THE UNIVERSITY OF MANITOBA
METABOLISM OF THE INSECT GROWTH REGULATOR, METHOPRENE BY JAPANESE QUAIL (*Coturnix coturnix japonica*)

by

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ABSTRACT

(5-^{14}C)-Methoprene was administered to Japanese quail at rates of 18-73 mg/kg body weight in three ways: as a single oral dose, as a single intraperitoneal injection, and as a continuous dose of 25 ppm in the daily diet. $^{14}CO_2$ expiration constituted 13-17% of the oral dose, and 6-13% of the intraperitoneal injection. ^{14}C in the excreta constituted 56.5% of the oral dose, 33.8% of the intraperitoneal injection, and 56.0% of the total quantity of methoprene ingested during the 192 hours of continuous feeding. ^{14}C in the excreta appeared largely associated with highly polar unidentified natural products. The remainder of the administered ^{14}C was found to reside in body tissues, with the quantity in whole liver reaching a maximum of 5.0% of the intraperitoneal injection, and the quantity per gram of subcutaneous fat never exceeding 1.5% by any route of administration. Eggs laid over the 192-hour period of continuous feeding of methoprene contained up to 10.4% of ingested ^{14}C.

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INTRODUCTION

The recent advent of insect growth regulators as marketable insecticides has necessitated the determination of their toxicity to non-target organisms, a phenomenon at present poorly defined. Two of the intended uses of the insect growth regulator, methoprene (Altosid®, isopropyl-(2E,4E)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate), are as a mosquito larvicide and as a feed-through fly-control agent in the manure of poultry and cattle. The deliberate feeding of methoprene to poultry, and the accessibility of methoprene to wild birds feeding on the maggots in cattle manure, make exposure of these birds to methoprene imminent. In the face of scanty information available on the metabolic fate of methoprene in birds, a study was undertaken to determine the ability of Japanese quail (Coturnix coturnix japonica) to cope metabolically with the various modes of entry of methoprene into the body--by single oral dose, single intraperitoneal injection, and continuous ingestion via the feed. Such information is basic to the determination of the toxicity of methoprene to birds, and serves as a basis for further studies.

LITERATURE REVIEW

Insecticidal Properties and Relative Toxicity of Methoprene

The insecticidal properties and toxicity of methoprene have been extensively characterized (Zoecon Corporation, a). Methoprene is an amber liquid, with a molecular weight of 310, specific gravity of 0.9261 g/ml, vapor pressure of 2.37×10^{-5} mm Hg at 25°C, and a water solubility of 1.39 ppm. It is commercially available in technical form, containing greater than 90% active ingredient (vide infra), or as:

(1) an emulsifiable concentrate, containing 3% active ingredient and formulated to provide 5 lb per gallon of formulation

(2) a 2% granular formulation

(3) a 5% slow release flowable formulation

(4) a 10% slow release formulation

(5) a 10% slow release flowable formulation

(6) a feed pre-mix formulation, containing 10% active ingredient.

Methoprene exists in several isomeric forms, the most active isomer and active ingredient being the 2E (2-trans) form (vide Appendix A, page 74)[1]. Its rapid breakdown following its use is due to degradation by dealkylation, deesterification, and oxidation to form the metabolites in Appendix A. The half-life of methoprene in water is less than 2 days, degradation being promoted by elevated temperature, sunlight and aquatic organisms. In soil, it does not leach below the surface layer,

[1]Unless otherwise stated, all references to methoprene in this dissertation are to the technical form (>90% active ingredient).

and has a half-life of less than 10 days at an exaggerated application rate of 1 lb/acre. Wheat does not accumulate residues from soil, and the half-life in alfalfa is less than 1 day. Under conditions of low moisture and limited sunlight, its half-life in stored grains is greater than 12 months (Zoecon Corporation, a).

The efficacy of methoprene on target insects is given in Appendix B. Its mode of action is to physiologically mimic natural juvenile hormone. Application to the developing insect embryo, nymph, larva or pupa results in retention of juvenile characteristics upon moulting, or formation of supernumerary intermediate stages. This leads to maturation as sterile adults, or death. Application of methoprene to normal adult insects induces sterility and reversion of the integument to the larval form. Its toxicity to target insects is order specific, and as a result, it is of low toxicity to non-target insects at low concentrations. The toxicity of methoprene to other non-target organisms ranges from greater than 0.1 ppm in estuarine mud crabs to an acute oral LD_{50} of greater than 34,000 mg/kg in the rat. Complete LD_{50}, LC_{50} and TL_{50} values are given in Appendix C. Twenty-one-day subacute inhalation of 20 mg/l had no effect on the rat. No teratogenicity or dominant lethal mutagenicity were observed at daily intakes of 1,000 and 2,000 mg/kg for five days, and no mammalian steroid mimicking activity occurred. Methoprene is not an optic or dermal irritant to rabbits. No effect was elicited during dermal exposure to 400 mg/kg for 21 days, nor was there any teratogenicity at a daily intake of 500 mg/kg. A dietary level of 30 ppm had no effect on the reproductive capacities of Bobwhite quail and mallard ducks (Zoecon Corporation, a).

Methoprene in the Aquatic Environment

Persistence and metabolism in water

Metabolic breakdown of methoprene in the aquatic environment is promoted by photodecomposition. Schaefer and Dupras (1973) demonstrated the persistence of methoprene in water to be dependent in part upon the degree of exposure to sunlight, and to a lesser extent on temperature. Tap water containing 0.1 ppm active ingredient (AI) lost 98% of this initial concentration either after eight hours of exposure to direct sunlight at 38°C, or after 120 hours of darkness at the same temperature. Similar results were obtained with distilled water. Under natural conditions, technical methoprene, the emulsifiable concentrate, and the 10% slow release flowable formulations applied at a rate of 0.1 lb AI/acre, formed a layer on the surface of ponds and pasture pools immediately after application, thereby enhancing exposure to sunlight. Application of the 10% slow release flowable formulation at a rate of 0.1 lb AI/acre resulted in an immediate concentration range of 0.022-0.096 ppm. After 24 hours, no methoprene was detectable.

Quistad et al. (1975d) found the half-life of 2 mg (5-^{14}C)-methoprene at a concentration of 0.5 ppm in sterile water exposed to sunlight to be less than one day. Fourteen days after commencement of exposure of the solution to sunlight, no methoprene (as the parent compound) was detectable, and twenty-one days after commencement of exposure to sunlight, 3.4% of the (5-^{14}C)-methoprene in the 0.5 ppm solution was recovered as $^{14}CO_2$. The dienoate moiety of methoprene was readily attacked and degraded by photocatalytic degradation to form

7-methoxycitronellic acid.

Because of the limited water solubility of methoprene (1.4 mg/l), and the subsequent difficulty in collecting sufficient of each photoproduct from the large amounts of treated water for detailed nuclear magnetic resonance spectral analysis, a larger mass of methoprene (400 mg) than the previous mass (2 mg) was exposed to sunlight in the form of an aqueous emulsion (Quistad et al., 1975d). Although the distribution of photoproducts in the 2 mg and 400 mg solutions differed, sunlight irradiation of 400 mg of methoprene in water for seven days produced four major photodecomposition products: 7-methoxycitronellal (representing 9% of applied (5-^{14}C)-methoprene), 7-methoxycitronellic acid (7%), methoprene epoxide (4%), and methoprene methyl ketone (4%), plus 46 other decomposition products each accounting for no more than 2%. No methoprene was detectable seven days after sunlight irradiation. In addition, Schaefer and Dupras (1973) obtained a 1:1 isomeric mixture of 2E and 2Z forms four hours after irradiating 0.1 ppm methoprene (70% 2E) in tap water.

To study microbial action, samples of natural and autoclaved pond water (pH = 8.3, BOD = 1.2 mg/l, COD = 8 µg/l, 450 mg sediment/sample) were treated with (5-^{14}C)-methoprene (97% 2E) to obtain a concentration of 0.65 ppm (Schooley et al., 1975a). After 312 hours of exposure to sunlight, no methoprene was detectable in the natural pond water, and only 48% of the applied label was left in the sediment and solution, of which 29% was 7-methoxycitronellic acid. The authors attributed the loss of label to ^{14}C-metabolite volatilization and evolution of $^{14}CO_2$ produced by catabolism. The autoclaved water sample contained 10-20%

of the applied material as methoprene, plus an additional 78% of the applied label still in solution. Thin layer chromatographic treatment of natural pond water containing 0.42 ppm (10-^{3}H)-methoprene and exposed to sunlight for 66 hours, gave three bands as follows (vide Appendix A, page 74, for structures):

(1) a 1:1 mixture of 2E:2Z methoprene (60% of the applied label) plus an ethyl-ester photoproduct of methoprene

(2) the hydroxy-ester and an hydroxy-ethyl-ester photoproduct of methoprene (7%), plus the methoxy-acid metabolite of methoprene (5.7%)

(3) the hydroxy-acid metabolite of methoprene (2.6%).

At the intended dose rates for mosquito control, 0.01 and 0.001 ppm AI methoprene in samples of pond water exposed to sunlight, had respective half-lives of forty and thirty hours.

Effects on and metabolism by target aquatic insects

Midges--Mulla et al. (1974) studied the toxicity of methoprene to several chironomid species of the Subfamily Chironominae, and species of the Subfamily Tanypodinae. Slightly higher concentrations of methoprene were required to produce 100% mortality in organophosphate resistant strains than in susceptible strains. Adult emergence of species of both subfamilies was completely inhibited with 0.1 ppm AI methoprene, using the emulsifiable concentrate or slow release flowable formulations. The 5% slow release formulation performed best, providing the greatest initial and overall mortalities, and the longest residual action. Four

separate applications of the 5% and 10% slow release flowable formulations of methoprene to a lake surface caused complete inhibition of adult emergence of Procladius spp., Chironomus spp. and Tanytarsus spp. for up to nine days post-treatment.

Black flies--Complete inhibition of adult emergence of black flies (Simulium spp.) was shown by McKague and Wood (1974), and Cummings and McKague (1973), using a 0.1 ppm concentration of the 10% slow release flowable and granular formulations of methoprene. Application of 10 ppm methoprene to Simulium pictipes (Hagen) larvae by Garris and Adkins (1974) resulted in 94% mortality within four days post-treatment. Pupation was totally suppressed at the 100 ppm level.

Mosquitoes--Methoprene produced 100% mortality of all larval instars of Culex pipiens fatigans (=quinquefasciatus) (Say), at concentrations of 1.0 ppm in water (Jakob, 1972; Schaefer and Wilder, 1972). According to Schaefer and Wilder, late fourth instar larvae were the most susceptible to methoprene exposed to sunlight, giving 100% mortality after 72 hours of sunlight exposure. Methoprene application to ponds at a rate of 0.25 lb AI/acre-surface produced 97% mortality of fourth instar larvae (Schaefer et al., 1973a). Dunn and Strong (1973) obtained 100% control of larvae of C.p. fatigans with polyurethane foam impregnated with 1% and 3% methoprene. Quistad et al. (1975c) showed the ability of larvae of C.p. fatigans to inactivate methoprene to nonactive polar residues, and suggested that O-dealkylation of methoprene is a more important metabolic reaction in the larvae than deesterification. Culex tarsalis (Coquillett) has been found to be less susceptible to methoprene than other dipterans tested so far (Schaefer and Wilder, 1973b). In

the presence of the synergist, triorthocresylphosphate (TOCP), larval mortality of C. tarsalis increased fivefold at 0.0001-0.001 ppm concentrations of methoprene (Quistad et al., 1975c).

The relative potency of methoprene to emerging adults of Aedes aegypti (Linnaeus) was observed to be 1,000 fold greater than that of natural juvenile hormone (Henrick et al., 1973). A. aegypti larval mortality was 100% in methoprene concentrations of 0.01-0.25 ppm (Jakob, 1972; Schaefer and Wilder, 1973). Quistad et al. (1975c) found fourth instar larvae of A. aegypti to be the stage most susceptible to methoprene applied to the water, ingesting ca. 30% of the dose in fifteen hours after treatment, and retaining this largely as unmetabolized methoprene and polar conjugated metabolites. Polar metabolites in the water increased with larval age. The older larvae were the most sensitive to an increased rate of methoprene metabolism, implying the presence of a bioactive metabolic product. None of the metabolites of methoprene (vide Appendix A, page 74) have been shown to possess this degree of larvicidal activity. The authors suggested the involvement of factors besides metabolism (e.g., selective cuticle permeability) in the regulation of the acute toxicity of methoprene. Schaefer and Wilder (1972, 1973b) observed an LC_{50} at 0.000008 ppm methoprene for Aedes nigromaculis (Ludlow), and 100% mortality of fourth instar larvae at a concentration of 0.001 ppm in the laboratory, and 0.5 ppm in field and pasture pools. Application of the 10% slow release flowable formulation at a rate of 0.1 lb AI/acre resulted in 100% mortality of all larval instars. Aedes melanimon (Dyar) and Aedes taeniorhynchus (Wiedeman) showed similar or greater susceptibilities to methoprene.

Jakob (1972) established LC_{95} values for third instar larvae of Anopheles albimanus (Wiedeman) and Anopheles stephensi Liston of 0.0025 ppm and 0.05 ppm respectively.

Effects on non-target aquatic animals and aquatic plants

Miura and Takahashi (1973, 1974) observed that a methoprene concentration of 0.1 ppm in tap water had no effect on algae (Pithaphora oedogonia (Mont.)Wittr., Spirogyra sp., Hydrodictyon reticulatum (L.) Lagerh., Anacystis sp.) and the diatom, Diatoma vulgare (Bory). Acute toxicity tests on several invertebrates revealed a high degree of tolerance to methoprene in Triops longicaudatus LeConte (LC_{50}^{24h} = 5.0 ppm) and other predators of mosquitoes, while Daphnia magna Straus was least tolerant (LC_{50} = 0.90 ppm). Aquatic dipterans, including Brachydeutera argentata (Walker) with a 70% mortality at 0.01 ppm, Chironomus stigmaterus Say and Pericoma sp., were highly susceptible to methoprene, while pond snails (Physa spp.) and Aulophorus sp. produced no mortalities at 100 ppm. There was no effect on daily population fluctuations of Daphnia magna and Cyclops sp. at a concentration of 0.1 ppm of the 10% slow release flowable formulation, nor on Corisella decolor (Uhler) and Notonecta unifasciata Guerin. Further treatment of N. unifasciata and Buenoa spp. three times over two months using the same formulation at an application rate of 0.1 lb AI/acre, had no effect. Field applications of 0.1 lb AI/acre of the emulsifiable concentrate and 10% slow release flowable formulations to ponds and irrigated pastures produced no effect on sixteen non-target invertebrates, including nematodes and oribatid mites.

Gomez et al. (1973) showed that methoprene concentrations of up to 0.50 ppm in seawater had no effect on the metamorphosis of the acorn barnacle Balanus galeatus (L.).

Miura and Takahashi (1974) found no mortality in mosquito fish, Gambusia affinis (Baird) Girard, subjected to two treatments of 0.1 lb AI/acre methoprene, applied on days one and fourteen of a forty-day trial.

Behaviour in an aquatic ecosystem

A study on the behaviour of methoprene in an outdoor model ecosystem was conducted by Bionomics Inc. (1973). Three applications of ^{14}C-methoprene were made over a fifteen-day period, to soil, bear rush plants (Rynchospora cephalantha) and bluegill fish (Lepomis macrochirus) in circular metal pools containing well water. Analysis of the ^{14}C-content in components of the ecosystem after each application showed a translocation of radiolabel from water to plants > fish > soil. As the post-treatment times after each application increased, ^{14}C concentrated in the soil, decreased in the fish, and remained constant in the plants. Characterization of ^{14}C-containing compounds in the fish on the twenty-eighth and forty-second days after the first application revealed the presence of small amounts of methoprene, its methoxy-acid metabolite and hydroxy-acid metabolite (Zoecon Corporation, a). Most of the compounds containing ^{14}C were highly polar and largely unextractable from fish tissue. The bulk of the extractable radiolabel was concentrated in the head, skeleton and fins, with lesser quantities in the edible muscle portions. This layer chromatography of these compounds did not

move them from the origin, and the compounds remained unidentified.

Methoprene in the Terrestrial Environment

Photodegradation of methoprene

Quistad et al. (1975d) observed a half-life of 6 hours for a 0.1 μ-thick film of technical methoprene (97.9% 2E) on glass, exposed to sunlight. After 27 hours, 13% of the initial methoprene was recovered in the vapor phase as 7-methoxycitronellal (4%), $^{14}CO_2$ (6%), methoprene (0.2%), and the remainder unidentified.

Plants contain naturally occurring photosensitizers. To evaluate their possible effect on the photodegradation of methoprene, Rose Bengal and anthraquinone were studied by the same authors for their effect as photosensitizers. Rose Bengal increased the rate of photolytic breakdown by 32%, to 56% in six hours, while anthraquinone increased the rate to 86%. Whereas 7-methoxycitronellal was a major photolytic product (10-14%) in the absence of photosensitizers, none was produced in their presence. Methoprene was stable to photodecomposition in methanol, showing only 10% decomposition in 25 days. In the presence of Rose Bengal, 47% of the 2E isomer was unreacted in four days, while 30% had formed the 2Z isomer.

