REPORT FOR THE MINISTRY OF HEALTH

Environmental and health impacts of the insect juvenile hormone analogue, S-methoprene

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Abbreviations

IGR	insect growth regulator
Bt	*Bacillus thuringiensis*
Bti	*Bacillus thuringiensis israelensis*
ppm, ppb	parts per million, parts per billion
JH	juvenile hormone
JHA	juvenile hormone analogue

1. Summary

- Recent interceptions of exotic mosquitoes with the potential to vector serious mammalian diseases has highlighted the need for agents for use in control and/or eradication programmes.

- Methoprene is a larvicide and is not effective against adult mosquitoes. It kills by disrupting metamorphosis and most mortality occurs during the larval and pupal moults. As well as indirect lethal effects, methoprene can cause a number of effects in insects at sublethal doses, such as reduced fecundity, abnormal morphologies and altered pheromone production.

- Methoprene is toxic to a range of insects from 12 orders, including Diptera, Lepidoptera and Coleoptera. Methoprene also kills some mite species. It is most toxic to Diptera, but has been used in the field against a number of pests such as mosquitoes, biting flies, hornflies, ants, hemipteran pests and termites. The lethal dose required to kill common mosquitoes is generally around 1 part per billion. Field application rates used against mosquitoes would be unlikely to be lethal to many other insects.

- In the field, methoprene is effective in controlling a number of mosquito species. When adult emergence is measured, methoprene generally performs as well or better than organophosphates and *Bti*. Methoprene has been used in the localised eradication of ants and fleas in hospitals and public areas. The choice of methoprene for these eradication campaigns was largely influenced by the perceived environmental safety of this agent.

- Methoprene is available in a number of formulations, including sustained release pellets, boluses and briquettes. Various formulations have improved persistence of methoprene, especially in water. Unformulated methoprene has a short half-life in water and soil (<10 days), but with the use of sustained-release formulations, activity against mosquitoes has been detected for over 100 days in water. Persistence is affected by water quality, salinity and temperature. UV light rapidly degrades methoprene.

- Several methods have been developed for detecting methoprene in environmental samples, based on high performance liquid chromatography, selected extraction and/or immunoassays (ELISA). These techniques can detect methoprene at below 1 ppm. However, methoprene is effective at controlling mosquitoes at levels below 2 ppb, well beneath the limit of detection.

- Extensive studies have shown that methoprene breaks down quickly in the environment, spares non-target organisms and poses little hazard to humans. Methoprene has little phytotoxicity, very low toxicity to mammals, however it is moderately toxic to warm-water, freshwater fish and slightly toxic to cold-water fish. Examination of benthic communities after application against

mosquitoes has detected negative impacts on some organisms, however recovery after application was rapid.

- In 1991, methoprene was viewed by the EPA as a biochemical insect growth regulator with low toxicity, posing very little hazard to people and most non-target species. While acutely toxic to some estuarine invertebrates, there appears to be few lasting effect after treatment. The extensive literature review compiled below supports this appraisal. Methoprene will have some non-target impacts, but breaks down rapidly after application and should cause less environmental disruption than most available mosquitocidal chemicals.

- A controversy has arisen involving the discovery of deformed frogs, firstly in Minnesota and subsequently in many areas of North America. Although no definitive cause has been identified, contamination of the environment with pesticides has been suggested. A group of chemicals, retinoids (which includes methoprene), have been suggested as possible causal agents. One laboratory research has indicated a link between sunlight-exposed methoprene and deformities in frogs, although the results are vigorously debated in recent literature and subsequent studies have not found the same effect.

- The development of insect resistance to methoprene has been demonstrated, including in mosquitoes in the laboratory and has recently been found in the mosquito, *Aedes taeniorhynchus,* populations in Florida. Some insects have shown cross-resistance to methoprene when resistant to other chemical pesticides. The development of resistance remains a strong possibility if methoprene is used extensively or heavily in a limited area.

- Comparison with the bacterium *Bacillus thuringiensis israelensis* (*Bti*) which produces toxins active against mosquitoes, suggests there are advantages in the use of methoprene. Methoprene has longer residual activity, but is toxic to a greater range of species than *Bti*.. However, the use of more than one agent during mosquito control is advisable, considering the risks of resistance developing and both methoprene and *Bti* should be considered.

2. Introduction

Methoprene is an insect growth regulator which acts as a juvenile hormone mimic to disrupt normal development of insects. It is used extensively overseas against insects, in particular Dipteran pests. Previously, methoprene has been recommended as an environmentally safe mosquitocidal agent for use in New Zealand. This report examines the known information on methoprene in relation to environmental effects and health.

2.1. Background

Recent discovery of potential disease vectoring mosquitoes in northern New Zealand has highlighted the likelihood of serious mosquito-vectored disease incursions in the near future. In the light of such introductions, it would be prudent to develop strategies to respond to introductions of unwanted mosquitoes. As part of this process, the Ministry of Health commissioned the preparation of a "National Pest Management Strategy for Exotic Mosquitoes of Public Health Significance" (Cowley *et al.* 1998). This strategy outlines methods of exclusion, surveillance and response activities to combat the threat posed by mosquitoes to New Zealand. As part of the response to mosquito incursions, the strategy reviewed mosquitocidal agents and recommended several agents be registered for use in New Zealand as a priority. In particular, the report recommended that the mosquito-pathogenic bacteria *Bacillus thuringiensis israelensis* (*Bti*) and *B. sphaericus* and insect growth regulators should be cleared for use in New Zealand. A thorough knowledge of potential controls for mosquito vectors, including their efficacy and environmental impacts, will be essential for effective control.

Few products are currently registered for mosquito control in New Zealand. In general, the use of chemical insecticides is declining in New Zealand, as a result of increasing concern over negative environmental impacts such as non-target mortality and mammalian toxicity. Such concerns are exacerbated when pest control measures are required in densely populated urban environments, possibly requiring large scale aerial application. This concern was reflected in the choice of mosquitocidal agents suggested for priority registration by Cowley *et al.* (1998). *Bti* is currently widely used overseas and has been registered in New Zealand by NuFarm NZ Ltd. An environmental and health assessment was completed for the Ministry of Health (Glare and O'Callaghan 1998), which found little environmental risk in the application of this agent for mosquito control in New Zealand. However, due to lack of residual control and possible efficacy problems under some conditions, it would be prudent to consider additional agents.

The insect juvenile hormone analogue, methoprene (the isopropyl ester of the 11-methoxy acid), has been widely used in mosquito control around the world. It has more prolonged residual activity than *Bti* and is considered by many authors to be more environmentally benign than most chemicals in use against mosquitoes. For example Norland and DeWitt (1975) reporting on use of methoprene against mosquitoes stated it is non-toxic to man and vegetation, and makes only mild impacts on non-target organisms.

