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.