Methoprene and target insects

Effects of methoprene--Miller and Eubel (1974) added various levels of methoprene to fresh manure from dairy cattle. At 0.5 ppm, 98.6% mortality occurred in face fly larvae, Musca autumnalis DeGeer. At 100 ppm, 97.4% mortality occurred in larvae of the house fly, Musca

domestica. Mortalities for the two fly species were 50% and 12%, respectively, in untreated manure. Administration of methoprene to dairy cattle via the feed for eight days resulted in a face fly mortality of 100% at the 2.5 mg/kg body weight level, and a house fly mortality of 80% at the 10 mg/kg body weight level.

Harris et al. (1973) using gelatin capsules, fed methoprene to cattle daily for fourteen days. The efficacy of methoprene in cattle manure seeded with laboratory-reared house flies, horn flies, Haematobia irritans (L.), and stable flies, Stomoxys calcitrans, is presented in Table I. In a subsequent study by Harris et al. (1974), ten Angus

Table I. Mortalities of horn flies, Haematobia irritans (L.), stable flies, Stomoxys calcitrans, and house flies, Musca domestica, seeded in the manure of methoprene-treated cattle (Harris et al., 1973)

Body weight of steer (kg)	Dose mg/day	Fly species	% Mortality
404	0.35	H. irritans	93
336	0.70	H. irritans[a]	100
336	10	S. calcitrans	52
336	100	S. calcitrans[b]	100
286	1000	M. domestica	72

[a]Still 100% effective three days after termination of feeding.

[b]Still 97% effective ten days after termination of feeding.

heifers and a bull were given access to mineral blocks containing methoprene at concentrations of 0.01, 0.12 or 0.94%. After nine weeks, the methoprene content dropped from 0.94% to 0.62%. Blocks maintained in storage lost slightly less. The average daily intake per animal was 640 mg of methoprene. The doses and resultant efficacies listed in Table I indicate that a daily intake of 640 mg methoprene is sufficient for control of H. irritans and S. calcitrans. In actual practice, the average number of flies per cow (ca. 270) was no different than on twenty Hereford heifers and a bull used as control animals, since the two herds were not isolated from each other. Alternate feeding of blocks containing 0.01 and 0.12% methoprene was insufficient for control of S. calcitrans, but was 100% effective on H. irritans.

Inhibition of development of the cattle biting louse, Bovicola limbata Gervais, was obtained by Chamberlain et al. (1973), using 50 ppm methoprene in the diet.

Strong and Diekman (1973) found 5 ppm methoprene in the feed to be fatal to the cigarette beetle, Lasioderma serricorne (F.), lesser grain borer, Rhyzoptera dominica (Fabricius), Indian meal moth, Plodia interpunctella (Hubner), almond moth, Cadra cautella (Walker), saw-toothed grain beetle, Oryzaephilus surinamensis (L.), and merchant grain beetle, Oryzaephilus mercator (Fauvel). It was not effective on immature stages of the confused flour bettle, Tribolium confusum Jacquelin duVal, red flour beetle, Tribolium castaneum (Herbot), or on adults of the granary weevil, Sitophilus granarius (L.), and rice weevil, Sitophilus oryzae (L.). It was also ineffective on any stage of the dermestid beetle, Trogoderma inclusum LeConte, at levels up to 50 ppm. Application

of methoprene to pear trees (Westigard, 1974) at a rate of 40-48 lb AI/tree had no effect on the number of adult pear psylla, Psylla pyricola Forster, per tree. The egg:nymph ratio increased, indicating a reduction in hatchability. Simultaneously, there was no effect on the two-spotted spider mite, Tetranychus urticae Koch, pear rust mite, Epitrimerus pyri (Nalepa), Metaseiulus occidentalis Nesbitt, or on predators of P. pyricola, the lacewing, Chrysopa sp., and Deraeocoris brevis piceatus Knight. Decreases occurred in the European red mite, Panonychus ulmi Koch, and codling moth, Carpocapsa pomonella (L.). Henrick et al. (1973) established ID_{50} values of 1.1 µg/pupa and 0.0054 µg/pupa for the greater wax moth, Galleria mellonella (L.), and yellow mealworm, Tenebrio molitor (L.) respectively. Yin and Chippendale (1973, 1974) found that increasing the juvenile hormone titre of the Southwestern corn borer, Diatraea grandiosella Dyar, by exposing it to methoprene caused a decrease in the number of larvae completing larval-pupal ecdysis, and further caused a sustained diapause. Application to mature, non-diapause larvae about to enter pupal ecdysis, caused them to revert in development. Troisi and Riddiford (1974) observed 100% mortality in colonies of the fire ant, Solenopsis invicta richteri Forel, exposed to 100 ppm methoprene in the feed. Methoprene affected caste differentiation, and the authors suggested that since the observed change was from worker to alate male ants, the effect was exerted either on the queen or on the sex of the resulting progeny.

Metabolism of methoprene--In a study conducted by Weirich and Wren (1973), it was concluded that haemolymph esterases of the tobacco hornworm, Manduca sexta Johannson, and of Tenebrio sp. were unable to hydrolyze

the isopropyl-ester group of methoprene. Only slight amounts of acid metabolite were formed when methoprene was present in excess. Quistad et al. (1975c) suggested that deesterification and O-dealkylation were major metabolic reactions of methoprene in larvae of the house fly, Musca domestica (L.). Two days after topical application of 0.36 μg to third instar larvae, 64% of the dose was metabolized, and an additional 24% of the biologically active 2E dose had been isomerized by the larvae to the less active 2Z form. The authors believed 2Z isomerization to be a major route of detoxification (the 2Z isomer being 1,000 times less active in Aedes mosquitoes than the 2E isomer).

The ability of methoprene to induce or inhibit metabolite-producing enzymes in Musca domestica was investigated by Terriere and Yu (1973). One percent methoprene in the diet of adult females for three days slightly induced heptachlor epoxidase, and had no effect on the levels of p-nitroanisole O-demethylase and DDT-dehydrochlorinase. After topical treatment of pupae, the authors found no correlation between the morphogenetic potency of methoprene and its induction or inhibition of metabolizing enzymes, in relation to the other juvenilizing compounds examined: juvenile hormone of the Cecropia moth, Hyalophora cecropia (L.)[1]; MDP-Juvenile hormone[2]; piperonyl

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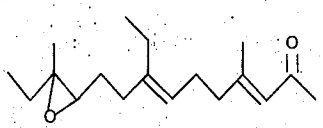
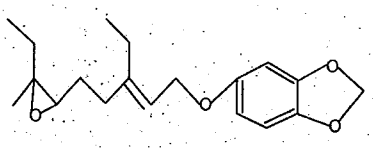
[1]Methyl-10,11-epoxy-7-ethyl-3,11-dimethyl-2,6-tridecadienoate.



[2]6,7-epoxy-3,7-diethyl-1-(3,4-(methylenedioxy)phenoxy)-2-octene.



butoxide[1]; and ethyl-3,7,11-trimethyl-2,4-dodecadienoate[2]. Extensive examination of the effects of methoprene on microsomal enzymes revealed it to be a poor inducer of enzyme activity, and because of its high stability *in vivo*, its potency as a morphogenetic agent is directly proportional to its inability to act as an inducer. The involvement of cytochrome P_{450} in the oxidative metabolism of both the 2*E* and 2*Z* isomers was suggested by the 66-77% decrease of *in vitro* metabolism of methoprene by microsomes after their treatment with carbon monoxide. In the presence of paraoxon, a potent inhibitor of β-esterases, there was no change in the extent of methoprene metabolism, indicating a lack of esterase involvement. Flies treated with phenobarbital, dieldrin or juvenile hormone from *Hyalophora cecropia* prior to microsomal extraction, produced microsomes with much greater methoprene-metabolizing ability than untreated flies.

Synergism and antagonism of methoprene--Solomon and Metcalf (1974) found varying degrees of synergism and antagonism of methoprene in insects. A 200-fold range in concentration existed between minimum and maximum responses of fifth instar nymphs of the yellow mealworm, *Tenebrio molitor*. This range was not a result of differences in rates of inactivation of methoprene. The activity was only slightly

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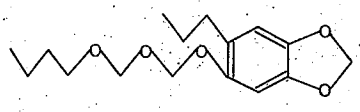
1



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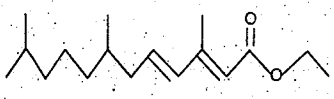


synergized by piperonyl butoxide or TOCP. The two synergists promoted the formation of an unknown product, less in amount than only methoprene. In addition, TOCP promoted O-demethylation of methoprene to increase the level of hydroxy-ester metabolite; piperonyl butoxide blocked the reaction. By contrast, in identical tests carried out on fifth instar nymphs of the milkweed bug, Oncopeltus fasciatus (Dallas), a twenty-fold concentration range existed between minimum and maximum responses to treatment. Piperonyl butoxide and TOCP severely antagonized methoprene by suppressing the maximum obtainable response over the twenty-fold concentration range. In the absence of the two compounds, the hydroxy-ester metabolite was four times as active as methoprene. The authors suggested that, since the antagonists strongly inhibited the juvenilizing activity of methoprene, the formation of hydroxy-ester metabolite was being blocked, and that methoprene acted by its activation to the hydroxy-ester metabolite by O-demethylation. Since the activity of the hydroxy-ester metabolite was not decreased by the two antagonists, they further suggested that its formation was a final activation process. The hydroxy-acid metabolite was six times more active than the methoxy-acid metabolite, also indicating that metabolism to the hydroxyl moiety was important to the activity of methoprene.

Quistad et al. (1975c) found piperonyl butoxide and TOCP to have a slight synergistic effect on larvae of Musca domestica. Applications of piperonyl butoxide (100 µg/g) along with methoprene (0.15 µg/g) to larvae increased larval mortality fourfold to 87%.

Resistance to methoprene--Cerf and Georghiou (1972), Jakob (1973), and Plapp and Vinson (1973) tested methoprene against susceptible and

resistant strains of Musca domestica, producing the results in Table II. Cerf and Georghiou stated that pupae require higher doses for mortality than larvae or adults, and suggested that a low sensitivity to juvenile hormone was associated with a high capacity for metabolism and elimination of xenobiotics. This is supported by Plapp and Vinson, who found cross-resistance to be highest in strains with high microsomal oxidase activity and high DDT-dehydrochlorinase activity. Terriere and Yu (1973) verified this by subjecting microsomes from high oxidase-containing strains and DDT-resistant strains of larvae and adults to methoprene. These strains possessed a high metabolic capacity, with the high oxidase-containing strain metabolizing methoprene at twice the rate of low oxidase-containing strains, in both larvae and adults. The authors believed resistance to methoprene to be possible among some strains of Musca domestica.

Metabolism of methoprene in soil and plants

Schooley et al. (1975a) treated sandy loam (aerobic, anaerobic, and autoclaved) soil and silt loam (aerobic) soil with (5-^{14}C)-methoprene in the laboratory at an application rate of 1 kg/ha. The order of disappearance of radiolabel from the different soil treatments, the half-lives of methoprene, and the degree of degradation of methoprene on days seven and sixty post-treatment are given in Table III.

Sandy loam (aerobic) soil was exhaustively analyzed for methoprene recovery, $^{14}CO_2$ production, content of extractable metabolites, quantities of bound ^{14}C compounds in soil (humic and fulvic acids), and quantity of unextractable ^{14}C-residue (humin fraction). On the fourteenth

Table II. Cross-resistance to methoprene in Musca domestica. Susceptibility of different stages of different strains (Cerf and Georghiou, 1972; Jakob, 1973; Plapp and Vinson, 1973)

Strain	Stage	Dose	% Mortality
		ppm in diet	
NAIDM - susceptible	first larval instar	10	100
Bethesda - malathion-resistant	"	50	100
Thompson - dimethoate/organophosphate-resistant	"	125-150	92
Roberds - DDT/dieldrin-resistant	"	10	100
Rutgers - diazinon/organophosphate-resistant	"	50	100
		µg/vial[a]	
Orlando regular - susceptible	third larval instar	0.02	
Stubby wing; brown body; ocra eye - susceptible	"	0.11	
R-Fc - DDT/carbamate/organophosphate-resistant	"	0.15	
R-Baygon; brown body; ocra eye - carbamate/organophosphate/DDT/cyclodiene-resistant	"	0.63	
		µg/pupa	
NAIDM - susceptible	pupal	0.25	100
DDT/lindane-resistant	"	"	100
parathion-resistant	"	"	78
chlorthion-resistant	"	"	71
fenthion-resistant	"	"	64
OMS-12-resistant	"	"	30
OMS-15-resistant	"	"	19
dimethoate-resistant	"	"	14

[a]Values are LC_{50}.

Table III. Persistence of ^{14}C-residues and degradation of methoprene in treated and untreated soils (Schooley et al., 1975a)

Soil type	Half-life in soil (days)	% Degradation of methoprene	
		Day 7	Day 60
Sandy loam (anaerobic)	7	15	32
Silt loam (aerobic)	11	8	85
Sandy loam (aerobic)	12	8	60
Sandy loam (autoclaved)	43	0	5

day post-treatment, 30% of the applied radiolabel was contained in the hydroxy-ester metabolite of methoprene, and 35% was contained in $^{14}CO_2$. On the sixtieth day post-treatment, the $^{14}CO_2$ collected was 56% of the applied radiolabel, the unextractable fraction contained 56% of the applied radiolabel, bound residues contained 9% of the applied label, and extractable methoprene and metabolites decreased almost to zero. In the sixty days of testing, greater than 50% of the radiolabel was recovered as $^{14}CO_2$. The authors suggested that the lack of presence of metabolites and the rapid rate of disappearance of methoprene, indicate that degradation of metabolites of methoprene was as rapid as that of methoprene itself. On day 35 post-treatment, fulvic acid contained 2.5% of the applied radiolabel; on day 45, humic acid contained 9%; and on day 60, methoprene metabolites constituted 1% of the applied label.

Quistad et al. (1974b) applied (5-^{14}C)-methoprene as a 77%

emulsifiable concentrate to alfalfa and rice at a rate of 11 $\mu g/cm^2$ of leaf (ca. 1 lb/acre). The plants were maintained in dim indoor light, and samples were taken for thirty days. The half-life of methoprene on alfalfa was 2 days, and on rice it was 0.5 days. Three days after treatment, the primary nonpolar metabolites found in rice plants were the hydroxy-ester (0.1%), 7-methoxycitronellic acid (0.2%), the methoxy-acid metabolite (0.9%), and 7-hydroxycitronellic acid (0.9%). No hydroxy-acid metabolite was detectable. The metabolites were maximized by three days post-treatment, and became stabilized by seven days at somewhat lower levels. There was 5% isomerization of the 2E form to the 2Z form in three days, and 14% in seven days. Analysis of radioactive constituents on the seventh day revealed an extensive degradation of methoprene, with subsequent incorporation of the radiolabel into high molecular weight, lipophilic natural products in the plant. In rice, chlorophyll contained 0.8% of the applied radiolabel, and a yellow carotenoid contained 1%. In alfalfa, similar quantities were found in seven yellow and green natural plant pigments. Of the 10-20% residual ^{14}C, up to 1% was contained in ^{14}C-glucose and ^{14}C-cellobiose. Enzymatic cleavage of polar conjugates produced the hydroxy-acid metabolite (7.4%) in quantities greater than the non-conjugated metabolite, as well as the hydroxy-ester metabolite (2.2%), 7-methoxycitronellic acid (0.8%), and 7-hydroxycitronellic acid (3.1%). Loss of label by volatilization in rice after four days amounted to 13% of the applied material as 7-methoxycitronellal, and 0.7% as $^{14}CO_2$. After seven days, 14% was lost as methoprene. Loss by volatilization from alfalfa was 7% of the

applied material after seven days. Fifteen days after treatment, 0.4% of the applied dose was recovered as methoprene in rice, and thirty days after treatment, 1% was recovered as methoprene in alfalfa. No label was found in the roots of rice.

Effects on and metabolism by mammals

Siddall and Slade (1974) subjected Swiss-Webster mice to 5,000 mg/kg methoprene in a single gastric lavage, using vegetable oil as a carrier. They subsequently observed no lesions of the lungs, heart, endocrine organs, liver and kidneys on gross inspection. Glucose, blood urea nitrogen, sodium, and alkaline phosphatase levels were all normal, as were hemoglobin, hematocrit, total leucocyte and differential blood cell counts. There was no effect on weight gain. Methoprene was a mild skin irritant to rats at a topical application rate of 10,200 mg/kg, and exhibited mild irritation to the conjunctiva of rabbits. The dermal and optic LD_{50} in rabbits was greater than 5,000 mg/kg.