This report collates available information on environmental impacts of methoprene from overseas published data. The purpose of this document is to consider the environmental and health impacts of methoprene, including potential non-target effects, to assist the Ministry of Health in making recommendations regarding methoprene use in mosquito control in New

Zealand. The document may also support eventual application for registration against mosquitoes in New Zealand, including any ministerial exemption under the Biosecurity Act for use in emergency situations before full registration is approved. As such, extensive referencing is made to the original source of material used in preparing this report.

2.2. Insect growth regulators

Endogenous hormones influence metamorphosis and development of insects. These insect growth regulators or juvenile hormones are found in relatively high concentrations in the haemolymph during certain stages of larval insects, where their function is to maintain the larval stage or prevent metamorphosis. During normal insect development, the concentration of juvenile hormone decreases in the final larval instar stage, allowing development of pupal and adult stages.

Identification of the function of juvenile hormone in insects gave impetus for the search and development of synthetic juvenoids. The general class of biochemicals capable of disrupting insect development are called Insect Growth Regulators (IGRs). These compounds are structurally divided into two classes, ie, terpenoids and nonterpenoids. Initially, IGRs were analogs of cecropia juvenile hormone (Wright 1976). Subsequently, other compounds with analogous juvenile hormone activity have been classed as IGRs. There are a number of IGRs in common use as pesticides, including fenoxycarb, hydroprene and diflubenzuron. Dimilin (diflubenzuron) is a common growth regulator (chitin inhibtior, not juvenile hormone analog) used in New Zealand for insect control. Dimilin has been used overseas for mosquito control, although Cowley *et al.* (1998) did not recommend its use against mosquitoes in New Zealand because of questions regarding mammalian and non-target safety, such as carcinogenic breakdown products. Dimilin has not been registered in the United States for general mosquito control, but has a special use permit in California and Florida for use in waters that have no out flow to open water. As a chitin-inhibitor, Dimilin has a much broader effect on non-target organisms and is unlikely to be approved for use in open water.

S-methoprene, a juvenile hormone analog (JHA), is possibly the most attractive alternative to the bacterial mosquito control agent, *Bti,* currently used against mosquitoes overseas. Methoprene disruption of the mosquito growth cycle allows it to be defined as a biochemical pesticide, rather than a conventional pesticide (EPA, 1991).

2.3. Methoprene

Methoprene is a long chain hydrocarbon ester active as an insect growth regulator. Methoprene (1, isopropyl 2E, 4E-11 methoxy-3,7,11-trimethyl-2, 4-dodecadienoates) is a terpenoid and is considered to have higher potency and better field stability than do naturally occurring juvenile hormones (Henrick *et al.* 1976). Methoprene is especially effective against dipteran insects and has been widely used for control and eradication of numerous pests and insects that affect humans and livestock and in the storage of various agricultural products (Garg and Donahue 1989). The World Health organisation has approved its use in drinking water for control of mosquitoes. It was first registered as a biological pesticide by the EPA in the USA in 1975 and was subsequently re-classified by the EPA as a biochemical pesticide.

Formulations used include slow-release briquettes, sprays, foggers and baits (see next section).

TABLE 1: Physical properties of methoprene.

Appearance	Technical methoprene is a amber or pale yellow liquid with a faint fruity odor
Chemical Name	ispropyl(E,E)-(R,S)-11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate [1]
CAS Number	40596-69-8
Molecular Weight	310.48
Water Solubility	1.4 mg/L @ 25 C [1]
Solubility in Other Solvents	Miscible in organic solvents [1]
Melting Point	Not Available
Vapor Pressure	3.15 mPa @ 25 C [1]
Partition Coefficient	Not Available
Adsorption Coefficient	Not Available

[1] Kidd and James 1991

3. Methoprene-based products

A number of different formulations of methoprene are available, including charcoal formulations, micro-encapsulated products and briquettes for slow release. The various products have been aimed at different target pests, with the most common targets of products being mosquitoes, horn flies, ants and fleas. Some products used in product evaluations in the literature are now out of production.

Methoprene has been available in commercial products since the early 1970s. Access to the literature on methoprene is assisted by knowledge of the many trade names, products and experimental formulations which have contained methoprene over the years (Table 2). According to the Florida Agricultural Information Retrieval System (University of Florida, Institute of Food and Agricultural Sciences Cooperative Extension Service), as of 1997, the methoprene-based products available included: ZR-515, Altosid SR-10, XR-G and CP-10, Apex 5E, Diacan, Dianex, Kabat, Minex, Pharorid and Precor.

Among the products available are a number of formulations which improve stability, persistence or targeting against certain pests. Sand granule formulations have been used with success against mosquitoes (Rathburn and Boike 1975; Kline 1993). A field evaluation of methoprene (Altosid Liquid Larvicide) on Biodac (an inert granular carrier) against 3rd instar *Ae. sollicitans* larvae, conducted in a saltmarsh at Bombay Hook Wildlife Refuge, Delaware, USA, resulted in 50% adult emergence inhibition. Methoprene on Biodac presented no problems in terms of formulation or application and appears economically attractive relative to other granular larvicides (Wolfe *et al.* 1995).

Many of the formulations for use against mosquitoes and simuliids are slow release, to extend the effective control, such as Altosid SR-10. Microencapsulation is used as a slow release mechanism in Altosid SR10, CP10 and PS10, while briquette formulations are also common. Against *Cx. p. pallens* Noguchi and Ohtaki (1974) found that a slow-release formulation of methoprene was more potent than a concentrated methoprene solution against larvae of *Cx. p. pallens*.

Formulation can also be used to assist targeting of hosts. *Culex* mosquitoes are often difficult to kill because they feed on or near the surface, whereas most mosquitocidal agents settle quickly out of water. Formulations which remain on the surface longer are more effective against *Culex.* Schaefer *et al.* (1974) examined the distribution of Altosid in artificial ponds of an encapsulated formulation and found that the toxicant accumulated near the sides and bottom of the ponds, with little remaining near the water surface after 2-3 days. The settling effect was even more marked when a second formulation with particles of larger average diameter (100 μm) was used. When, however, a formulation on a charcoal base, Altosid 515225, was applied, more of the toxicant remained near the surface and the surface water was active against larvae of *Cx. tarsalis* for a correspondingly longer period. In field tests against larvae of *Ae. nigromaculis* and *Ae. melanimon*, Altosid 515225 at 0.0125 lb toxicant/acre was found to be more effective than the encapsulated formulation at 0.02-0.025 lb/acre (0.022-0.028 kg/ha)(Schaefer *et al.* 1974).

