and α-oxidation of the ethyl group are significant routes in the metabolism of R-20458 by rats and mice (Gill et al., 1974; Hoffman et al., 1973), and metabolites resulting from each of these biotransformation pathways were identified in the steer. However, no metabolites resulting from β-oxidation of the ethyl group were observed in the excreta of the steer. Certain of these products, some of which were available as metabolite standards in this study (Table I), were reported as minor metabolites in urine and feces of mice treated orally with low levels of R-20458 (Gill et al., 1974) and as major metabolites in excreta of rats treated intraperitoneally with R-20458 at very high doses (Hoffman et al., 1973).

Hoffman et al. (1973) identified several R-20458 metabolites in the feces of rats that contained a 6,7-double bond. However, they speculated that these products might have been a result of metabolism of the ethyldiene-^{14}C present as an impurity in the original R-20458-^{14}C preparation, since conversion of the 6,7-epoxide to an olefin is difficult to explain mechanistically. Diene metabolites of R-20458 were not detected in studies by Gill et al. (1974), but the current studies with a steer indicate that diene metabolites of R-20458 may indeed be generated in mammals. Although the ethyldiene-^{14}C was a minor contaminant (1%) in the R-20458-^{14}C preparation used in the current studies, it seems unlikely that the impurity could have accounted in total for the apparent ethyldiene observed in feces, as appreciable quantities of this metabolite were also seen in feces samples collected 2 and 3 days after the oral R-20458 treatment. The enzymatic conversion of an epoxide moiety to an olefin is apparently without precedence, and additional studies are underway in this laboratory which involve animal treatment with higher levels of purified R-20458 in an attempt to obtain spectral confirmation of these findings.

Several of the available metabolite standards (Table I) would seem likely R-20458 mammalian metabolites, but they were not observed in the current studies. However, it is probable that some of these products were generated as short-lived intermediates in the metabolism of R-20458 in the steer. Previous studies have shown that several compounds, including the ethyl diepoxide, aceto epoxide, α- and β-hydroxyethyl epoxides, and *cis*- and *trans*-ethyltetrahydrofurandiols, are formed during in vitro incubation of R-20458 with hepatic microsomal enzyme preparations of rats and mice, yet they are not observed in the excreta of living animals treated with R-20458. Thus, if these metabolites were formed in the steer, they would likely have undergone more extensive metabolism before being excreted.

Studies in which R-20458 was applied dermally to a steer indicated that the compound can be absorbed through the skin in appreciable quantity. Of the total applied ra-

diocarbon, more than 30% was absorbed within 7 days after treatment, and movement of the compound into the body was apparently continuing because appreciable amounts of radioactivity were still being eliminated in urine and feces at the time of sacrifice. About one-third of the absorbed radioactivity was eliminated through the feces, which strongly indicates that biliary excretion is involved to a significant extent in the elimination of R-20458 and/or its metabolites from the body. Previous studies with rats administered R-20458-^{14}C intraperitoneally have also demonstrated appreciable radiocarbon excretion through the feces (Gill et al., 1974; Hoffman et al., 1973). Although the proportion of excreted radiocarbon appearing in feces was considerably greater after the dermal treatment than after the oral dose, there is no reason to believe that accidental contamination of feces accounts for this difference. The treated areas of skin on the stanchioned animal were well forward of the area where fecal matter was deposited, so contamination from the treatment area seems unlikely. No attempts were made to define the chemical nature of the radiocarbon appearing in urine or feces of the steer after dermal treatment, but it seems probable that the dermal dose, once absorbed, would be metabolized much the same way as after oral exposure.

About 40% of the dermally applied radioactivity remained at the application site after 7 days. About 30% of the dose was unaccounted for, which presumably was loss by volatility, at least in part. TLC studies with the residual radiocarbon on the hair and skin 7 days after treatment indicated that R-20458 was quite stable under these conditions (Figure 4). Although low amounts of the ethyldiene were observed in the methanol wash of treated hair and skin, this almost certainly represents the ethyldiene impurity in the original treatment solution. It may be that the ethyl-6-hydroxy-2,7-diene that was observed in very minor quantities in the dermal wash (Figure 4) was not a breakdown product of the ethyl epoxide, but rather of the ethyldiene impurity. Studies by Gill et al. (1974) have shown that this allylic alcohol is a sunlight photodecomposition product of the ethyldiene, but not of the ethyl epoxide. Although the ethyl diepoxide is a photoproduct of the ethyl epoxide (Gill et al., 1974), the diepoxide was not observed in the hair wash, even though it was present in trace amounts in the original treatment mixture. It should perhaps be noted that the dermally treated animal was maintained indoors, under incandescent lighting, but received brief exposure to sunlight (<0.5 hr) immediately before sacrifice.

The current studies with steers have shown that R-20458 is a highly biodegradable compound in these animals and that it will not show appreciable tendency toward accumulation of residues within the body. Because this juvenile hormone mimic offers excellent control of certain insect pests and is exceedingly low in mammalian toxicity, it may well be a useful alternative to conventional insecticides in some situations.

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