Chamberlain et al. (1975) administered 50.86 mg (5-^{14}C)-methoprene (92 μCi) to a guinea pig weighing 1,050 g. $^{14}CO_2$ collected over a 24-hour period post-dose contained 17.2% of the administered radiolabel, urine 24.3%, and feces 9.1%. Component analysis of urine by thin layer chromatography using benzene:ethyl acetate:acetic acid (100:50:5) revealed an hydroxy-acid metabolite content of 2-4%, a combined hydroxy-ester and methoxy-acid metabolite content of 0.5-1%, and a methoprene content of less than 0.1%. Upon cleavage of urine conjugates with β-glucuronidase, these values became 7.1% for the hydroxy-acid metabolite, 8-10% for the combined hydroxy-ester and methoxy-acid metabolites,

and 0% for methoprene. The elevated metabolite values indicated a large degree of biliary conjugation of metabolites. Fecal analysis produced 2-3% hydroxy-acid metabolite, 8-10% combined hydroxy-ester and methoxy-acid metabolites, and 79% methoprene.

Subsequent to the guinea pig study, the same researchers administered 2 g of (5-^{14}C)-methoprene, containing 3.9 mCi, to a Hereford steer weighing 277 kg. The steer was maintained in a metabolism unit for fourteen days, during which time $^{14}CO_2$ expiration, and ^{14}C-contents of urine and feces were recorded. Tissue samples were taken upon sacrifice, fourteen days post-dose. Total radiolabel recovered as $^{14}CO_2$ in the fourteen days of recording was 22% of the dose, with peak production thirty hours post-dose.

In the steer study, the total radiolabel recovered in urine in the fourteen days post-dose was 22% of the dose, and in feces was 39% of the dose. Peak excretion of label in urine occurred 24-36 hours post-dose. In this time interval, 18% of the dose was excreted in urine. Thin layer chromatographic analysis of a 24-hour urine sample using benzene:ethyl acetate:acetic acid (100:50:5), produced very little methoprene, 1.5% of the total 24-hour radiolabel content as hydroxy-acid metabolite, and 0.9% of the total 24-hour radiolabel content as combined hydroxy-ester and methoxy-acid metabolites. B-glucuronidase treatment of the urine elevated these metabolite values to 3.6% and 7.7%, respectively. In contrast to urinary excretion, peak excretion of radiolabel in feces occurred 36-48 hours post-dose. Feces collected during this time interval contained 19% of the dose, and consisted of large amounts of methoprene and its metabolites. As the post-dose time increased

from zero to 48 hours, the quantities of fecal methoprene and combined hydroxy-ester/methoxy-acid metabolites recovered from thin layer chromatographic plates also increased, reaching peak values of 42% and 23% of the radiolabel extracted from 48-hour feces. The hydroxy-acid metabolite comprised 20% of the radiolabel extracted from feces sampled 24 hours post-dose.

Steer tissues examined by Quistad et al. (1975a) were blood, gall bladder, kidney, adrenal, spleen, lung, kidney fat, subcutaneous fat, muscle, bone, and liver. Total recovery of radiolabel from all tissues except blood on the fourteenth day was 15% of the dose. The blood level reached a maximum at 72 hours post-dose (4 µg methoprene equivalents/ml), dropping to half this value eleven days later. The order of magnitude of radiolabel associated with some of these tissues was lung > liver > subcutaneous fat > muscle. ^{14}C-Cholesterol made up 16-88% (depending on the tissue) of the nonpolar metabolite extraction fraction. The order of magnitude of ^{14}C-cholesterol content was lung > subcutaneous fat > liver > muscle. Less than 0.1 ppm methoprene or any primary metabolite was present in subcutaneous fat, muscle, liver, lung and blood in the fourteenth-day tissue samples. A lipophilic moiety, less polar than methoprene, was also found to be 11-33% of the nonpolar metabolite fraction, and was contained in tissues of the lung > muscle > liver > fat. In blood, this moiety contained radiolabeled cholesteryl esters of linoleic, myristic and palmitic fatty acids. Greater than 98% of the label was associated with the sterol of the conjugated fatty acids. Of the 52% unextractable ^{14}C-residue from the liver, 83% was extracted upon treatment with Pronase, of which the authors believed

75% to be associated with amino acids. Bile contained 72% of the fourteenth-day radiolabel predominantly as cholic acid (39%) and deoxycholate (32%), with some cholesterol (1%). In an effort to verify (5-^{14}C)-methoprene incorporation into natural products, Quistad et al. (1974a) degraded ^{14}C-cholesterol and ^{14}C-deoxycholate to randomly labeled ^{14}C-acetate, 1-^{14}C-acetate and 2-^{14}C-acetate. The bulk of the labeled acetate was present as 2-^{14}C-acetate, derived by α, β, β-oxidation of methoprene in a sequence similar to the degradation of branched fatty acids. Random labeling and 1-^{14}C-labeling constituted 20% of the total ^{14}C-acetate.

Quistad et al. (1975b) gave a lactating cow 0.74 mCi of (5-^{14}C)-methoprene. A milk sample was taken 44 hours post-dose for analysis, and blood taken at 48 and 168 hours. Tissue samples were taken upon sacrifice 168 hours after dosing. The 44-hour milk sample contained 8% of the dose, of which 32% was identifiable as:

(1) 22% radiolabeled fatty acids (2.1% saturated, 9.5% monoenoic, 8% dienoic but not methoprene)

(2) 2.5% radiolabeled casein

(3) 3.8% radiolabeled lactalbumin

(4) 1% radiolabeled methoprene

(5) 2.7% radiolabeled lactose

(6) 64% unidentifiable ^{14}C-residue in polar products and aqueous phases produced in the various analytical procedures.

The 48-hour blood sample contained 18,400 dpm/15 ml. Of this, 8% was randomly labeled ^{14}C-acetate, 11% was volatile fatty acids, and 78% was associated with precipitated proteins. The 168-hour blood sample

contained 85,600 dpm/100 ml. Precipitated proteins contained 88.4% of this (versus 56% for the steer, fourteen days after dosing). The organic extract contained 7.8%, of which 68% was cholesterol, and 18% cholesteryl esters. The aqueous portion of extracted blood contained 3.8% of the label in the 168-hour blood sample.

Effects on quail reproductive capacity

Bobwhite quail, Colinus virginianus, were given 3 or 30 ppm methoprene in the diet for twenty weeks in a study conducted by Hazleton Laboratories, Inc. (1973). No effects were observed on body weights taken during the first ten weeks of feeding, nor at the termination of the experiment. Similarly, no differences from controls were noted in the number of eggs laid by birds receiving methoprene, nor in the number of eggs cracked or embryonated. The eggshell thickness, the number of live three-week-old embryos, the percent hatch, and the number of fourteen-day-old chicks surviving were also the same as controls.

EXPERIMENTAL

Test Species--Rearing and Maintenance

Experiments in this study were conducted with Japanese quail (Coturnix coturnix japonica), which were multigeneration progeny of a breeding colony maintained in the Department of Entomology, University of Manitoba. Eggs collected from the breeding colony were held in a Crelab model 1212 biological cabinet at 15°C for up to ten days. The eggs were incubated in a laboratory-constructed incubator, with a fully automated, four-cycle, egg-turning system, a thermostatically controlled atmospheric temperature of 36.0 ± 0.5°C, and a relative humidity of 85%. On the sixteenth day of incubation, the eggs were transferred to perforated metal cages within the incubator, and the relative humidity elevated to 90% in preparation for the ensuing hatch.

Newly-hatched chicks were placed in a wooden brooder measuring 6 ft long, 2 ft wide and 1 ft high (Figure 1), where they were maintained for 3½-4 weeks. Water and a basal diet of commercial chick starter crumbles (Feed-Rite Mills, Ltd.), containing 21% protein, 2% fat and 5% fibre were provided ad libitum. A brooder temperature of 39°C, and an elevated humidity during the first week post-hatch were gradually reduced to ambient levels by the fourth week. At four weeks of age, the birds were transferred to a Petersyme brood-unit (model 2SD) pending experimentation, or were immediately subjected to preconditioning for subsequent testing.

Figure 1. Brooder used to rear newly-hatched quail chicks

Experimental Materials

In all experiments the quail were fed a diet of commercial chick starter (vide supra, p. 27). Birds were housed in a Petersyme brood-unit for acute toxicity, and oral or intraperitoneal dosing studies. All other experiments were conducted by isolating individual birds in rat cages (Hoeltge, Inc.), measuring 9½ inches long, 7 inches wide and 7 inches high.

Technical grade methoprene (94.8% 2E) was used in all experiments. The radiochemical purity of (5-^{14}C)-methoprene (specific activity 58 mCi/mmol) used in metabolic studies was either 97.9% or 95.3% 2E. Labeled and unlabeled methoprene and its chromatographically pure

metabolites were provided by the Zoecon Corporation, Palo Alto, California. Other chemicals were of reagent, pesticide or scintillation grade. Solvents were redistilled prior to use in thin layer chromatography.

Procedures in the metabolism studies involving trituration of collected excreta or tissue homogenization were carried out with a Sorvall omni-mixer, model 17150 (Ivan Sorvall, Inc.). Extraction of ^{14}C-constituents from excreta and tissues was accomplished with the aid of micro- and macro-soxhlet extraction tubes, fitted with either West or Allihn condensers. Whatman single-thickness cellulose extraction thimbles were used in all soxhlet extractions. A model VE50 rotary evaporator (Rinco Instrument Co., Inc.) assisted in concentrating organic extracts. Thin layer chromatographic plates employed in the identification of ^{14}C-constituents in excreta were laboratory-prepared with silica gel GF_{254} (Brinkmann Instruments, Ltd.). Solubilization of fat and liver tissue samples was carried out with NCS Tissue Solubilizer, 0.6N in toluene (Amersham/Searle Corp.), utilizing a Temp-Blok Module Heater, model 2090 (Lab-Line Instruments, Inc.).

Preliminary Investigations

Acute toxicity determination

An experiment was conducted to establish the acute oral LD_{50}^{48h} of methoprene. Six-week-old quail were separated into five groups, each consisting of three males and three females. A single oral dose of methoprene was administered to each bird via a gelatin capsule (Eli Lilly and Co.), at intakes from 625 to 5,000 mg AI/kg (vide Table IV).

Table IV. Single oral administration of technical methoprene to six-week-old Japanese quail. Average weight of male = 90 g. Average weight of female = 100 g. Parameters of response are described in text

Dose (mg/kg)	Response
control	no effect
625	no effect
1,250	no effect
2,500	no effect
5,000	no effect

Control birds were given empty gelatin capsules. Food and water were provided ad libitum. Over a period of 48 hours post-dose, the birds were observed for visible symptoms of toxicity, manifested by a change in food and water consumption, nervousness, hypersensitivity to noise, tremoring, ataxia, convulsions, blindness or death. The birds were subsequently sacrificed.

No quail died during the 48-hour test period, and no visible symptoms of toxicity were observed up to a maximum dose of 5,000 mg/kg (vide Table IV).

Establishment of experimental dose

Due to the apparent lack of visible response observed in the acute toxicity determination, a level of methoprene toxicity could not be established upon which metabolism studies could be based. At the time

of initiation of these studies, it was thought that a methoprene concentration of 25 ppm in the daily diet of poultry was required to obtain feed-through fly control in poultry manure (cf. Appendix B, page 75). Also, an output concentration of 25 ppm was required for feed-through fly control in cattle manure (Zoecon Corporation, 1974). Based on the premise that this concentration was intended to appear in the diet of galliform birds, and in the manure of cattle which galliform birds have access to when feeding on maggots, all subsequent experiments on Japanese quail (as galliform birds) were carried out with a maximized dose rate of 25 mg/kg body weight.

Metabolism Studies

Part 1. Methods

Three methods of (5-^{14}C)-methoprene administration were utilized in determining its metabolic fate in the quail: (1) single oral dosing; (2) single intraperitoneal injection; and (3) continuous ingestion with the feed. After the administration of an oral or intraperitoneal dose of methoprene, or after initiation of its ingestion by continuous feeding, total ^{14}C was quantitated from excretory and expiratory exit routes, and in body tissues. The presence of $^{14}CO_2$ in the expired air of a guinea pig and a steer (vide page 22 et seq.), and in chickens (Zoecon Corporation, 1974) prompted the same analysis in quail. Outputs of ^{14}C in urine and feces were determined collectively as outputs in excreta, since colostomy of the minute ureter in Japanese quail is a delicate surgical procedure. ^{14}C in the excreta was characterized and quantitated

as methoprene and four of its metabolic products: (1) the hydroxy-ester metabolite; (2) the methoxy-acid metabolite; (3) the hydroxy-acid metabolite; and (4) methoxycitronellic acid (vide Appendix A, page 74). The tissue ^{14}C content was measured in subcutaneous fat samples, liver, whole eggs, and collectively in all other body tissues. The procedure followed in examining ^{14}C excretion and tissue deposition is presented in Figure 2.

Preparation and administration of doses

Three preparations of methoprene were administered to the quail:

(a) encapsulated oral doses, each containing a weighed quantity of methoprene, and each corrected for variations in the weights of replicate birds

(b) stock intraperitoneal injection solutions

(c) methoprene-spiked feed for continuous ingestion by the quail in the daily diet.

Each of the preparations were made up within 24 hours of use, and stored at 4°C until administered to the quail.

Oral doses were prepared in gelatin capsules. Bird weights were taken, and volumetric amounts of (5-^{14}C)-methoprene in benzene, containing the desired quantities of ^{14}C, were dispensed into capsules. The benzene carrier was driven off with dry nitrogen gas, and appropriate quantities of technical grade methoprene were weighed into the capsules to make up a 25 mg/kg dose. The capsules were force-fed to the birds, and washed down with water. Control birds were each given empty capsules.

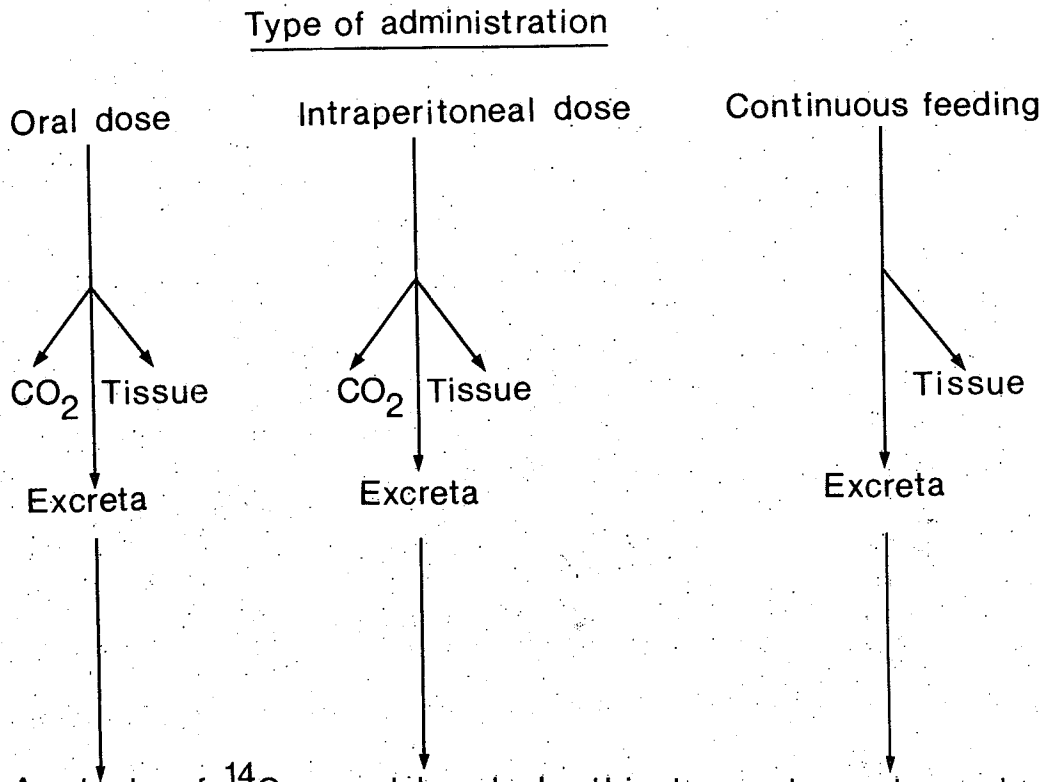


Figure 2. Procedure used to determine ^{14}C excretion by and tissue deposition in Japanese quail administered (5-^{14}C)-methoprene

Stock injection solutions for intraperitoneal administration were prepared in septum vials by: (1) dispensing (5-^{14}C)-methoprene in benzene into the vial; (2) removing all but a trace of the benzene under dry nitrogen gas; (3) adding technical grade methoprene, followed by a 2% (w/v) surfactant mixture of polyethylene sorbitan mono-oleate (Tween 80) in physiological saline; (4) evaporating the trace of benzene under a gentle stream of nitrogen gas; and (5) shaking to emulsify. Intraperitoneal injections were made with glass Luer-Lok hypodermic syringes, using 24-gauge stainless steel needles. Prior to each withdrawal from the stock injection solution, the vial was shaken vigorously for 30 seconds to ensure emulsion uniformity. Extreme care was exercised in the weighing and delivery of accurate doses into the peritoneal cavity. Control birds received 0.5 g injections of 2% Tween 80 in physiological saline.