Another form of methoprene with extended persistence is formulation in boluses (cylindrical shaped mass of compounds for curative treatment of livestock). In studies in several states in

the USA in 1977-78, sustained-release boluses containing methoprene provided long-term control of the development of both *Haematobia irritans* and *Musca autumnalis* in the faeces of treated cattle. A 3% methoprene bolus inhibited the development of *H. irritans* in the faeces of a treated herd for 28-32 weeks. In other tests, 10% methoprene boluses provided 80-90% inhibition of the development of *M. autumnalis* in faeces for 10-12 weeks. The results indicated that bolus formulations could be an effective and practical method of administering methoprene to cattle for the control of the two flies (Miller *et al.* 1979).

TABLE 2: Methoprene products (historical, not all currently available)

Product	Target	Formulation	Company/Reference
Poultex 5E	mosquitoes		Farghal *et al.* 1988
Altosid 4E			Zoecon (company)
Altosid SR-10 and CP-10	Mosquitoes	10% methoprene microencapsulated	Zoecon (company)
Altosid PS10	Simuliids	Microencapsulation	Thompson and Adams 1979
Altosid 10F		10% methoprene, slow-release	Kikuchi *et al.* 1992
Altosid SR-10F	Mosquitoes and flies	Powdered charcoal	Spencer *et al.* 1979
Altosid XR Briquets	Mosquitoes	Slow release briquette 1.8% methoprene	Weathersbee and Meisch 1991
Altosid EC4			Zoecon (company)
Altosand	Mosquitoes	Sand	Schaefer and Dupras 1980
Altosid San 810	Mosquitoes		Romanowski *et al.* 1994
Altosid Pellet			
Altosid Liquid Larvicide	Mosquitoes	Liquid	Ali 1991
Altosid XR-G	Mosquitoes	Extended residual granule	Zoecon (company)
Apex 5E	sciarid flies		Zoecon (company)
Diacon			
Dianex	Coleoptera?		Klein and Burkholder 1984
Duplex		Methoprene + *Bti*	Zoecon (company)
Inhibitor	horn fly	3% methoprene	Fincher 1991
Juvenon (Cuba)			Ambros-Ginarte and Montada-Dorta 1992
Kabat	?		http://hammock.ifas.ufl.edu/txt/fairs/15424
Lafarex N, Lafarex N 86	Ants		Ryba *et al.* 1998
MoorMan's 650-B	Horn flies	0.02% methoprene mineral blocks	Moon *et al.* 1993
Pharorid (USA)	Ants	Commercially available ant bait	Williams and Vail 1993
Precor	Fleas	Aerosol, 0 075% methoprene, 0.5% permethrin, designed to use on carpets, furniture, pet beds etc.	http://www.killabug.com/about.html
Precor Plus Fogger	Fleas	0.075% methoprene, 0.5% permethrin	http://www.killabug.com/about.html
Viodat (Hungary)	Ants	Commercially available ant bait	Ryba *et al.* 1998
Viodat 10 MG	Mosquitoes	microgranules	Eross 1988
ZR-515	Flies		Zoecon (company)

3.1. Application rates

The United States EPA registered products recommend a minimum and maximum application rate, based on many years of field and laboratory studies using these specific formulations. Use of Altosid products at below label rates has led to periodic failures in mosquito control (D. Sullivan, pers. comm.).

TABLE 3: Recommended maximum and minimum application rates for Altosid products

Product	**Low rate**	**High Rate**	**Low a.i.** lb/acre (kg/ha)	**High a.i.** lb/acre (kg/ha)	**a.i./day** lb/acre (kg/ha)
Altosid ALL ounce/acre	3	4	0.01 (0.011)	0.0134 (0.015)	0.0014-0.0019 (0.0016-0.0022)
Concentrate	0.75	1	0.01 (0.011)	0.0134 (0.015)	0.0014-0.0019 (0.0016-0.0022)
30-day Briquets 1/100ft^2	1	1	0.0094 (0.105)	0.0094 (0.0105)	0.00031 (0.00035)
150 day XR 1/100ft^2	1	1	0.00145 (0.0016)	0.00145 (0.0016)	0.00001 (0.00001)
Pellets lbs/acre 30-day	2.5	10	0.1 (0.1)	0.4 (0.45)	0.0033-0.013 (0.0037-0.015)
XR-G lbs/acre 21-day	5	20	0.125 (0.14)	0.3 (0.34)	0.0059-0.014 0.0066-0.016)

Note: All Altosid products are S-methoprene except the 30-day briquette which is r,s-methoprene. Typically, the Altosid Pellets and XR-G granules remain effective for longer than the stated 30-days and 21-days, respectively.

4. Activity of methoprene

Methoprene and other IGRs are not generally directly toxic to insects, but have a delayed effect, usually expressed late in the life-cycle. Methoprene applied against mosquito larvae will inhibit adult emergence. Methoprene has toxicity to eggs in some cases. As well as causing mortality, sublethal effects from methoprene application include reduced fecundity, abnormal morphologies/development, alterations in pheromone production and altered behaviour.

4.1. Mode of action and effect of methoprene treatment

Methoprene, as a JHA, is not immediately toxic to insects. It disrupts the development of the insect and so causes death or reproductive failure at a specific time in the life-cycle, usually not the stage treated. Thus, treated larvae rarely die as larvae and are more likely to die as adults or during pupation. For example, mosquito larvae are the target stage for methoprene, but the mortality is not seen until lack of adult emergence. Fourth instar *Culex tarsalis* treated with methoprene were often unable to escape from the larval exoskelton during larval-pupal moult, or were unable to detach the legs and wings from the pupal exuvia when trying to emerge as adults, and so died (Arias and Mulla 1975).

During insect development, insects undergo changes at specific times (such as pupation) which are mediated by endogenous hormones. Juvenile hormone expressed at certain specific times leads to metamorphosis, however if present at other times, the presence of JH leads to morphogenetic abnormalities. This is the basic theory behind the use of methoprene and other JHAs. Similar influences can affect embryonic development. Morphogenetic abnormalities are usually irreversible and the most readily observed effect of IGRs. The extent and character of the response varies between insects, but generally it is the last instars of the larval or nymph form, or pupae, which are most affected. As the various life stages are affected differently, the longer the duration of exposure, the more complete is the inhibition of development (Staal 1975).

Methoprene causes various morphogenetic and biochemical changes in susceptible hosts, but as with other JHAs, its exact mode of action is not completely understood. Many specific factors have been attributed to the mortality caused by methoprene. In mosquitoes, methoprene appeared to interfere with lysis and re-absorption of old endocuticle, prohibiting the synthesis and deposition of new, well-structured procuticle by the epidermal cells. Disrupted mitochondria and numerous vesicles in other tissues examined were suggestive of possible changes in membrane selectivity and permeability (Cocke *et al.* 1979).