Spiked feed, used for continuous feeding studies, was prepared by: (1) triturating a sample of the feed ration (vide page 27) to a coarse powder; (2) blending the powder with acetone (75 ml acetone in 100 g powder), containing technical and (5-^{14}C)-methoprene; (3) air drying to evaporate the acetone, and retriturating the spiked feed sample; and (4) mixing the sample with a bulk sample of triturated feed. All birds were preconditioned on untreated, but triturated feed for *ca*. seven days prior to each experiment. Upon initiation of each trial, the untreated feed of the test birds was replaced with treated feed. The daily intake of feed by all birds in each trial was determined by weighing each feeder every 24 hours.

The administered doses of methoprene varied with the weight of the

bird and route of administration. The oral doses given to the individual birds ranged from 19-73 mg AI/kg, containing 3.65-4.37 μCi. Intraperitoneal doses varied from 19-46 mg AI/kg, containing 2.19-3.38 μCi. In continuous feeding experiments, the average daily intake of methoprene per bird ranged from 0.2454 mg AI containing 0.003 μCi, to 0.8127 mg AI containing 0.006 μCi.

The specific activities of the stock injection solutions were determined by placing an accurately weighed amount of each solution in a scintillation vial, adding 10 ml dioxane scintillation fluid, and liquid scintillation counting (LSC). Quantities of ^{14}C in treated feed preparations were determined by the Biochemistry Section of the Zoecon Corporation. Samples of feed were mixed with an equal weight of cellulose powder, pelletized, and totally combusted in an oxygen flask sample oxidizer (Packard Instrument Co., model 305). The $^{14}CO_2$ evolved as a product of combustion was trapped in ethanolamine, and quantitated by LSC (vide infra).

Radioassay techniques

In all metabolic studies, ^{14}C was quantitated with the aid of a Nuclear-Chicago Mark II liquid scintillation counter (Nuclear Chicago Corp.). The scintillation fluid used for most ^{14}C quantitations consisted of 0.5% 2,5-diphenyloxazole (PPO), 0.01% 1,4-Bis(2-(5-phenyloxazoyl))benzene (POPOP), and 12% naphthalene in 1,4-dioxane. For radioactive samples not soluble in this scintillation fluid, or in order to obtain a higher efficiency of counting, a scintillation fluid consisting of 0.5% PPO and 0.03% POPOP in toluene was used. Efficiencies

of counting varied from 32-93%, and quench corrections were made by external standard ratio or channels ratio methods. Methanol (1-5 ml) was used as a water solubilizing agent in high moisture $^{14}CO_2$-ethanolamine solutions, and to desorb ^{14}C-containing compounds from the silica gel of thin layer chromatographic plates, prior to the addition of scintillation fluid.

$^{14}CO_2$ in ethanolamine, as a product of combusted ^{14}C in feed and excreta, was quantitated by the addition of Permafluor $^{T.M.}$ scintillation fluid (Packard Instrument Co.), and counting in a Tri-carb liquid scintillation spectrometer (Packard Instrument Co.).

Measurement of $^{14}CO_2$ evolution

Expired $^{14}CO_2$ was quantitated from four-week-old female quail given oral doses and five-week-old birds given intraperitoneal doses of (5-^{14}C)-methoprene. Two birds (average weight 89 g) were placed in separate metabolism units (Figure 3) modeled after Findlay (1969), for a twelve-hour conditioning period. The units contained food and water, and were kept in semi-darkness to dampen external stimuli. Each unit had a steady incoming flow of house-compressed air at a rate of 105-135 ml/min, and an outgoing flow of expired gas. After twelve hours, the birds were each orally dosed with ^{14}C-methoprene (24 mg AI/kg, 4.37 µCi), and immediately returned to the metabolism units. The expired gases in the two units were simultaneously and independently monitored by bubbling parts of the effluents through fresh 5 ml aliquots of ethanolamine for two-minute intervals at selected periods of time. Expired carbon dioxide not passed through the ethanolamine in the monitor

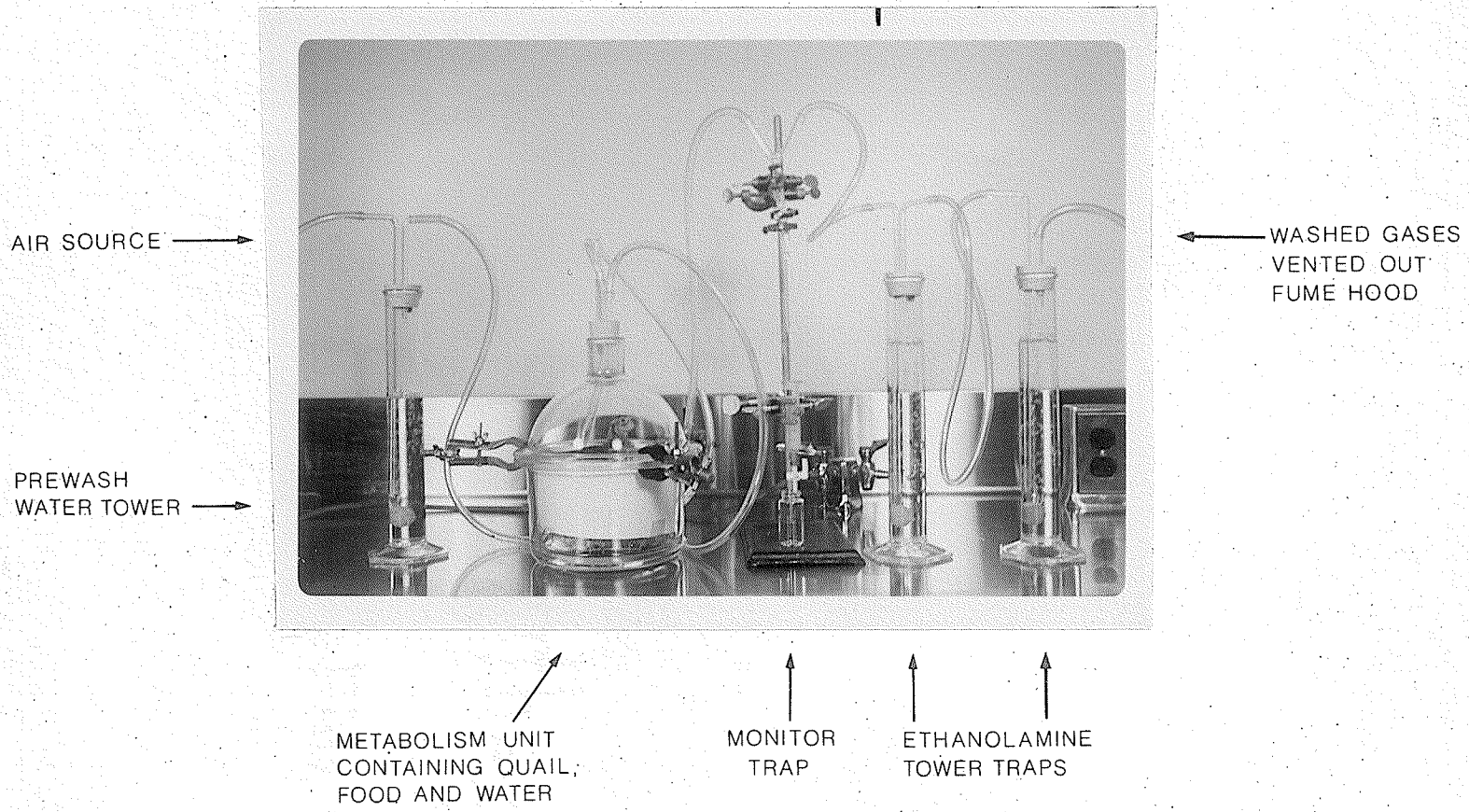


FIGURE 3. APPARATUS USED IN THE COLLECTION OF EXPIRED $^{14}CO_2$ FROM JAPANESE QUAIL (FINDLAY, 1969)

trap was trapped by bubbling through two consecutive towers, each containing 150 ml ethanolamine. The towers were changed twice during the four-day trail. The radioactivity collected in the towers was quantitated by adding 10 ml dioxane scintillation fluid to 5 ml aliquots of the $^{14}CO_2$-ethanolamine solution, followed by LSC. Those $^{14}CO_2$-ethanolamine solutions not soluble in the scintillation fluid because of their high moisture content, were solubilized with 1-5 ml methanol. The same procedure was used for the two birds (average weight 82 g) dosed intraperitoneally with methoprene, one at 19 mg AI/kg (2.87 μCi), and the other at 24 mg AI/kg (3.35 μCi).

Quantitative assessment of ^{14}C in excreta

Twelve, five-week-old female quail (average weight 72.6 g) were preconditioned for three days in individual rat cages containing food and water. Four birds subsequently received an average methoprene oral dose of 33 mg AI/kg (3.81 μCi), four others were given an average intraperitoneal injection of 35 mg AI/kg (0.381 μCi), and the remaining four controls each given an empty gelatin capsule orally or an intraperitoneal injection of 2% Tween 80 in physiological saline. Food and water were provided ad libitum, and the birds were weighed every 24 hours. Total quantities of excreta were collected 2, 4, 6, 8, 24, 48, 72, 96 and 120 hours post-dose, weighed, air-dried, reweighed, and triturated to a coarse powder. Accurately weighed subsamples (ca. 0.5 g) were then subjected to a ten-cycle soxhlet extraction using 10 ml methanol. The extracts were quantitatively transferred to scintillation vials, and after reducing the extract volume to ca. 5 ml under a

gentle stream of nitrogen gas, dioxane scintillation fluid (10 ml) was added and the ^{14}C content determined by LSC.

This procedure was repeated with four, five-week-old female quail (average weight 69.2 g) receiving methoprene continuously via the feed (25 ppm AI, 0.003 μCi/g), and four more as controls. The average daily ingestion of methoprene per bird ranged from 0.2454-0.4068 mg AI. Excreta were collected every 24 hours for 192 hours, and analyzed as above.

Unextractable ^{14}C in the excreta was quantitated by the Biochemistry Section of the Zoecon Corporation, using the same procedure as for the quantitation of ^{14}C in feed (vide page 35).

Identification of ^{14}C constituents in excreta

The radiolabeled components in the excreta were qualitatively separated by thin layer chromatography (TLC), and partially identified. Triturated excreta were subsampled from the 48-hour collection from an orally dosed bird, the 6-hour collection from an intraperitoneally dosed bird, and the 192-hour collection from a bird continuously fed (5-^{14}C)-methoprene, and were extracted as in the quantitation of ^{14}C in excreta (vide supra). The extraction volume was reduced to 10 ml under nitrogen gas. A 20 or 25 μl aliquot was taken for LSC quantitation of radiolabel, and the volume further reduced to 0.5 ml. The concentrated extract was then passed through a glass wool plug to remove precipitated materials, and along with the washings, the volume was again adjusted to 0.5 ml. A second aliquot was taken for radiolabel quantitation, and the extract subjected to TLC.

TLC plates were 0.5 mm thick, activated at 110°C for two hours, and stored in a desiccating cabinet until used. The plates were reactivated for thirty minutes immediately before use. Two-dimensional development of the methanolic extract of excreta (100 µl) was carried out with hexane:ether:acetic acid (60:40:1), followed by benzene:ethyl acetate: acetic acid (100:30:6). Reference side-markers, consisting of a standard mixture of methoprene, its primary metabolites and cholesterol, were run with the extract in each dimension. Migrated spots on the plate were visualized by charring at 110°C with 1% ceric ammonium sulfate in 10% sulfuric acid. After identification with the corresponding side-marker, the visualized areas were scraped off, transferred to scintillation vials, and the isolated component desorbed from the silica by shaking with 2 ml methanol for fifteen minutes. Dioxane scintillation fluid (10 ml) was added, and radioactivity determined by LSC.

Co-chromatographic mobility of the hydroxy-ester metabolite of methoprene with cholesterol and its excretable products (coprosterol, β-cholestanol) precluded the isolation of either component. Therefore, the sterol moiety was derivatized to form a precipitatable digitonide complex by a method used by Quistad et al. (1975a). The bulk of the concentrated extract not used in the initial TLC procedure was quantitatively transferred to a 25 ml pear-shaped flask. After adding 1 ml 95% ethanol, 0.016 g digitonin was dissolved in the extract. Acetone (5 ml) was added to this solution, and the mixture was heated for 10 min at 55°C until a silky white precipitate appeared. The solvent mixture was evaporated to dryness under nitrogen gas, and the precipitate resuspended in ca. 1 ml acetone. After the precipitate had settled,

the acetone supernatant was analyzed by TLC as above. Visualization and quantitation of radiolabel were also performed (vide page 40). Hydrolysis of the digitonin-sterol complex was effected by the addition of 1 ml glacial acetic acid, and maintaining at 55°C for fifteen minutes. The redissolved sterol moiety in acid was neutralized in a 125 ml separatory funnel by dilution with 15 ml water and the slow addition of 40 ml saturated potassium bicarbonate. The aqueous phase was extracted twice with 50 ml portions of chloroform, and the combined chloroform extracts reduced to 0.5 ml with the aid of a rotary evaporator and nitrogen gas. Persistent emulsions at the interface of the partitioning bicarbonate-chloroform layers were broken by the addition of 10-15 ml ethanol. A 25 µl aliquot of the concentrated extract was taken for LSC quantitation of radiolabel, and 100 µl subjected to TLC analysis as before.

Tissue quantitation of ^{14}C

Quantitative assessment of ^{14}C in tissue was conducted to verify that ^{14}C not found in excreta and expired gases was accounted for in the body of the quail. To meet this objective, it was necessary to perform analyses only at strategic times corresponding to certain levels of ^{14}C in the excreta and expired gases. Whole body quantitation was carried out, with separate determinations of ^{14}C in liver and subcutaneous fat.

Six-week-old female quail were separated into three groups of five each in a Petersyme brood-unit, and provided with food and water ad libitum in preparation for receipt of oral and intraperitoneal doses.

After three days of preconditioning, (5-^{14}C)-methoprene doses were administered. The mean oral dose given to one group (average weight 85.1 g) was 37 mg AI/kg (3.81 μCi), and the mean intraperitoneal injection given to another group (average weight 89.1 g) was 25 mg AI/kg (1.99 μCi). Control birds (average weight 84.2 g) were the third group. Two control birds each received an empty gelatin capsule, and the other two control birds each received an intraperitoneal injection of 2% Tween 80 in physiological saline.

One bird from each group was sampled 2, 8, 24, 48 and 120 hours post-dose. After decapitation, the liver and a subcutaneous fat sample were removed and stored at -26°C pending separate analysis. The rest of the body was homogenized with 2 x 150 ml methanol in a Sorvall omni-mixer homogenizer. The combined homogenates were filtered through Whatman #2 filter paper, and the residual tissue washed with methanol. Aliquots (2 ml) of the methanolic filtrate and washings were placed in scintillation vials, 10 ml dioxane scintillation fluid added, and the radioactivity determined by LSC. An accurately weighed subsample of the residual tissue (ca. 30 g wet weight) was subjected to an exhaustive sixteen-hour soxhlet extraction with 150 ml methanol, and 5 ml aliquots of the extract were scintillation counted as above.

Whole body quantitation of ^{14}C was repeated during administration of methoprene via the feed (25 ppm AI, 0.0003 μCi/g) to fully mature, thirty-week-old female quail. Six test birds (average weight 117.5 g) and six controls were maintained in separate rat cages, and provided with food and water ad libitum. After a preconditioning period of ten days, treatment birds were given (5-^{14}C)-methoprene via the feed. Average

daily dietary intake of methoprene ranged from 0.3975 mg AI/kg (0.003 μCi) to 0.8127 mg AI/kg (0.006 μCi). Quail and feed weights were taken daily, and birds were sampled 24, 48, 120, 144, 168 and 192 hours after initiation of methoprene feeding. Subsequent analysis was performed as for orally and intraperitoneally dosed birds.

Eggs were collected from birds in production. In the case of treatment birds, all eggs collected were quantitated separately for radiolabel at each sampling time by blending in a Sorvall omni-mixer. After solubilizing 1 g of the blender product in 4 ml NCS solubilizer at 50°C for 24 hours, 10 ml toluene scintillation fluid was added, and the ^{14}C content quantitated by LSC.

Liver and subcutaneous fat samples that were excised from the quail at sampling times were frozen at -26°C. At a later date, the tissues were thawed to 4°C, and 0.5 g subsamples solubilized in 4 ml NCS solubilizer at 50°C for 24 hours. Toluene scintillation fluid was then added, and the radioactivity determined by LSC.

Part 2. Results and Discussion

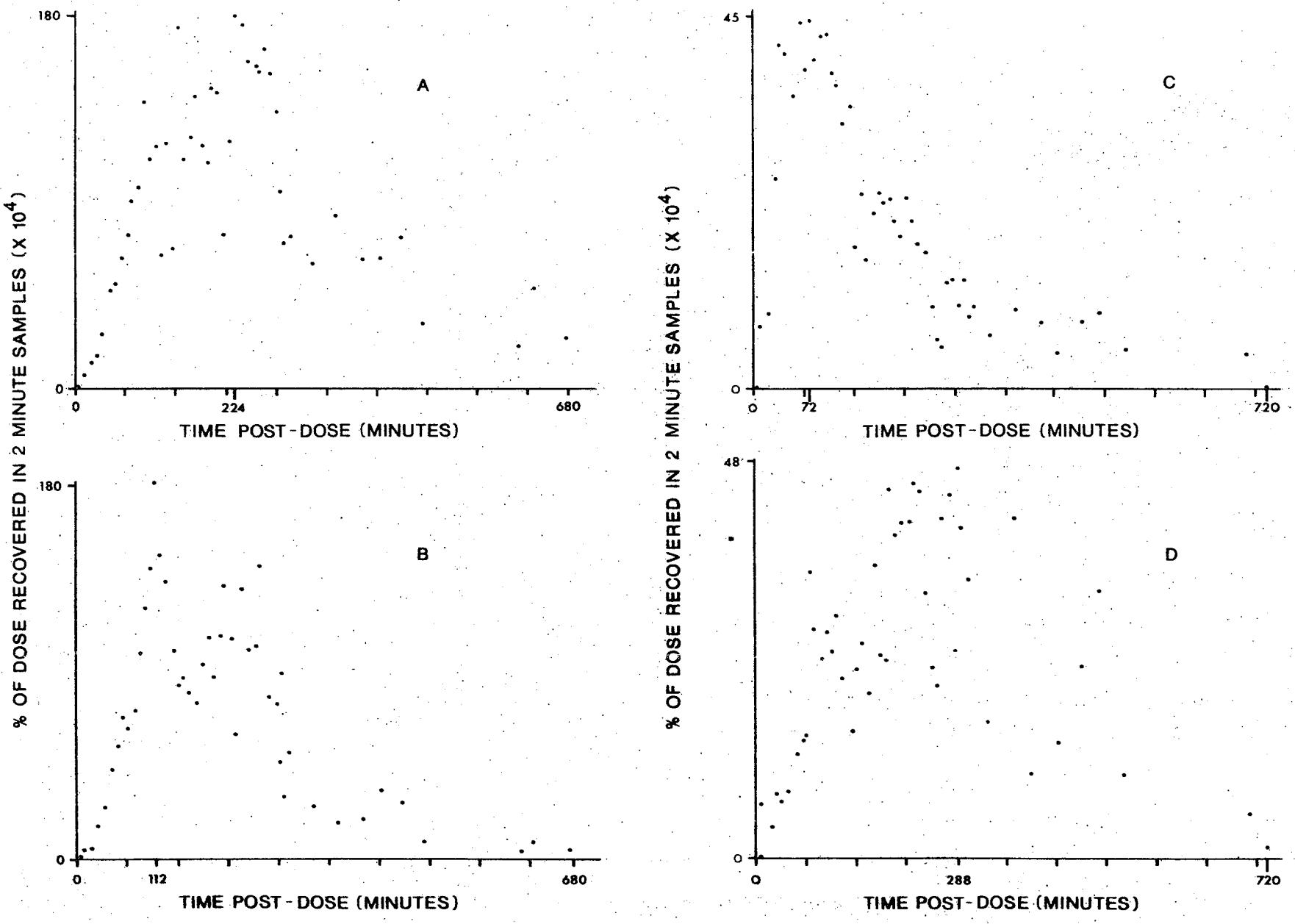
With the one exception of tissue analysis of quail continuously ingesting (5-^{14}C)-methoprene via the feed, all tests were, by design, conducted on female birds that were approaching, but had not yet reached maturity and egg production. The period of maturation (four to seven weeks of age) is critical to the growth and development of reproductive tissues and optimum hormone levels. Exposure to an xenobiotic at this time has the potential to interfere with normal development of the reproductive mechanism. In addition, at four to seven weeks of age the

absolute quantities of food consumed increase, so that the possibility of ingesting greater amounts of an xenobiotic is enhanced. At the same time, the overall growth rate begins to decline, causing the concentration of an ingested xenobiotic per unit of body biomass to increase, before egg production at eight weeks of age provides an added route for removal from the body.

$^{14}CO_2$ evolution

Expired $^{14}CO_2$ from Japanese quail receiving oral and intraperitoneal doses of (5-^{14}C)-methoprene was trapped for 75 hours, with monitoring of relative amounts of $^{14}CO_2$ output being conducted in the first 11.5 hours using the monitor traps. Throughout all $^{14}CO_2$ expiration determinations, the temperature within the metabolism units remained at 24.5 $\pm$ 0.5°C, and the humidity in the units became elevated to condensation point by *ca*. 350 minutes. The units were separately isolated under subdued light at all times. Activities of the birds during the preconditioning and trial periods consisted of resting intervals, sleeping, eating, drinking and occasionally moving about. The consistencies and wet weights of excreta produced were comparable to those of birds in the holding cages.

Plots of expired $^{14}CO_2$ collected from orally or intraperitoneally dosed birds in monitor traps are presented in Figure 4. The quantity of $^{14}CO_2$ expired by quail dosed orally or intraperitoneally increased rapidly, then declined slowly. The sensitivity of the monitor trapping system was such that changes in the activity of the quail being monitored, minor fluctuations in the flow rate of air through the system, and changes in the moisture content in the system were sufficient to produce


A
B
C
D
180
0
224
680
112
45
72
720
48
288
TIME POST-DOSE (MINUTES)
% OF DOSE RECOVERED IN 2 MINUTE SAMPLES (X 10⁴)

fluctuations in the amount of $^{14}CO_2$ collected from one sampling time to the next. In addition, changes in the flow rates and moisture contents of the two monitor traps were of different magnitudes, so that at any given sampling time, the conditions under which $^{14}CO_2$ passed through the monitor traps were not comparable. These factors precluded the averaging of monitor trap values for the two orally dosed replicate birds, and the two receiving intraperitoneal injections.

The greatest proportion of the oral dose collected in one two-minute trapping of expired $^{14}CO_2$ in the monitor traps occurred 112 minutes post-dose for one replicate bird, and 224 minutes post-dose for the second replicate bird (vide Figure 4). The greatest proportion of the intraperitoneal dose collected as $^{14}CO_2$ occurred 72 and 288 minutes post-dose for the two replicate birds, respectively (vide Figure 4). The post-dose times of expiration of the greatest proportion of the dose by the two orally dosed birds are not significantly different from those expired by the two intraperitoneally dosed birds ($F = 2.37$).

All expired $^{14}CO_2$ not shunted into a monitor trap was collected in ethanolamine tower traps (vide page 36). The amounts of expired $^{14}CO_2$ collected in the towers during extended and continuous intervals are presented in Figures 5 and 6. The cumulative plots of $^{14}CO_2$ output in these Figures are additive values, each consisting of the $^{14}CO_2$ outputs at all previous sampling times. The two birds dosed orally expired 12.8% and 16.8% of the dose respectively in 76.1 hours, while the two birds dosed intraperitoneally expired 6.4% and 13.1% of the dose in 77.3 hours. There is no statistical difference between the total quantities of $^{14}CO_2$ expired by orally and intraperitoneally dosed birds ($F = 1.7$).

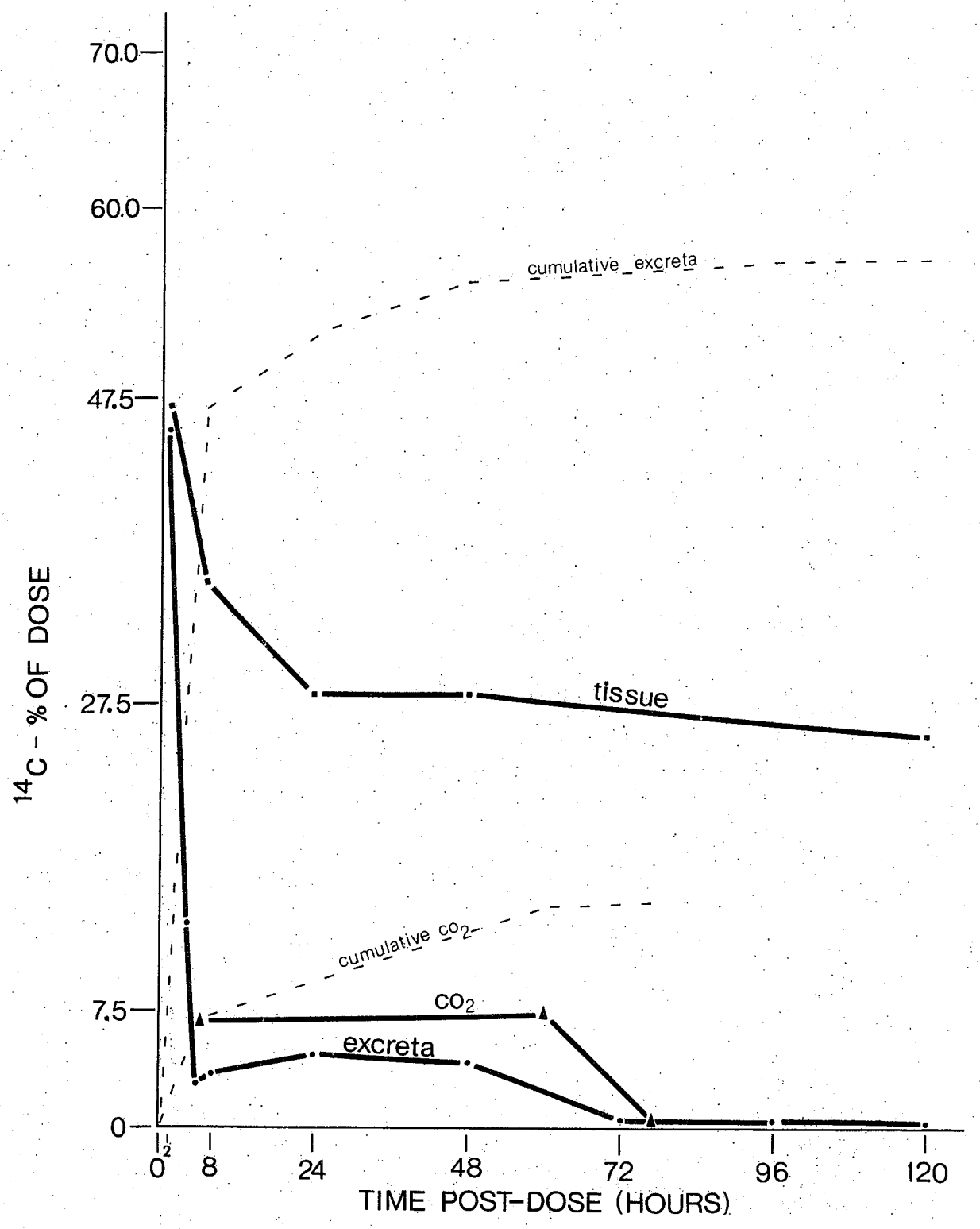


Figure 5. Distribution of ^{14}C in expired CO_2, excreta and whole bodies of quail after single oral dosing with (5-^{14}C)-methoprene

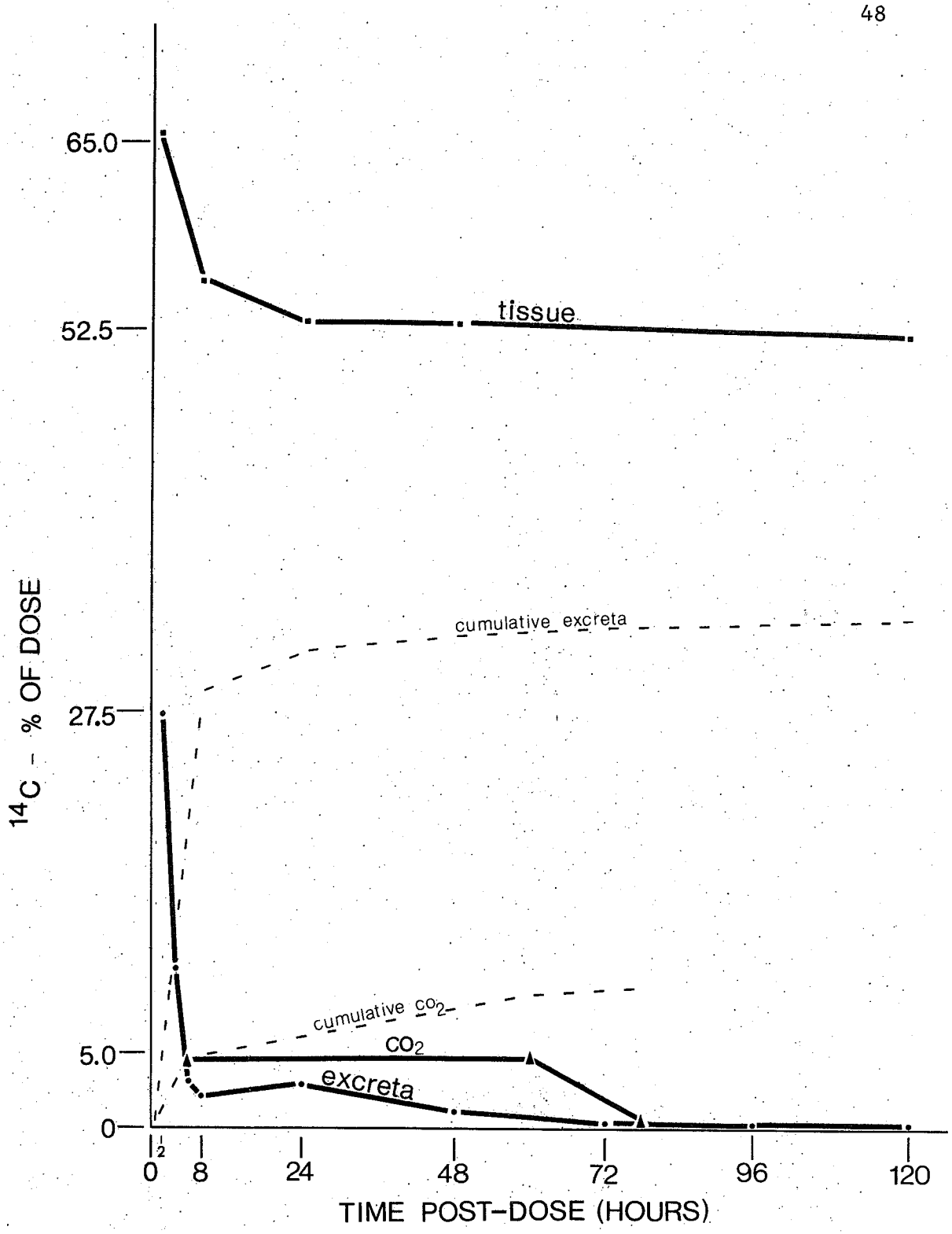


Figure 6. Distribution of ^{14}C in CO_2, excreta and whole bodies of quail after single intraperitoneal injections of (5-^{14}C)-methoprene

Very small quantities of $^{14}CO_2$ were being expired at 77 hours post-dose following either method of dosing.

The lack of difference in quantities of $^{14}CO_2$ expired by orally and intraperitoneally dosed birds might be due, in part, to the variation in metabolic performance among different test animals, as well as to the metabolic fate of methoprene once in the bloodstream. With a heat production of 23 kcal/day, quail have a comparatively high rate of metabolism (Giaja and Males, 1928). Minor variations in metabolic rate from one test animal to another, as well as metabolic changes induced by environmental stimuli aggravate the variability of outputs such as $^{14}CO_2$ expiration, resulting from metabolic processes. In addition, parenteral administration of methoprene by intraperitoneal injection bypasses the partial intestinal absorption of an orally administered dose, and subsequent portal shunt of absorbed material directly to the liver for its metabolism. The intraperitoneally injected compound enters the blood vascular system by lymphatic drainage, from whence it gains access to all body tissues. In contrast to the oral dose, the intraperitoneally administered dose must pass in its entirety into the bloodstream before it reaches the liver, and therefore there is a greater tissue distribution of methoprene and/or its metabolic products because of its slower metabolism to $^{14}CO_2$. This is supported by the tissue levels of ^{14}C found after administration of (5-^{14}C)-methoprene (vide Figures 5 and 6).

The expired radiolabel values are comparable to those of other animals receiving smaller or larger oral doses of (5-^{14}C)-methoprene. A guinea pig expired 17.2% of an oral dose in 24 hours, while a steer expired 22% of an oral dose in 96 hours (Chamberlain et al., 1975).

In comparison with the quail in the present study, the rate of $^{14}CO_2$ expiration by the guinea pig was twice that of the quail per unit of body weight, and by the steer was one-third that of the quail per unit of body weight. The quail and steer exhibited an initial peak $^{14}CO_2$ expiration (112 and 224 minutes for the two quail; 36-72 hours for the steer) to reflect the administered pulse dose. Subsequent secondary peaks or a gradual tailing off of $^{14}CO_2$ release occurred over an extended time period (> 3 days). Sequential breakdown of absorbed methoprene proceeds through α, β, β-oxidation, releasing ^{14}C-acetate in a fashion similar to fatty acid degradation. The pulsatile addition of ^{14}C to the acetate pool upon ^{14}C-methoprene degradation, preceded its pulsatile appearance as metabolic $^{14}CO_2$. Further incorporation of ^{14}C-acetate into natural biological products such as fatty acids and cholesterol (Quistad et al., 1974a), would account for the extended release of $^{14}CO_2$ in smaller quantities. Once the radiolabel has been incorporated into a common biological precursor as ubiquitous and universal as acetate, its release as metabolic $^{14}CO_2$ is dictated by metabolic rates and turnover times of acetate carbons within the organism.