Application of methoprene up to 24 h after blood-feeding completely and irreversibly inhibited follicle maturation in the mosquito, *Aedes aegypti* (Judson *et al.* 1976). Normal functioning and degeneration of the nurse cells and follicular epithelium of the follicle was blocked in treated females. The compounds also caused an increase in the amount of protein contained in the ovaries of non-blood fed females, although the follicles did not mature. Downer *et al.* (1976) observed that glycogen reserves were depleted 48h after treatment of mosquito pupae, unlike in untreated pupae. This reduced energy reserves available to newly emerged adults which may contribute to premature mortality

Palaniswamy and Sivasubramanian (1977) found that, depending on the dose applied to flies, various morphogenetic effects were noticed on the abdomen, such as the failure of rotation of the male genitalia, reduction in the number of bristles and microtrichiae, irregular orientation of the bristles and inhibition of differentiation of the genitalia. When the affected flies were examined histologically, it was found that the muscles of the genitalia and abdomen had failed to develop, while the thoracic muscles exhibited dystrophic changes. Eventually, such changes resulted in the inhibition of adult eclosion. The head and thorax of *Sarcophaga bullata* larvae were resistant, but the abdomen was highly sensitive (Palaniswamy and Sivasubramanian 1977). At low doses, Sehnal and Zdarek (1976) reported incomplete rotation of male genitalia and deformation of the ovipositor; at higher doses the effects gradually spread from the tip of the abdomen towards the middle of the body of flies. In pupae of cyclorrhaphous flies, juvenoids impeded the proliferation and differentiation of the imaginal disks and of abdominal histoblasts. The highest doses of methoprene influenced the entire abdomen, size and pigmentation of the eyes, and development of hairs and sclerotisation of the integument on the head and thorax (Sehnal and Zdarek 1976).

4.2. Sublethal effects

In many cases, organisms receive sublethal doses of methoprene, which can have substantial effects on tissues, reproduction and behaviour. Such sublethal effects need to be considered when evaluating the safety of methoprene application in the environment. Sublethal effects have been reported in both susceptible and non-susceptible organisms.

4.2.1. Sublethal effects in mosquitoes

Low doses can cause reduced blood-feeding success (Ritchie *et al.* 1997), impaired ability of the completely emergent mosquito to fly (Kramer *et al.* 1993), and reduced fecundity (Firstenberg and Sutherland 1982; Sithiprasasna *et al.* 1996). Sublethal concentrations of methoprene used on larvae resulted in reduced glycogen reserves in adult *Ae. aegypti* and reduced longevity in females (Sawby *et al.* 1992). Sex ratios on *Ae. dirus* treated with sublethal doses of methoprene were changed from fewer to more males. (Sithiprasasna *et al.* 1996).

4.2.2. Effect on morphology/development

JHs have been shown to be responsible for many morphological changes in insects, including wing dimorphism and vitellogenesis. In many cases, experiments determining effects are conducted using methoprene as an analog of JH. Such changes may be artificially induced in non-target insects after broadcast application of methoprene. For example, methoprene application to the delphacid *Nilaparvata lugens* increased the proportion of brachypters (winged forms) when topically applied to female nymphs near the penultimate instar and stimulated vitellogenesis in (presumptive) macropters, which usually initiated the process about 24 h later than brachypters. (Iwanaga and Tojo 1986). For the caterpillar, *Spodoptera frugiperda,* sublethal levels of methoprene increased larval growth (Ross and Brown 1982).

Methoprene has been used extensively to study morphological development in social insects. For example, during laboratory studies in the USA, topical application of methoprene induced

soldier development in the ant *Pheidole bicarinata* (Wheeler and Nijhout 1981). Soldier induction took place if methoprene was present during a period of sensitivity that occurred during the last larval instar. The control of worker dimorphism appeared to be accomplished by controlling the timing of metamorphosis. In the social wasp *Polybia occidentalis*, methoprene application accelerated the rate of age polyethism and reduced longevity (O'Donnell and Jeanne 1993).

Methoprene application to *Diatraea grandiosella* affected the circadian system controlling the adult eclosion rhythm, with specific changes in photoperiod response, earlier eclosion and accelerated adult differentiation and emergence in both sexes (Yin *et al.* 1987).

Methoprene (ZR-515) had specific effects on ecdysone-induced metamorphic differentiation of cell cultures from *Drosophila* sp. The number of vesicles containing imaginal cuticular structures was reduced to 10% of control levels. Similarly, the differentiation of adult fat-body was partly inhibited by methoprene (Milner and Dubendorfer 1982).

4.2.3. Effect on behaviour

Methoprene exposure increased the long term flight behaviour of both male and female *Hippodamia convergens* and stimulated ovarian development in females (Rankin and Rankin 1980). Similarly methoprene treated chrysomelids, *Diabrotica virgifera,* flew both trivial and sustained flights that were significantly longer in duration and distance than those of untreated females. In this case, there seems to be a definitive window of migratory flight activity that can be temporally displaced by methoprene treatment (Coats *et al.* 1987). For the wasp, *P. occidentalis,* behavioural tasks such as nest maintenance and foraging occurred later in methoprene treated individuals than untreated (O'Donnell and Jeanne 1993).

4.2.4. Effect on pheromones

In several insects, JH (including methoprene) possibly mediates pheromone synthesis (Bridges 1982; Dickens *et al.* 1988). It has been shown in some hosts that application of methoprene enhanced pheromone production (Pierce *et al.* 1986). JH may also play a role in controlling chemosensillar sensitivity (Angioy *et al.* 1983). In *Anthonomus grandis,* methoprene application decreased the sensitivity of antennal olfactory receptors (Dickens *et al.* 1988).

Topical application of 10 µg methoprene to adult females of the lepidopteran, *Choristoneura fumiferana,* significantly reduced their electroantennogram (EAG) responses to their own synthetic female sex pheromone, provided the recordings were performed at least 10-15 h after treatment (Palaniswamy *et al.* 1979). Newly emerged moths were more sensitive to methoprene treatment than older ones.

4.2.5. Effect on reproduction and sex ratios

Sterility and reduced fecundity commonly result from methoprene treatment (eg. Naqvi *et al.* 1978) and methoprene has been used in the study of many insect processes, such as vitellogenin synthesis (eg. Couble *et al.* 1979; Raikhel and Lea 1990), ovarian development (eg. O'Meara and Lounibos 1981) and diapause (eg. Mitchell 1981).

Laboratory investigations in Victoria, Australia, were conducted on sublethal effects of methoprene on insect pests of stored products (Amos *et al.* 1978). The productivity of adults of *Tribolium castaneum* that were reared in treated flour was found to be impaired, depending on the concentration of the compounds, whether or not the individual was morphologically deformed, and its sex. The authors suggested that these sterilising effect enhanced the potential of the compounds as protectants for stored products.

Sublethal doses of methoprene can cause changes in sex ratios. For example, horn fly parasites, *Spalangia cameroni,* were largely unaffected by methoprene but exposure did change the sex ratio in their progeny (Roth 1989).

Topical application of methoprene (ZR-515) to the fly *Calliphora vomitoria* caused acceleration of ovarian growth (Trabalon and Campan 1984) while topical application of sublethal concentrations of methoprene to pupae of the scarab coconut pest, *Oryctes rhinoceros*, adversely effected the reproductive system of adult males (Jacob 1989).

4.3. Developmental stage affected

Methoprene is generally used against larval/nymph forms, although the effect may be seen in pupae and adults. As methoprene interferes with the natural development processes and stops metamorphosis occurring, larvae never turn into adults, and the insects cannot reproduce. However, methoprene does not affect the same development stage in all insects. Methoprene might be ineffective against late larvae of one species, methoprene may kill eggs of the same species. Methoprene is not usually effective against adult insects and is therefore sometimes used in combination with an adulticide. For example, methoprene and pyrethrin combination products have been used in premise applications to control dog and cat fleas (Garg and Donahue 1989).

4.3.1. Ovicidal activity

Few studies have included treatment of insect eggs with methoprene. Where eggs have been directly treated, inhibition of hatching has been found, but effects have also been noted in the fitness of the surviving insects. Treating eggs of the mosquito, *Ae. aegypti,* continuously for 7 days with methoprene resulted in 13-79% inhibition of hatching, but also caused 20-100% mortality in resulting pupae from surviving eggs (Naqvi *et al.* 1976). At low doses (<0.5 ppm), methoprene had little effect on metamorphosis and adult emergence of *Ae. aegypti* but significantly reduced fecundity and fertility (Naqvi *et al.* 1976). The percentages of females laying eggs were 53, 48 and 39.5 at 0.004, 0.04 and 1 ppm, respectively, and egg viability was reduced to 58.9, 26.5 and 8.2% at these dosages as compared with 93% in those from untreated females. Sterility caused by 0.004, 0.04 and 1.0 ppm was 41.1, 73.5 and 91.8%, respectively. Against the mushroom pest, *Lycoriella mali,* methoprene was slightly ovicidal to 24-h-old eggs (22.4-27.1% mortality compared with 11.2% in the untreated control) but not 48 h after oviposition (Keil and Othman 1988).

Gonen and Schwartz (1979) studied the ovicidal activity of methoprene on 0-24 hr old eggs of the lepidopteran, *Ephestia cautella.* The eggs were exposed on filter papers treated with 0.6 to 73.7 mg methoprene/m^2. Effects were not seen on eggs, but in emerging larvae and adults. At the higher doses of 44 to 73 mg/m^2, there was an almost 60% reduction in moth

emergence. In another study on the same insect, exposure of young (pre-blastokinetic) eggs of *E. cautella* to very high concentrations of methoprene (10g/m^2) for 24 hours resulted in non-viable first instar larvae, while older eggs were less affected (Shaaya and Pisarev 1986). Methoprene in wheat flour showed strong ovicidal action against coleopteran eggs (*Oryzaephilus surinamensis, Rhyzopertha dominica, Tribolium castaneum*)(Mian and Mulla 1982a).

A comparative test against *Gryllus bimaculatus* (Gryllidae: Orthoptera) dipping eggs in three different IGRs showed that methoprene (Altosid) was over 20 times as effective as an ovicide as hydroprene (Altozar), which in turn was about 5 times as effective as farnesol [3,7,11-trimethyl-2,6,10-dodecatrien-1-ol] (Crochard 1975).

4.3.2. Larvicidal and pupicidal activity

Methoprene is commonly used against larvae or nymphs, especially for dipterans such as mosquitoes. The effect of use as a larvicide is most often seen in the pupal mortality and inhibition of adult emergence. With mosquitoes, the lack of direct larvicidal activity is the key to preserving the natural aquatic food chain, since mosquito larvae can be a food source for other organisms.

When fourth instar larvae of *Culex pipiens fatigans* were exposed to methoprene, effects were not manifested in treated larvae but only in the resulting pupae and adults (Georghiou and Lin 1975). Methoprene treatment of *Anopheles stephensi* did not cause larval mortality, but 75% of pupae could not shed their exuviae and died within 2-3 h. Further development of the remaining 25% varied according to the dosage of methoprene applied (Raj *et al.* 1978). Adult emergence from pupae decreased with increasing treatment rate.

When third instar larvae of *Haematobia irritans* were exposed to methoprene in cow dung at a concentration of 0.2 ppm in studies in Texas, subsequent adult emergence was inhibited by 94.5% (Gingrich and Hopkins 1977). However, there was no effect when first or second instar larvae or pupae were exposed to the same treatment and no accumulative effect was noted. Apparently, third instar larvae absorbed enough methoprene to cause inhibition of adult emergence.

In some cases, methoprene used against larvae has resulted in larval mortality. Farghal and Temerak (1981) found that methoprene was directly toxic to the mosquito *Culex molestus* larvae, but also prolonged larval and pupal developmental periods, inhibited adult emergence and affected the sex ratio. In the field, toxic effects on larvae and inhibition of adult emergence were observed in *Cx. molestus, Culiseta longiareolata* and *Eristalis* sp. (Farghal and Temerak 1981).

Susceptibility of mosquitoes and other insects to methoprene gradually increases during larval development, but pupae are more resistant than larvae (Amin and White 1984; Noguchi and Ohtaki 1974; Naqvi *et al.* 1976; 1978). Susceptibility of *Culex pipiens pallens* gradually increased during larval development until pupation. The EC_{50}s of methoprene against *Cx. p. pallens* were 0.03 ppm for late third-instar larvae, 0.02 ppm for late fourth-instar larvae, 0.0006 ppm for pharate pupae and 1 ppm for day-old pupae. The gradual increase in susceptibility to the compound until pupation and sudden decrease after pupation accords well with theories on the secretion and action of juvenile hormone (Noguchi and Ohtaki 1974). Exposure of fourth instar *Ae. aegpyti* larvae for 24, 72 or 120 h to 0.0001-1 ppm methoprene

showed that the oldest ones were the most susceptible (Naqvi *et al.* 1976). Georghiou and Lin (1974) showed that *Culex* mosquitoes were most sensitive to methoprene 10-30h before pupation. In other hosts, similar results are reported showing later instars are more susceptible than early instars (eg. Mian and Mulla 1982a). In simuliids, methoprene caused pupal rather than larval mortality (Thompson and Adams 1979).

In contrast, laboratory tests with methoprene (Altosid) showed no differences in susceptibility between third and fourth instar larvae, of *Ae. taeniorhynchus* found to be considerably more susceptible to methoprene than was *Cx. nigripalpus*. No difference in kill was noticed between second and third instar larvae in the small-plot field tests (Rathburn and Boike 1975).