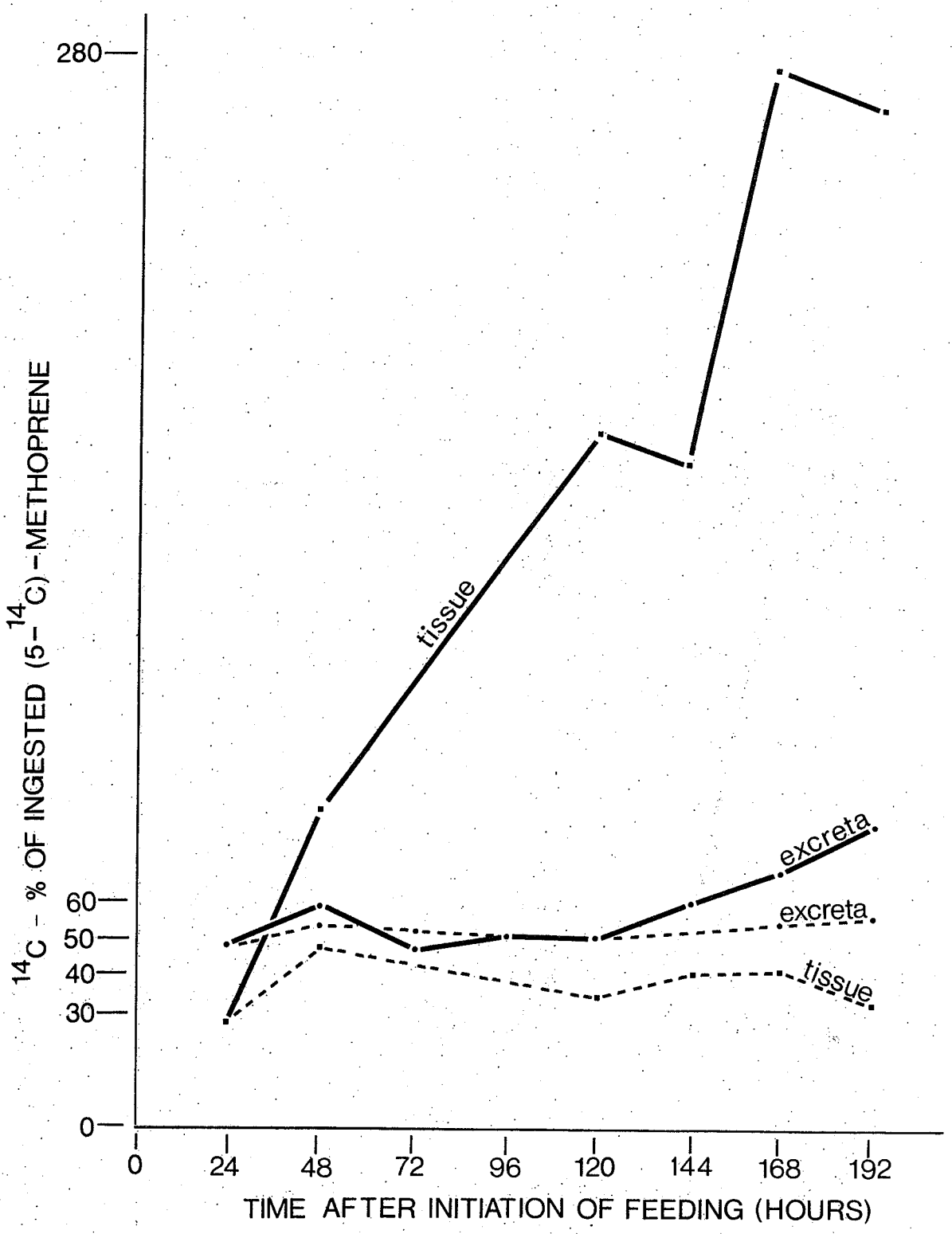
^{14}C content in excreta

All quail used in the determination of ^{14}C outputs in excreta produced similar quantities of excreta throughout the test periods, and no differences in daily feed consumption and weight gains were noted between control and test birds. Sufficient amounts of excreta for two-, four- and six-hour radiolabel quantitations were obtained only from one control bird, one orally dosed bird and two intraperitoneally dosed

birds. At eight hours post-dose, all four birds in each treatment group produced enough excreta for radiolabel quantitation.

Extraction recoveries of ^{14}C from the excreta varied with the hour of sampling, since the metabolite composition of the excreta was changing as a function of time. Extractable radiolabel from the excreta of orally dosed birds ranged from 58% of the two-hour sample, to 36% of the 48-hour sample. Values for intraperitoneally dosed birds ranged from 56% of the two-hour sample, to 35% of the 120-hour sample, while those for birds receiving methoprene continuously via the feed averaged 58%. Outputs of ^{14}C in the excreta of birds receiving (5-^{14}C)-methoprene orally, intraperitoneally or continuously via the feed are presented in Figure 5 (page 47), Figure 6 (page 48) and Figure 7 (page 52) respectively. Plotted values are averages of outputs of excreta from replicate birds. The cumulative plots of ^{14}C outputs in excreta in Figures 5 and 6 are additive values, each consisting of the ^{14}C outputs at all previous sampling times.

Elimination of ^{14}C in the excreta was 56.5% of the oral dose by 120 hours post-dose, and 33.8% of the intraperitoneal dose by 120 hours post-dose; no ^{14}C was detectable in the excreta of control birds within the zero to 120-hour post-dose sampling period. After oral or intraperitoneal dosing, most of the radiolabel was eliminated in the excreta within the first four hours. Much of the remainder was excreted during the subsequent 68-hour period, and trace amounts were still being detected 120 hours post-dose (vide Figures 5 and 6). The 23% greater cumulative elimination of radiolabel via the excreta following oral dosing may represent elimination without prior intestinal absorption.


280
60
50
40
30
0
^{14}C - % OF INGESTED (5-^{14}C)-METHOPRENE
tissue
excreta
excreta
tissue
0
24
48
72
96
120
144
168
192
TIME AFTER INITIATION OF FEEDING (HOURS)

Quail receiving 25 ppm (5-^{14}C)-methoprene continuously via the feed eliminated 56.1% of the ingested material during 192 hours of feeding (vide Figure 7). The quail were capable of continuously excreting a constant proportion of a sublethal quantity of chronically ingested methoprene. The following facts support this conclusion:

1. Throughout the entire 192 hours of feeding, the birds were able to maintain a constant balance between input and output of ^{14}C (vide broken line plot in Figure 7), even with variable daily doses dictated by variable amounts of feed consumed per day (vide page 39).
2. Birds receiving ^{14}C-methoprene continuously via the feed excreted a similar proportion of ingested ^{14}C (48.5%) to that of birds receiving a single oral dose (51.4%), twenty-four hours after dosing or feeding initiation. This occurred even though the four birds receiving ^{14}C-methoprene via the feed consumed a lesser average quantity of ^{14}C over the initial 24 hours of feeding than that given to birds as a single oral dose (4.51 mg AI/kg versus 33 mg AI/kg).
3. A similar effect was observed 120 hours after administering ^{14}C-methoprene by these two methods. From 120 to 192 hours, 50-56% of the ^{14}C consumed from feeding initiation was excreted, and 56% of a single oral dose was excreted in 120 hours post-dose. This suggests that smaller quantities, ingested over an extended time period, will meet a similar metabolic fate to that of a single pulse dose.

The constant input of ^{14}C-methoprene, but increased output of ^{14}C from 120 hours after feeding initiation (vide solid line plot of excretory outputs in Figure 7) suggests the addition to the excreta of labeled natural products which have metabolically incorporated ingested ^{14}C. This is supported by the presence of ^{14}C-cholesterol and/or its derivatives (vide infra) in the excreta 168-192 hours after initiation of the continuous feeding, and their absence in the excreta eliminated 8-24 hours after a single oral dose of ^{14}C-methoprene (vide page 55, and Table V, page 56). Quistad et al. (1974a) found that biodegradation of (5-^{14}C)-methoprene produces ^{14}C-labeled, two-carbon fragments, which become incorporated into cholesterol, and in turn are derivatized to cholesterol elimination products (e.g. β-coprostanol, cholestanol). This would account for the presence of the ^{14}C-sterols in the excreta.

After administration of single oral doses of methoprene to the quail and to a Hereford steer (Chamberlain et al., 1975), the proportion of the dose eliminated as ^{14}C in the excreta of the quail (56.5% in 120 hours post-dose) was similar to the proportion excreted by the steer in the urine and feces (60.4% in 336 hours post-dose). The quail eliminated this proportion in a shorter time interval, and had a shorter post-dose time of maximum elimination rate (2-4 hours for the quail; 24-48 hours for the steer).

Identification of ^{14}C constituents in excreta

The constituent makeup of ^{14}C-containing compounds in the excreta were quantitatively and qualitatively assessed in a 6-hour sample of

excreta from an intraperitoneally does bird, 24- and 48-hour samples of excreta from two orally dosed birds, and a 192-hour sample of excreta from a bird receiving methoprene continuously via the feed. These sampling times were selected because of sample availability, and because of their importance in the time course of metabolic elimination of methoprene. The accuracy of the results, shown in Table V, is limited by the uncorrectable recovery losses in the initial methanol extraction (vide page 51) and subsequent TLC procedure. Up to 58% of the ^{14}C content in excreta was unextractable in methanol, depending on the sampling time, and up to 30% of the plated radiolabel was unaccounted for. Thin layer plates containing control samples were devoid of radioactivity.

The values listed in Table V are greatest for ^{14}C-containing compounds at the origin and vicinity, for all methods of dosing and for all sampling intervals. The radiolabel in this region is associated with highly polar substituents which were immobile in the developing solvent systems used. This, along with the intense green-brown coloration defining the area on the TLC plate, suggests the presence of oxidized bile pigments, as well as cholyl, glucuronide, and sulfonide conjugates of methoprene metabolites and/or natural products, which incorporated the radiolabel. Cholic acid and its derivatives, formed from cholesterol, incorporate labeled carbon atoms from a (2-^{14}C)-acetyl CoA precursor, readily obtainable by α, β, β-oxidation of (5-^{14}C)-methoprene (Quistad et al., 1974a).

With one exception, because the total amount of ^{14}C was low in each of the excreta samples selected for ^{14}C-constituent analysis ($< 5.2\%$ of the dose), the amounts of ^{14}C contained in each of the

Table V. ^{14}C constituents in the excreta of quail receiving (5-^{14}C)-methoprene, as determined by two dimensional thin layer chromatographic analysis of methanol extracts of excreta

	% of dose as ^{14}C constituents in excreta			
Dose form:	intraperitoneal	oral	oral	continuously via the feed
Post-dose (or post-initiation of feeding) time interval represented by sample:	4-6 hours	8-24 hours	24-48 hours	168-192 hours
Constituent:				
2E - methoprene	< 0.1%	< 0.1%	< 0.1%	0.7%[a]
hydroxy - ester metabolite/cholesterol	< 0.1	< 0.1	< 0.1	0.6
methoxy - acid metabolite	0.2	< 0.1	< 0.1	0.1
hydroxy - acid metabolite	--	--	--	1.0
methoxycitronellic acid	--	--	--	< 0.1
neutral lipid	< 0.1	--	--	0.6
unidentified compounds at the origin	1.3	2.3	1.1	3.4
others	< 0.1	< 0.1	< 0.1	1.0
Total:	1.6%	2.3%	1.3%	7.5%
Percent of plated ^{14}C not recovered:	0	20-30%	20-25%	0

[a]Values are expressed as ^{14}C contents in the 168-192 hour interval as percents of the ^{14}C intake from 0-192 hours. Structures for methoprene and its primary metabolites are given in Appendix A, page 74. Methoprene metabolites were not detected where no values appear.

constituents were themselves low. Larger amounts of methoprene, hydroxy-ester metabolite/cholesterol, hydroxy-acid metabolite, and natural lipids were present in the extract of 168-192 hour excreta, collected from birds continuously fed methoprene. These larger amounts are a result of the greater proportion of total radiolabel excreted in that time interval (7.5%, expressed as the quantity of ^{14}C eliminated from 168 to 192 hours after feeding initiation, as a percent of the ^{14}C intake from zero to 192 hours). The predominance of these compounds is not to be overlooked, since a large amount of the material ingested from feeding initiation was eliminated prior to the 168-192 hour time interval examined, and since during the latter 24-hour period (168-192 hours), the quantity of radiolabel ingested and eliminated was comparatively small.

The rigorous chemical treatment required to distinguish the hydroxy-ester metabolite from co-chromatographic cholesterol prohibited the quantitative separation of the two. From the cholesterol-digitonin preparation of the 24-48 hour sample of excreta of an orally dosed bird, 1.5% of the extracted radiolabel used in the preparation was hydroxy-ester metabolite. After cleavage of the digitonide complex, cholesterol comprised 0.9% of the extracted radiolabel. Similar proportions were found in the four to six hour sample of excreta from an intraperitoneally dosed bird. These values were found to be significantly different, and indicate that the combined hydroxy-ester metabolite/cholesterol values listed in Table V are predominantly hydroxy-ester metabolite. This also provides concrete proof that ^{14}C-acetate derived from α, β, β-oxidation of (5-^{14}C)-methoprene by the quail is incorporated into cholesterol,

which is consistent with the results of Quistad et al. (1974a).

The results of the component analysis of ^{14}C in excreta after single oral dosing of the quail are not the same as those from guinea pig and steer studies (Chamberlain et al., 1975). Samples of excreta taken from the two mammals 24 and 48 hours post-dose contained highly significant quantities of methoprene and all of its primary metabolites in the feces, and lesser quantities in the urine. Methoprene comprised 77% of the radiolabel extracted from 24-hour feces of the guinea pig, while methoprene and the hydroxy-acid metabolite respectively comprised 24.5% and 26.1% of the radiolabel extracted from 24-hour steer feces. Still greater quantities were found in the 48-hour sample of steer feces. While urine and feces were not separated in the quail, the presence of methoprene or any of its primary metabolites at any times was less than 0.1% (vide Table V).