4.3.3. Adults

A few studies have reported successful application of methoprene against adults, although in some cases effects were not seen until abnormal egg laying by females. For example, incorporation of methoprene into the diet of adult insects caused substantial reductions in oviposition of both *Tribolium castaneum* and *T. confusum* (Loschiavo 1975).

In females of *Anopheles stephensi* fed 10% glucose solution mixed with 0.1-1% methoprene, before a blood meal, over 80% of the eggs in the batch laid after the first blood-meal were small, white, deformed and fragile, and no larvae hatched from them (Divakar and Rao 1975). Even the lowest concentration of methoprene resulted in the production of such abnormal eggs, which continued to a declining extent for 10 days. Divakar and Rao (1975) suggested the analogue acts on the follicle cells of the ovary, interfering with the normal formation of the chorion and the development of the oocyte. Direct adulticidal action was rare. The highest concentration caused some mortality among the *An. stephensi* females but the lowest rate had virtually no effect.

Occasionally, there is a difference in susceptibility between male and females adults. Spraying larval stages of the diaspids, *Chrysomphalus aonidum* and *Aonidiella auranti* with methoprene at 0.15-0.1% resulted in differential inhibition of emergence of males these species. Females of the two diaspids were not affected when subjected to methoprene as second instars (Peleg and Gothilf 1981).

5. Susceptible insect and mite species

Methoprene causes mortality among many species, including insects from 12 orders and mites. The majority of records are from dipteran species. Relative susceptibility varies greatly, however, among insect species. Lethal doses for mosquitoes are most often measured as ppb while other classes of insects, such as Lepidoptera, require dose levels around 100x higher for equivalent mortality. Many records of hosts other than Diptera show very low susceptibility to methoprene. In general, methoprene used at rates used in the field against mosquitoes is unlikely to affect insects outside the target host group.

5.1. Records of susceptible insects and mites

Methoprene is lethal to a wide range of insects and mites, and is particularly toxic to Diptera (e.g. Henrick *et al.* 1976). A summary of reports of mortality among insects and mites after methoprene application is given in Table 4. While the range of species showing susceptibility appears to be relatively broad, covering insects from 12 orders and mites, the relative susceptibility of each organism varies greatly between organisms (Table5). In some cases, high doses were required to achieve mortality, while in others very low doses gave comparable results (see also next section). Table 4 also takes no account of the method of application, which can influence susceptibility.

Diptera appear to be among the most susceptible order. In an extensive comparison of IGRs, Henrick *et al.* (1976) tested methoprene and other variants against Diptera, Lepidoptera, Coleoptera and Hemiptera representatives and found methoprene one of the most effective against *Ae. aegypti*, but still quite effective against the other insect groups. Relative susceptibility among mosquitoes varies and many workers have found that *Aedes* spp. are more susceptible to methoprene than *Culex* spp. (e.g. Rathburn and; Boike 1975; Norland and DeWitt 1975; Ritchie *et al.* 1997).

Relative susceptibility also varies between closely related species. Both the grain pests, *Sitophilus oryzae* and *S. granarius* are listed in 4, however larvae of *S. oryzae* proved more susceptible than those of *S. granarius* to methoprene incorporated into an artificial diet (Baker and Lum 1976). Pupal mortality of *S. oryzae* became significant after ingestion of the diet incorporating methoprene at 20 ppm or more, although concentrations of up to 150 ppm did not prevent adult emergence in *S. granarius*. Researchers using different formulations and application methods have not always agreed on susceptibility. *S. oryzae* was reported susceptible by Loschiavo (1976) and Baker and Lum (1976). However Daglish *et al.* (1995) and McGregor and Kramer (1975) found methoprene treatment of *S. oryzae* relatively ineffective, even at 10 ppm.

Methoprene is rarely recommended for use against lepidopteran pests, however it can be effective in the laboratory. Sehnal *et al.* (1976) found methoprene the most active compound among 32 juvenoids against two caterpillars, with *Autographica gamma* affected at doses of 0.05 µg/example and *Spodoptera littoralis* at 0.8 µg. Two other caterpillars required much higher doses: *Mamestra brassicae* at 6 µg and *Agrotis ipsilon* at 50 µg/example. Lepidoptera have the second largest number of species within one order recorded as methoprene sensitive in Table 4, although toxicity is low for lepidopterans compared with dipterans (Table 5).

The purpose of reviewing susceptibility records is to determine the level of specificity of methoprene. Methoprene is toxic to insects other than dipterans, however susceptibility decreases significantly in other orders. Therefore, field doses used against mosquitoes would be unlikely to be high enough to cause mortality in most terrestrial insects. Aquatic insects are discussed below (section 9). There are a few beneficial species among those listed in Table 4, such as the parasitoid *Microctonus aethiopoides* (Hymenoptera: Braconidae), *Eristalis* sp. (Diptera: Syrphidae), *Cucelatoria* sp. (Diptera: Tachinidae) and *Chrysoperla carnea* (Neuroptera: Chrysopidae). However, these make up a small percentage of recorded susceptible insects, which probably reflects the research aims rather than a true reflection of beneficial susceptibility.

TABLE 4: Insects and mites susceptible to methoprene (Compiled mainly through CAB abstracts).

Organism	Representative references
Coleoptera: Anobiidae	
Lasioderma serricorne	Marzke *et al.* 1977; Belles 1979; Manzelli 1982; Benezet and Helms 1994
Coleoptera: Bostrichidae	
Rhyzopertha dominica	McGregor and Kramer 1975; Amos and Williams 1977; Daglish *et al.* 1995[1]
Coleoptera: Bruchidae	
Callosobruchus maculatus	Hussein *et al.* 1982; Hussein 1983
Coleoptera: Chrysomelidae	
Dicladispa armigera	Hazarika and Baishya 1996; 1997
Coleoptera: Coccinellidae	
Chilocorus bipustulatus	Peleg 1983
Coccinella septempunctata	Kismali and Erkin 1984
Epilachna chrysomelina	Kinawy and Hussein 1987
Coleoptera:Curculionidae	
Sitophilus granarius	Loschiavo 1976; Baker and Lum 1976; Edwards and Short 1984
S. oryzae	Loschiavo 1976; Baker and Lum 1976; Daglish *et al.* 1995[1]
S. zeamais	Daglish *et al.* 1995[1]
Coleoptera: Dermestidae	
Trogoderma glabrum	Klein and Burkholder 1984; El-Sayed 1984
Coleoptera: Scarabaeidae	
Onthophagus gazellus	Blume *et al.* 1974
Oryctes rhinoceros	Dhondt *et al.* 1976
Coleoptera: Scolytidae	
Dendroctonus frontalis	Sambeek and Bridges 1980; Sambeek *et al.* 1981
D. pseudotsugae	Ibaraki and Sahota 1976
Coleoptera: Silvanidae	
Oryzaephilus mercator	Loschiavo 1976
O. surinamensis	McGregor and Kramer 1975; Loschiavo 1976; Daglish *et al.* 1995[1]
Coleoptera: Tenebrionidae	
Alphitobius diaperinus	Edwards and Abraham 1985
Tenebrio molitor	Solomon and Metcalf 1974; Styczynska 1979
Tribolium castaneum	Loschiavo 1976; Amos *et al.* 1977; Daglish *et al.* 1995[1];Hoppe 1981

T. confusum	McGregor and Kramer 1975; Loschiavo 1976; Amos *et al.* 1977
Dictyoptera: Blattellidae	
Blattella germanica	Edwards 1976
Periplaneta americana	Edwards 1992
Diptera: Anthomyiidae	
Delia radicum	Young and Gordon 1987; Young *et al.* 1987
Diptera: Agromyzidae	
Liriomyza trifolii	Parrella and Robb 1982; Parrella *et al.* 1982
Diptera: Culicidae	
Aedes aegypti	Pridantseva *et al.* 1978; Spencer and Olson 1979; Failloux *et al.* 1990
Ae. albopictus	Buei *et al.* 1975; Farghal *et al.* 1988; Marten *et al.* 1993
Ae. canadensis	Rodrigues and Wright 1978; McCarry 1996
Ae. cinereus	Rodrigues and Wright 1978
Ae. communis	Baldwin and Chant 1976
Ae. daitensis	Toma *et al.* 1990
Ae. detritus	Gradoni *et al.* 1976; Majori *et al.* 1977
Ae. dorsalis	Kramer *et al.* 1993
Ae. epactius	Spencer and Olson 1979
Ae. excrucians	Rodrigues and Wright 1978
Ae. fitchii	Rodrigues and Wright 1978; McCarry 1996
Ae. funereus	Ritchie *et al.* 1997
Ae. implicatus	Rodrigues and Wright 1978; McCarry 1996
Ae. intrudens	McCarry 1996
Ae. iriomotensis	Toma *et al.* 1990
Ae. melanimon	Norland and DeWitt 1975
Ae. nigromaculis	Norland and DeWitt 1975
Ae. notoscriptus	Ritchie *et al.* 1997
Ae. polynesiensis	Failloux *et al.* 1990
Ae.. provocans	McCarry 1996
Ae. riversi	Toma *et al.* 1990
Ae. sollicitans	McAlonan *et al.* 1976; Spencer and Olson 1979
Ae. stimulans	Baldwin and Chant 1976; Rodrigues and Wright 1978; McCarry 1996
Ae. taeniorhynchus	Giglioli 1975; Rathburn and Boike 1975; 1977; Kline 1993
Ae. togoi	Buei *et al.* 1975
Ae. triseriatus	Wells *et al.* 1975
Ae. vexans	Batzer and Sjogren 1986; Sanzone and Rupp 1995
Ae. vigilax	Ritchie *et al.* 1997
Anopheles annularis	Baruah and Das 1996
An. crawfordi	Baruah and Das 1996
An. dirus	Sithiprasasna *et al.* 1996
An. farauti	Ritchie *et al.* 1997
An. freeborni	Case *et al.* 1977
An. gambiae	Busvine *et al.* 1976
An. nuneztovari	Moreno and Scorza 1983
An. quadrimaculatus	Dame *et al.* 1976
An. stephensi	Hatakoshi *et al.* 1987; Raj *et al.* 1978
An. sundaicus	Imai *et al.* 1987
An. vagus	Baruah and Das 1996
Armigeres subalbatus	Buei *et al.* 1975; Toma *et al.* 1990
Culex annulirostris	Ritchie *et al.* 1997
Cx. fatigans	Moreno and Scorz 1983
Cx. fuscanus	Toma *et al.* 1990
Cx. gelidus	Baruah and Das 1996
Cx. infantulus	Buei *et al.* 1975
Cx. nigripalpus	Rathburn and Boike 1975; Dame *et al.* 1976

Cx. orientalis	Buei *et al.* 1975
Cx. peus	Pfunter 1978
Cx. pipiens	Ibrahim 1990
Cx. pipiens fatigans	Brown and Brown 1974
Cx. pipiens molestus	Pridantseva *et al.* 1979
Cx. pipiens pipiens	Pridantseva and Volkova 1976
Cx. pipiens pallens	Noguchi and Ohtaki 1974; Buei *et al.* 1975; Hatakoshi *et al.* 1987
Cx. pipiens quinquefasciatus	Axtell *et al.* 1975
Cx. quinquefasciatus	Farghal *et al.* 1988; Navarro-Ortega *et al.* 1991; Failloux *et al.* 1990
Cx. restuans	Knepper *et al.* 1992
Cx. salinarius	Dame *et al.* 1976
Cx. sitiens	Ritchie *et al.* 1997
Cx. tarsalis	Muira *et al.* 1978
Cx. tritaeniorhynchus	Noguchi and Ohtaki 1974; Buei *et al.* 1975; Toma *et al.* 1990
Cx. univittatus	Abdel-Aal 1995
Cx. vishnui group	Baruah and Das 1996
Culiseta incidens	Kramer 1990
Cs. inornata	Norland and Mulla 1975
Cs. longiareolata	Farghal 1987
Cs. melanura	Woodrow *et al.* 1995
Coquillettidia perturbans	Ranta *et al.* 1994; Sanzone and Rupp 1995
Mansonia spp.	Krishnamoorthy *et al.* 1993
Psorophora columbiae	Spencer and Olson 1979; Weathersbee and Meisch 1991
P. confinnis	Mulla and Darwazeh 1975; Steelman *et al.* 1975
Diptera: Calliphoridae	
Cochliomyia hominivorax	Wright *et al.* 1974
Diptera: Cecidomyiidae	
Heteropeza pygmaea	Hsieh and Hsu 1983
Diptera: Ceratopogonidae	
Culicoides circumscriptus	Takahashi *et al.* 1985
Culicoides variipennis	Apperson and Yows 1976
Diptera: Chironomidae	
Chironimids	Ali 1996
Chironomus attenuatus	Pelsue *et al.* 1974
C. californicus	Pelsue *et al.* 1974
C. stigmaterus	Mulla *et al.* 1974
C. yoshimatsui	Tabaru 1985; Kamei *et al.* 1982
Cricotopus sp	Pelsue *et al.* 1974
Procladius culiciformis	Pelsue *et al.* 1974
Tanypus grodhausi	Mulla *et al.* 1974
Tanytarsus sp.	Pelsue *et al.* 1974
Diptera: Drosophilidae	
Drosophila melanogaster	Wilson *et al.* 1987
Diptera: Hippoboscidae	
Melophagus ovinus	Hopkins and Chamberlain 1978
Diptera: Muscidae	
Musca autumnalis	Miller and Uebel 1974; Miller *et al.* 1979
M. domestica	Miller and Uebel 1974; Das and Vasuki 1992
Haematobia irritans	Harris *et al.* 1974; Paysinger and Adkins 1977
Stomoxys calcitrans	Wright and Jones 1976;Wright and Smalley 1977
Diptera: Oestridae	