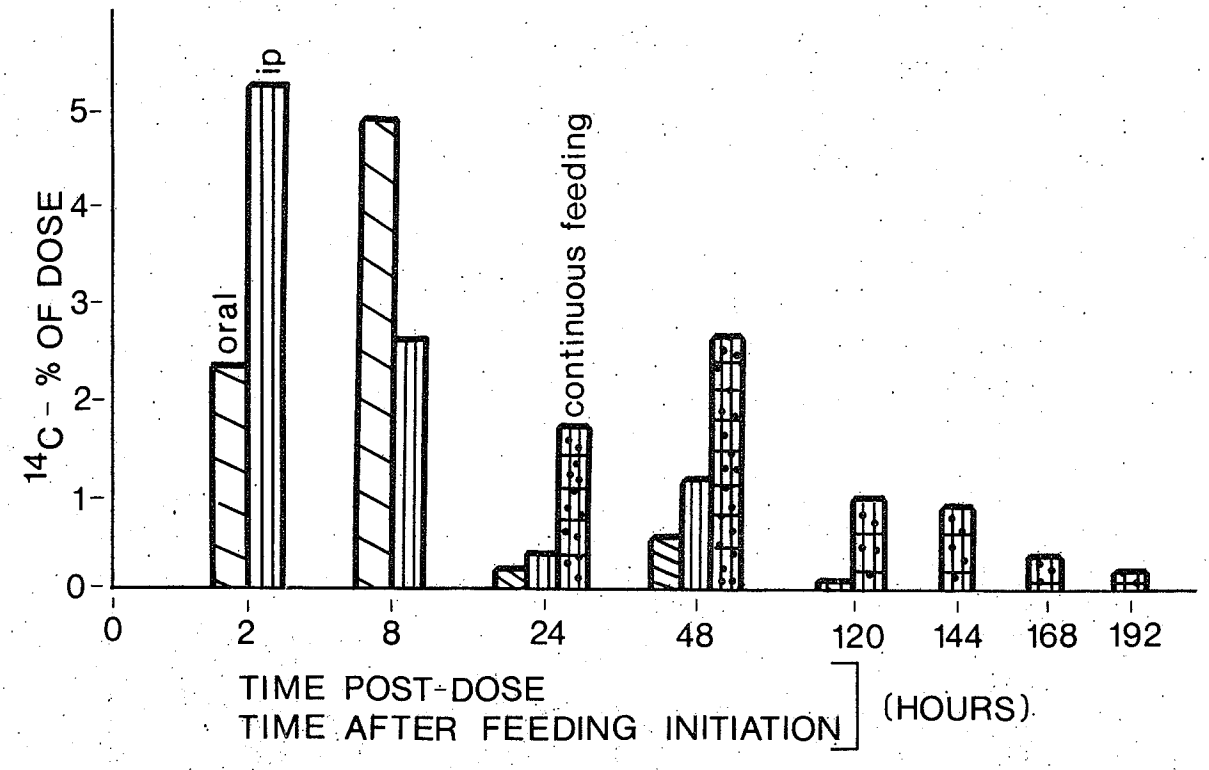
Tissue quantitation of ^{14}C

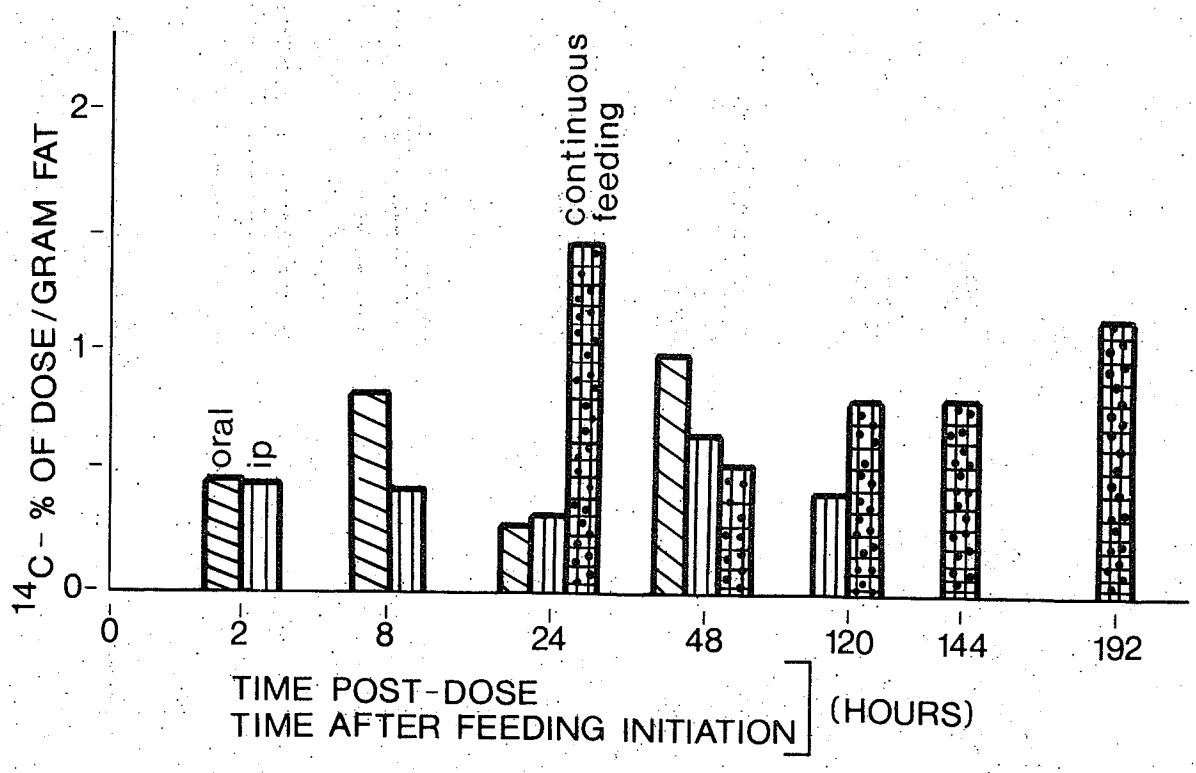
Tissue levels of ^{14}C after single oral dosing, intraperitoneal injection and initiation of continuous feeding of (5-^{14}C)-methoprene are presented in Figure 5 (page 47), Figure 6 (page 48) and Figure 7 (page 52), respectively. The tissue values are for whole birds, including feathers and eggs laid. The tissue ^{14}C values for birds receiving intraperitoneal injections uniformly remained 20-25% higher than for those receiving single oral doses. On the other hand, the ^{14}C levels in the excreta of birds receiving intraperitoneal injections uniformly remained 20-25% lower than from those receiving single oral doses (vide Figures 5 and 6). This suggests that methoprene and its metabolites

are more readily excreted and less readily deposited in tissues after oral dosing and absorption, than after intraperitoneal injection. Furthermore, the rapid post-dose appearance and disappearance of radiolabel in the excreta was accompanied by the prompt drop and stabilization of the tissue level (to 66.9% within two hours post-injection) at coincident times (vide Figures 5 and 6). This, along with the appearance of $^{14}CO_2$ within 16 minutes post-dose and the immobile nature of ^{14}C-labeled compounds on a TLC plate six hours post-dose, suggests a rapid biodegradation of methoprene in vivo, with subsequent tissue distribution of the radiolabel as natural biological compounds. In all analyses, control values were at background level.

Body retention of ^{14}C by birds given (5-^{14}C)-methoprene continuously via the feed contrasted with body retention after oral or intraperitoneal dosing. As depicted by the tissue plot (solid line) in Figure 7 (page 52), the rise to 280% by 192 hours after feeding initiation represents the ^{14}C content at sacrifice, expressed as a percent of the (5-^{14}C)-methoprene ingested in the 24 hours prior to sacrifice. This serves only to illustrate that ^{14}C is indeed accumulated in the body, and based on the large proportion of the radiolabel found at the origin of the TLC plate when an extract of 168-192 hour excreta was analyzed, the assumption is made that the majority of the ^{14}C accumulation was in the form of natural products.

The presence of ^{14}C-labeled compounds in liver (vide Figure 8) and in subcutaneous fat (vide Figure 9) attest to the general distribution of radiolabel in vivo. When the total body content of ^{14}C was 66.9% of the dose two hours after intraperitoneal injection, Figure 8 shows that the quantity of ^{14}C in liver (the major organ of metabolism) was only 5% of the dose,


^{14}C - % OF DOSE
0
1
2
3
4
5
oral
ip
continuous feeding
0
2
8
24
48
120
144
168
192
TIME POST-DOSE
TIME AFTER FEEDING INITIATION
(HOURS)


^{14}C - % OF DOSE/GRAM FAT
0
1
2
oral
ip
continuous feeding
0
2
8
24
48
120
144
192
TIME POST-DOSE
TIME AFTER FEEDING INITIATION
(HOURS)

dropping to less than 0.1% by 120 hours post-dose. Similar results, shown in Figure 8, were obtained after oral dosing, although the less direct route of access to the liver (absorption and enterohepatic cycling) delayed the time of maximum liver content of ^{14}C to eight hours post-dose. The levels of ^{14}C in subcutaneous fat, given in Figure 9, remained below 1% per gram of fat at all times after oral or intraperitoneal dosing. Over the entire trial period, no tissues from control specimens contained radiolabel at levels above background.

The liver content of ^{14}C in birds fed (5-^{14}C)-methoprene continuously, rose to a maximum of 3% of the ^{14}C intake from feeding initiation 48 hours after feeding initiation, and then fell, as illustrated in Figure 8. This corresponds to a simultaneous increase in the percent ^{14}C appearing in the excreta (vide page 54 and Figure 7, page 52), and emphasizes the importance of the turnover time. The quantity per gram of fat never exceeded 1.5% of the total (5-^{14}C)-methoprene ingested from feeding initiation (continuous feeding, Figure 9).

Four of the six birds used in the continuous feeding of (5-^{14}C)-methoprene trial were in full egg production. Whole eggs were analyzed separately for their radiolabel content at 24, 120, 144 and 168 hours after feeding initiation. Table VI shows that following 168 hours of continuous ingestion of ^{14}C, over 10% of the amount ingested to that time was deposited in the seven eggs produced. According to Watt and Merril (1963), eggs contain a high fat content (11.5 g/100 g whole chicken egg), which is partly cholesterol (550 mg/ 100 g whole chicken egg); therefore it is possible that a large portion of the ^{14}C-label in eggs was present in natural products.

Table VI. ^{14}C- content in whole eggs laid by quail ingesting (5-^{14}C)-methoprene continuously via the feed

Time of sacrifice (hours)	Number of eggs produced	% of ingested ^{14}C deposited in eggs
24	1	1.8%
120	2	1.1
144	6	7.9
168	7	10.4

The distribution of radiolabel among expired gases, excreta and body tissues upon ^{14}C-methoprene administration orally, intraperitoneally or continuously via the feed are given in Figure 10. The major difference in the results obtained from methoprene administration orally versus intraperitoneally is the percentage eliminated via the excreta. After oral intake, the greatest proportion of ingested ^{14}C appeared in the excreta (56.5%); after intraperitoneal injection, the greatest proportion of ^{14}C was retained in the body (52.4%). The proportion of radiolabel appearing in the expired gases of orally dosed birds was not significantly different from that of intraperitoneally dosed birds.

The acute oral toxicity of methoprene to Japanese quail is at least 200 fold greater than the level that would be environmentally accessible according to the contemplated use pattern. This applies to other members

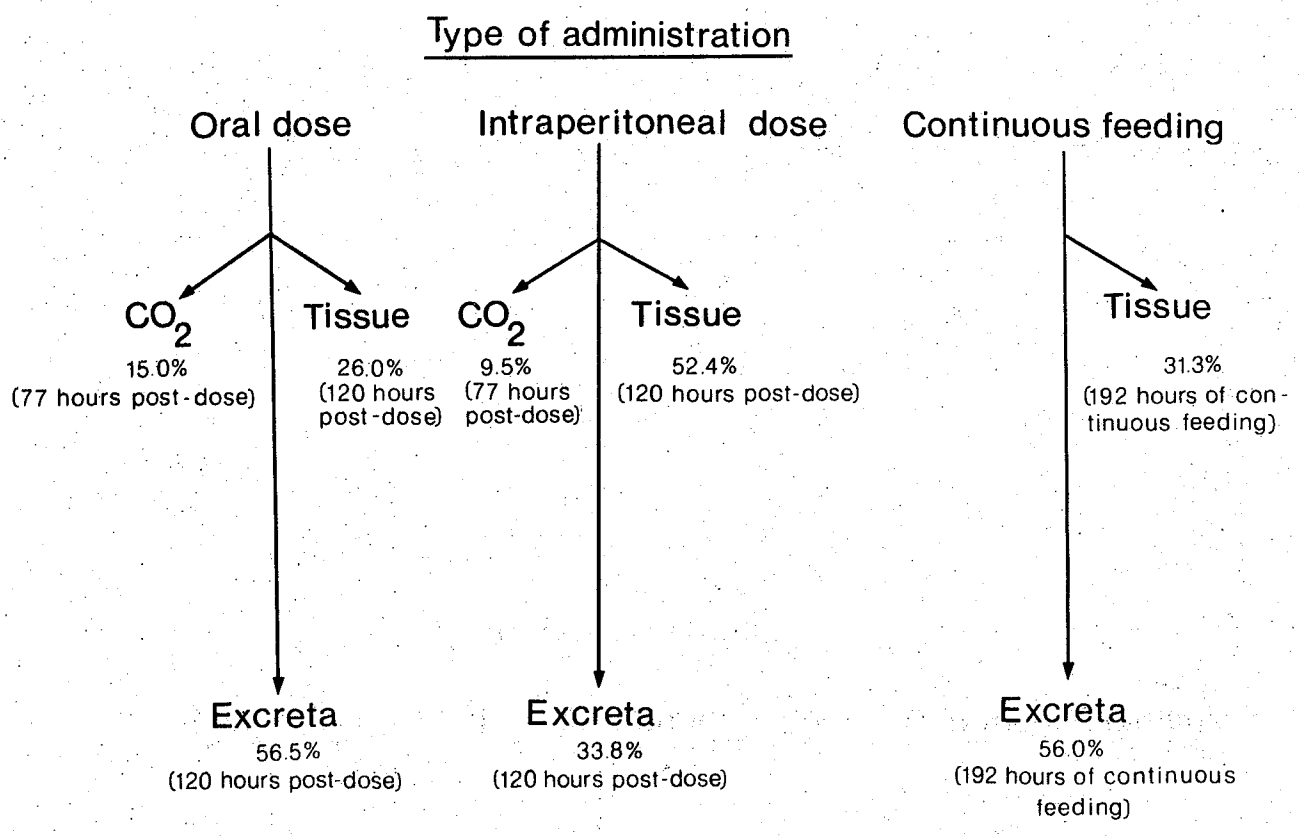


Figure 10. Proportion of ^{14}C intake appearing in expired gases, excreta and body tissues of Japanese quail administered (5-^{14}C)-methoprene.

of Galliformes including the chicken (Zoecon Corporation, a; Zoecon Corporation, 1974), to which methoprene is intended to be given for feed-through fly control. The 25 mg/kg given to the quail as oral or intraperitoneal doses (two and one-half times the maximum level required for fly control) did not affect the birds in any known way. The ability of the quail to handle both pulsatile and chronic doses of ^{14}C-methoprene is illustrated by the rapid removal of a large portion of a pulsatile dose from the body by expiratory and excretory routes, and by the stability of the quail's metabolic capability in eliminating a constant percentage of a continuously administered dose. The response to a pulsatile oral or intraperitoneal dose of ^{14}C-methoprene was manifested by a rapid increase and decrease in $^{14}CO_2$ expiration, as well as in ^{14}C appearing in the excreta and whole body tissues (especially the liver). The response to a continuously fed dose of ^{14}C-methoprene was manifested by a constant percentage of the ingested dose excreted within the 192-hour period examined. In quail continuously fed ^{14}C-methoprene, and after the pulsatile appearance and disappearance of ^{14}C in the excreta and expired gases of birds given pulsatile doses, tissue levels of ^{14}C remained stable, as reflected by the level in subcutaneous fat shown in Figure 9. This was due to the biodegradative treatment of methoprene as a "pseudo fatty acid", unique to the class of insecticidal compounds to which it belongs.

The ^{14}C distribution pattern of (5-^{14}C)-methoprene, administered as a pulse dose, was a direct consequence of the biodegradation of methoprene mentioned above. Tissue levels of ^{14}C-containing compounds after oral administration of a pulse dose of (5-^{14}C)-methoprene were

substantially higher than the levels of ^{14}C-containing compounds excreted from the body, and were concentrated in subcutaneous fat more than in the liver. This is not as apparent during chronic administration of methoprene. Larger quantities of ^{14}C were found in the excreta than in the body, and the presence of primary metabolites of methoprene in the excreta suggests that they may constitute part of the *in vivo* ^{14}C pool. Based on this observation, and in light of the fact that under normal use conditions methoprene would be continuously available to quail, the capacity of quail to metabolize methoprene is probably more accurately assessed by its continuous ingestion.

SUMMARY

The toxicity of methoprene (isopropyl-2E,4E-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate) to Japanese quail and its metabolic fate as a function of time were studied. In an acute toxicity determination, no effects of a single oral administration of 5,000 mg/kg methoprene were observed. In the metabolism studies, (5-^{14}C)-methoprene was given to the quail by three methods: (1) 19-73 mg AI/kg as a single oral dose; (2) 19-46 mg AI/kg as a single intraperitoneal injection; and (3) 25 ppm in the daily diet.

Orally dosed birds and those continually fed ^{14}C-methoprene eliminated the greatest proportion of the dose in the excreta (56%) in 120 hours and 192 hours respectively, while intraperitoneally dosed birds retained the greatest proportion of the dose in the body (53%) at 120 hours post-dose. Methoprene was metabolized to CO_2, hydroxy-ester and hydroxy-acid metabolites, cholesterol, neutral lipids and unidentifiable highly polar compounds present in the excreta. The rate of degradation of ^{14}C-methoprene to $^{14}CO_2$ and the quantity of expired $^{14}CO_2$ were the same for orally and intraperitoneally dosed birds (6-17% of the dose in 77 hours post-dose). Thin layer chromatographic analysis of the methanol extracts of the excreta showed predominantly highly polar, unidentifiable metabolites of methoprene, immobile on the thin layer plates. Extracts of excreta sampled during the 168-192 hour interval of continuous ingestion of ^{14}C-methoprene with the feed contained 2E-methoprene, its hydroxy-ester and hydroxy-acid metabolites, ^{14}C-cholesterol, and

^{14}C-containing neutral lipids in larger quantities than in extracts of excreta from orally and intraperitoneally dosed birds.

The proportion of the dose appearing in liver tissue reached a maximum earliest in intraperitoneally dosed birds (5.11% at the 2-hour sampling time post-dose). The maximum proportion in the livers of orally dosed birds (4.48%) occurred at the 8-hour sampling time, and that in the livers of birds continuously fed methoprene (2.73%) occurred 48 hours after feeding initiation. The levels of ^{14}C in subcutaneous fat were consistently less than 1.5% of the dose per gram of fat following all three methods of administration. The eggs of birds receiving ^{14}C-methoprene continuously via the feed contained 10.4% of the ingested ^{14}C following 168 hours of ingestion. Thus methoprene is metabolized to excretable metabolites.

CONCLUSIONS

Methoprene has a high degree of environmental non-persistence and biodegradability. As an insect growth regulator, it is acutely toxic to aquatic and terrestrial dipterans, and is of low toxicity to vertebrate organisms affected by dipterans. The exposure of Japanese quail to environmental levels of methoprene will have no metabolic effect over an eight-day period of exposure. Seventy-seven hours after single oral dosing with methoprene, 13-17% of the dose was eliminated as CO_2 in expired gases, and by 120 hours post-dose, 56.5% of the dose was eliminated via the excreta. After single intraperitoneal injection, 6-13% of the dose was expired in 77 hours, and 33.8% of the dose was excreted in 120 hours. Quail receiving methoprene continuously via the feed eliminated 56.0% of the ingested material in 192 hours from feeding initiation, and retained 31.3% in the same time period.

Since many galliform birds are edible, it is essential that more comprehensive investigations be carried out on the characterization of ^{14}C-containing compounds derived from ^{14}C-methoprene in tissues. In addition, elucidation of the physiological and metabolic effects of long-term chronic exposure to methoprene, and tissue residue levels after such exposure, are necessitated by the fact that these edible birds are upland game species which are native to areas where there is a potential use for methoprene in insect control. Such studies would serve as sensitive indices by which the consequences of the use of juvenoid compounds in general could be predicted.

BIBLIOGRAPHY

Bionomics, Inc. (1973). Kinetics of ^{14}C - Altosid in a Synthetic Aquatic Ecosystem. Report submitted to Zoecon Corp. 15 pp.

Cerf, D. C. and G. P. Georghiou (1972). Evidence of Cross-Resistance to a Juvenile Hormone Analogue in Some Insecticide-Resistant Houseflies. Nature 239:401.

Chamberlain, W. F., D. E. Hopkins and A. R. Gingrich (1973). Cattle Biting Louse: Evaluation of Compounds for Juvenile Hormone Activity. J. Econ. Entomol. 66:127.

_____, L. M. Hunt, D. E. Hopkins, A. R. Gingrich, J. A. Miller and B. N. Gilbert (1975). Absorption, Excretion and Metabolism of Methoprene by a Guinea Pig, a Steer and a Cow. J. Agr. Food Chem. 23:736.

Cummings, J. E. and B. McKague (1973). Preliminary Studies of Effects of Juvenile Hormone Analogues on Adult Emergence of Black Flies (Diptera: Simuliidae). Can. Ent. 105:509.

Dunn, R. L. and F. E. Strong (1973). Control of Catch Basin Mosquitoes Using Zoecon ZR-515 Formulation in a Slow Release Polymer - A Preliminary Report. Mosq. News. 33:110.

Findlay, G. M. (1969). The Metabolism of 1-^{14}C-Methyl Linoleate Hydroperoxide in the Rabbit. PhD thesis. University of Illinois, Urbana. pp. 22-24.

Garris, G. E. and T. R. Adkins, Jr. (1974). The Effects of Altosid, an Insect Developmental Inhibitor, on the Last Instar Larva of Simulium pictipes. Mosq. News. 34:335.

Giaja, J. and B. Males (1928). Sur la Valeur du Metabolisme de Base de Quelques Animaux en Fonction de leur Surface. Ann. Physiol. Physicochim. Biol. 4:875.

Gomez, E. D., D. J. Faulkner, W. A. Newman and C. Ireland (1973). Juvenile Hormone Mimics: Effect on Cirriped Crustacean Metamorphosis. Science. 179:813.

Harris, R. L., E. D. Frazer and R. L. Younger (1973). Horn Flies, Stable Flies, and House Flies: Development in Feces of Bovines Treated Orally with Juvenile Hormone Analogues. J. Econ. Entomol. 66:1099.

_____, W. F. Chamberlain and E. D. Frazar (1974). Horn Flies and Stable Flies: Free Choice Feeding of Methoprene Mineral Blocks to Cattle for Control. J. Econ. Entomol. 67:384.

Hazleton Laboratories, Inc. (1973). One-Generation Reproduction Study - Bobwhite Quail. Report submitted to Zoecon Corp. 8 pp.

Henrick, C. A., G. B. Staal and J. B. Siddall (1973). Alkyl 3,7,11-Trimethyl-2,4-dodecadienoates, A New Class of Potent Insect Growth Regulators with Juvenile Hormone Activity. J. Agr. Food Chem. 21:354.

Jakob, W. L. (1972). Additional Studies with Juvenile-Hormone-Type Compounds Against Mosquito Larvae. Mosq. News. 32:592.

_____ (1973). Insect Development Inhibitors: Testswith House Fly Larvae. J. Econ. Entomol. 66:819.

McKague, B. and P. M. Wood (1974). Effects of Insect Developmental Inhibitors on Adult Emergence of Black Flies (Diptera: Simuliidae). Can. Ent. 106:253.

Miller, R. W. and E. C. Eubel (1974). Juvenile Hormone Mimics as Feed Additives for Control of the Face Fly and House Fly. J. Econ. Entomol. 67:69.

Miura, T. and R. M. Takahashi (1973). Insect Developmental Inhibitors. 3. Effects on Non-Target Aquatic Organisms. J. Econ. Entomol. 66:917.

_____ ___ _____ (1974). Insect Developmental Inhibitors. Effects of Candidate Mosquito Control Agents on Non-Target Aquatic Organisms. Environ. Entomol. 3:631.

Mulla, M. S., R. L. Norland, T. Ikeshoji and W. L. Kramer (1974). Insect Growth Regulators for the Control of Aquatic Midges. J. Econ. Entomol. 67:165.

Plapp, F. W., Jr. and S. B. Vinson (1973). Juvenile Hormone Analogs: Toxicity and Cross-Resistance in the Housefly. Pestic. Biochem. Physiol. 3:131.

Quistad, G. B., L. E. Staiger and D. A. Schooley (1974a). Cholesterol and Bile Acids Via Acetate from the Insect Juvenile Hormone Analog Methoprene. Life. Sci. 15:1797.

_____ _____ ___ _____ (1974b). Environmental Degradation of the Insect Growth Regulator Methoprene (Isopropyl(2E,4E)-11-Methoxy-3,7,11-trimethyl-2,4-dodecadienoate). I. Metabolism by Alfalfa and Rice. J. Agr. Food Chem. 22:582.

_____ _____, B. J. Bergot and D. A. Schooley (1975a). Environmental Degradation of the Insect Growth Regulator Methoprene (Isopropyl (2E,4E)-11-Methoxy-3,7,11-trimethyl-2,4-dodecadienoate). VII. Bovine Metabolism to Cholesterol and Related Natural Products. J. Agr. Food Chem. 23:743.

_____ _____ and D. A. Schooley (1975b). Environmental Degradation of the Insect Growth Regulator Methoprene (Isopropyl (2E,4E)-11-Methoxy-3,7,11-trimethyl-2,4-dodecadienoate). VIII. Bovine Metabolism to Natural Products in Milk and Blood. J. Agr. Food Chem. 23:750.

_____ _____ __ _____ (1975c). Environmental Degradation of the Insect Growth Regulator Methoprene (Isopropyl (2E,4E)-11-Methoxy-3,7,11-trimethyl-2,4-dodecadienoate). V. Metabolism by Houseflies and Mosquitoes. Pestic. Biochem. Physiol. 5:233.

_____ _____ __ _____ (1975d). Environmental Degradation of the Insect Growth Regulator Methoprene (Isopropyl (2E,4E)-11-Methoxy-3,7,11-trimethyl-2,4-dodecadienoate). III. Photodecomposition. J. Agr. Food Chem. 23:299.

Schaefer, C. H. and W. H. Wilder (1972). Insect Developmental Inhibitors. A Practical Evaluation as Mosquito Control Agents. J. Econ. Entomol. 65:1066.

_____ and E. F. Dupras, Jr. (1973). Insect Developmental Inhibitors. 4. Persistence of ZR-515 in Water. J. Econ. Entomol. 66:923.

_____, E. F. Dupras, Jr. and W. H. Wilder (1973a). Pond Tests with ZR-515: Biological and Chemical Residues. Proc. pap. 41st Ann. Conf. Calif. Mosq. Contr. Assn., Inc. Jan. 28-31, 1973. p. 137.

_____ and W. H. Wilder (1973b). Insect Developmental Inhibitors. 2. Effects on Target Mosquito Species. J. Econ. Entomol. 66:913.

Schooley, D. A., B. J. Bergot, L. L. Dunham and J. B. Siddall (1975a). Environmental Degradation of the Insect Growth Regulator Methoprene (Isopropyl (2E,4E)-11-Methoxy-3,7,11-trimethyl-2,4-dodecadienoate). II. Metabolism by Aquatic Microorganisms. J. Agr. Food Chem. 23:293.

_____, K. M. Creswell, L. E. Staiger and G. B. Quistad (1975b). Environmental Degradation of the Insect Growth Regulator Methoprene (Isopropyl (2E,4E)-11-Methoxy-3,7,11-trimethyl-2,4-dodecadienoate). IV. Soil Metabolism. J. Agr. Food Chem. 23:369.

Siddall, J. B. and M. Slade (1974). Tests for Toxicity of Juvenile Hormone and Analogues in Mammalian Systems. In: Invertebrate Endocrinology and Hormonal Heterophylly. Ed. Walter J. Burdette. Springer-Verlag, New York. pp. 345-348.

Solomon, K. R. and R. L. Metcalf (1974). The Effects of Piperonyl Butoxide and Triorthocresyl Phosphate on the Activity and Metabolism of Altosid (Isopropyl 11-Methoxy-3,7,11-Trimethyl-dodeca-2,4-Dienoate) in Tenebrio molitor L. and Oncopeltus fasciatus (Dallas). Pestic. Biochem. Physiol. 4:127.

Strong, R. G. and J. Diekman (1973). Comparative Effectiveness of Fifteen Insect Growth Regulators Against Several Pests of Stored Products. J. Econ. Entomol. 66:1167.

Terriere, L. C. and S. J. Yu (1973). Insect Juvenile Hormones: Induction and Detoxifying Enzymes in the Housefly and Detoxication by Housefly Enzymes. Pestic. Biochem. Physiol. 3:96.

Troisi, S. J. and L. M. Riddiford (1974). Juvenile Hormone Effects on Metamorphosis and Reproduction of the Fire Ant, Solenopsis invicta. Environ. Entomol. 3:112.

Watt, B. K. and A. L. Merril (1963). Composition of Foods. USDA - ARS Agricultural Handbook No. 8. Washington, D.C.

Weirich, G. and J. Wren (1973). The Substrate Specificity of Juvenile Hormone Esterase from Manduca sexta Hemolymph. Life Sci. 13:213.

Westigard, P. H. (1974). Control of the Pear Psylla with Insect Growth Regulators and Preliminary Effects on Some Non-Target Species. Environ. Entomol. 3:256.

Yin, C.-M. and G. M. Chippendale (1973). Juvenile Hormone Regulation of the Larval Diapause of the Southwestern Corn Borer, Diatraea grandiosella. J. Insect Physiol. 19:2403.

_____ _____ _____ (1974). Juvenile Hormone and the Induction of Larval Polymorphysm and Diapause of the Southwestern Corn Borer, Diatraea grandiosella. J. Insect Physiol. 20:1833.

Zoecon Corporation. a. (n.d.). Technical Information.

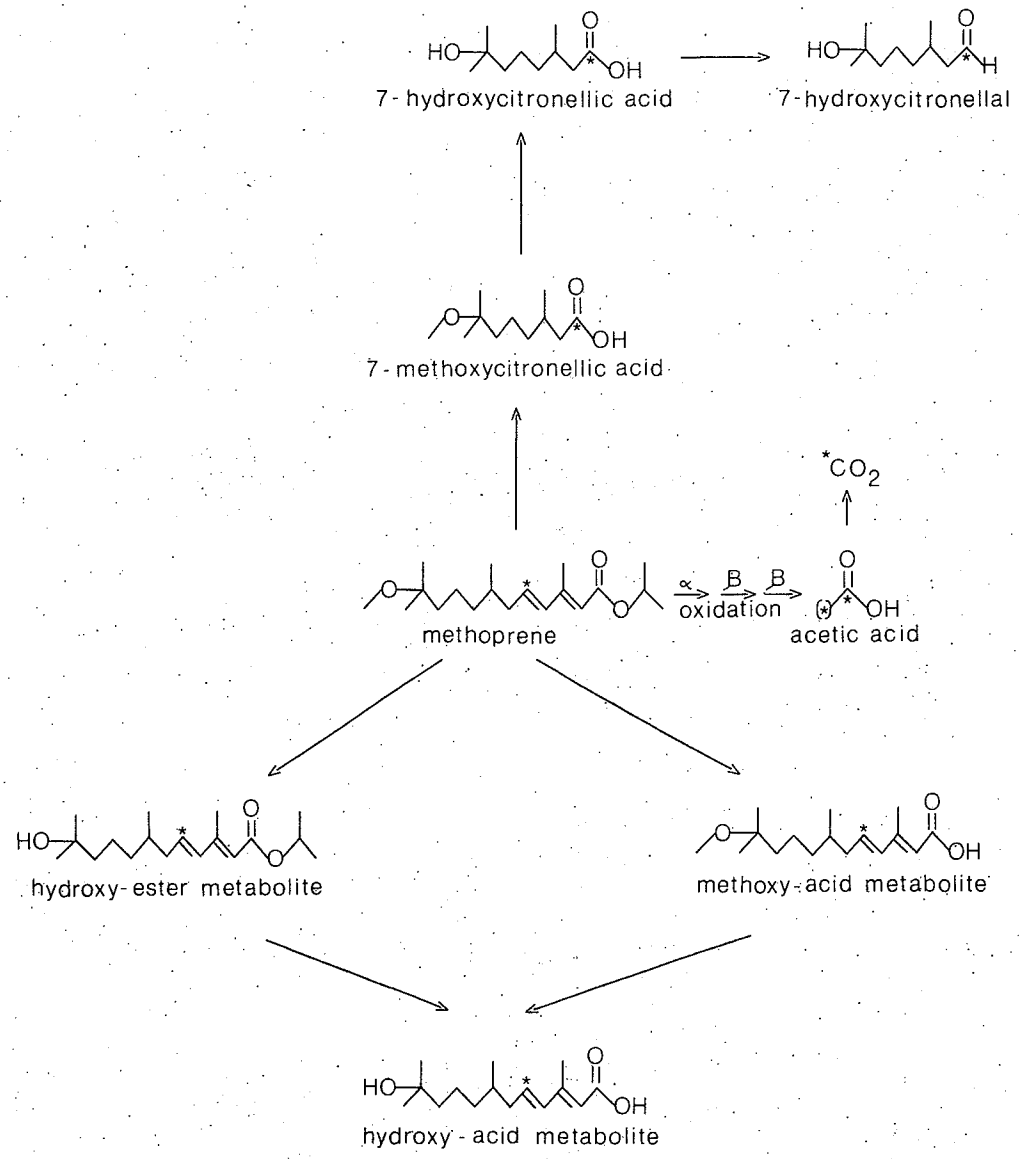
_____ b. (n.d.). Bluegill Fish Residues from Metabolism of Altosid.

_____ (1974). Personal communication.

APPENDICES

APPENDIX A

Degradation products of methoprene



* Indicates ^{14}C - label

APPENDIX B.

Efficacy of Methoprene on Target Insects

Insect	Formulation and rate of application	Mortality
Mosquitoes		
Aedes spp. (14)[a]		
Anopheles spp. (3)	Slow release formulation (10%)	
Culex spp. (6)	0.025-0.25 lb AI/acre	
Culiseta inornata		100%
Psorophora confinis		
Flies		
Musca spp. (2)	10% pre-mix in poultry feed and	
Haematobia irritans	in salt and mineral blocks of	
Stomoxys calcitrans	cattle. Intake = 0.003-10 mg AI/kg daily or 5-10 ppm in the diet.	100%
Pests of stored grain products (*vide* page 13)	5 lb/gal emulsifiable concentrate formulation; less than 10 ppm AI in grain.	100%

[a]Parenthetical figures are the numbers of species tested.

APPENDIX C.

Toxicity Values for Methoprene on Non-Target Organisms

Organism	LD_{50}, LC_{50}, TL_{50} (static)
freshwater shrimp	Greater than 100 ppm
saltwater shrimp	Greater than 100 ppm
white shrimp	Greater than 100 ppm
pink shrimp	Greater than 100 ppm
estuarine mud crabs	Greater than 0.1 ppm
crayfish	Greater than 100 ppm
channel catfish	Greater than 100 ppm
largemouth bass	Greater than 100 ppm
trout	3.30 ppm
bluegill sunfish	4.62 ppm
coho salmon	32 ppm
mallard duck	Greater than 10,000 ppm (continuous feeding)
bobwhite quail	Greater than 10,000 ppm (continuous feeding)
Japanese quail	Greater than 5,000 ppm (acute oral)
chicken	Greater than 4,640 ppm (acute oral)
mouse	Greater than 5,000 ppm (acute oral)
rat	Greater than 34,000 mg/kg (acute oral) Greater than 5,000 ppm (90 day continuous feeding) 210 mg/1 (acute aerosol inhalation)
rabbit	10,000 mg/kg (acute dermal)
guinea pig	Greater than 210 mg/1 (acute aerosol inhalation)
dog	5,000-10,000 mg/kg (acute oral) Greater than 5,000 ppm (90 day continuous feeding)