Oestrus ovis	Prasert *et al.* 1975
Hypoderma bovis	Barrett *et al.* 1978
H. lineatum	
Diptera: Phoridae	
Megaselia halterata	White 1979; Cantelo 1985
Diptera: Psychodidae	
Psychoda alternata	Kamei *et al.* 1993
Diptera: Sarcophagidae	
Sarcophaga bullata[3]	Loof *et al.* 1979
Diptera: Sciaridae	
Bradysia spp.	Hamlen and Mead 1979
Bradysia coprophila	Lindquist *et al.* 1985
B. tritici	Lin 1980
Lycoriella auripila	White 1979; Eicker and Ludick 1993
L. fucorum	Semenova *et al.* 1995
L. mali	Keil and Othman 1988
L. solani	Czajkowska *et al.* 1981
Diptera: Simuliidae	
Simulium arcticum	Cumming and McKague 1973
S. canadense	Cumming and McKague 1973; Thompson and Adams 1979
S. decorum	Cumming and McKague 1973; McKague and Wood 1974
S. hunteri	Cumming and McKague 1973
S. luggeri	Sanzone and Rupp 1995
S. pictipes	Garris and Adkins 1974
S. pugetense	Cumming and McKague 1973
S. tuberosum	McKague and Wood 1974
S. venustum	Cumming and McKague 1973; Thompson and Adams 1979
S. verecundum	Dove and McKague 1975
S. vittatum	Thompson and Adams 1979
Prosimulium mixtum	Thompson and Adams 1979
Diptera: Syrphidae	
Eristalis sp.	Farghal and Temerak 1981
Metasyrphus corollae	Ruzicka *et al.* 1974
Diptera: Tachinidae	
Eucelatoria sp.	Divakar 1980
Pales pavida	Riviere 1975
Diptera: Tephritidae	
Ceratitis capitata	Orphanidis 1976; Martinez-Pardo *et al.* 1979
Dacus oleae	Fytizas 1975; Orphanidis and Kapetanakis 1979
D. cucurbitae	Saul and Seifert 1990
D. dorsalis	Saul and Seifert 1990
Ephemoptera	
Callibaetis pacificus	Norland and Mulla 1975
Hemiptera: Aleyrodidae	
Aleyrodes proletella	Thompson and Goodwin 1983
Trialeurodes vaporariorum	Giustina 1975; Giustina *et al.* 1976
Hemiptera: Aphididae	

Lipaphis erysimi	Arora and Sidhu 1992
Myzus persicae[3]	Giustina 1975; Hamlen 1977; Kismali 1979
Hemiptera: Cimicidae	
Cimex lectularius	Takahashi and Ohtaki 1975
Hemiptera: Coccidae	
Ceroplastes floridensis	Peleg and Gothilf 1981
C. pseudoceriferus	Kamei and Asano 1976
Pseudococcus longispinus[3]	Hamlen 1977
Quadraspidiotus perniciosus	Kozar and Varjas 1976
Saissetia oleae	Peleg and Gothilf 1981; Lampson and Morse 1992
Hemiptera: Diaspididae	
Aonidiella aurantii	Boboye and Carman 1975; Peleg and Gothilf 1981
Chrysomphalus aonidum	Peleg and Gothilf 1981
Epidiaspis leperii	El-Kareim *et al.* 1988; 1989
Hemiptera: Lygaeidae	
Oncopeltus fasciatus	Solomon and Metcalf 1974; Brown *et al.* 1978
Hemiptera Piesmatidae	
Piesma quadratum	Lefevre 1976
Hemiptera: Pseudococcidae	
Phenacoccus solani[3]	Hamlen 1977
Planococcus citri	Hamdy 1984
Hemiptera: Psyllidae	
Cacopsylla pyri	Baldassari *et al.* 1997
Hemiptera: Pyrrhocoridae	
Dysdercus cingulatus	Zutshi *et al.*1979; 1980
D. koenigii	Nikhat *et al.* 1984
Pyrrhocoris apterus	Styczynska 1979
Hemiptera: Reduviidae	
Rhodnius prolixus	Pridantseva *et al.* 1978; Kul'-kova *et al.* 1983; Langley *et al.* 1990
Heteroptera: Naucoridae	
Ilycorcis cimicoides	Gelbic *et al.* 1994
Hymenoptera:Aphelinidae	
Aphytis mytilaspidis	El-Kareim *et al.* 1988
Hymenoptera: Bethylidae	
Parasierola nephantidis	Sundaramurthy *et al*. 1985; Jayaraj 1989
Hymenoptera: Braconidae	
Apanteles congregatus	Beckage and Riddiford 1982
Bracon brevicornis	Sundaramurthy *et al.* 1985; Jayaraj 1989
Microctonus aethiopoides	Flessel 1978
Oenonogastra microrhopalae	Oetting 1985
Hymenoptera: Chalcidoidea	
Coccophagus pulvinariae	Peleg and Gothilf 1980
Hymenoptera: Formicidae	
Camponotus pennsylvanicus	Fowler and Roberts 1982

Monomorium destructor	Edwards 1992
M. pharaonis	Edwards 1976, 1977; Edwards and Clarke 1978
Paratrechina fulva	Chacon-de-Ulloa *et al.* 1994
Pheidole megacephala	Breed *et al.* 1981; Horwood 1988
P. siniatica	Breed *et al.* 1981
Solenopsis invicta	Bigley and Vinson 1979
Wasmannia auropunctata	Ulloa-Chacon and Cherix 1989
Hymenoptera: Pteromalidae	
Nasonia vitripennis	Fashing and Sagan 1979
Hymenoptera: Vespidae	
Polybia occidentalis	O'-Donnell and Jeanne 1993
Vespula maculifrons	Parrish and Roberts 1983
Isoptera: Rhinotermitidae	
Coptotermes formosanus	Su *et al.* 1985; Haverty *et al.* 1989
Reticulitermes flavipes[4]	Haverty and Howard 1979; Howard 1980
Reticulitermes virginicus	Haverty and Howard 1979
Isoptera: Termitidae	
Odontotermes guptai[3]	Varma 1982
Lepidoptera: Arctiidae	
Earias insulana	Hussain and Askari 1975
Spilosoma obliqua	Qamar *et al.* 1994
Lepidoptera: Bombycidae	
Bombyx mori	Gaaboub *et al.* 1990
Lepidoptera: Gelechiidae	
Pectinophora gossypiella	Abdel-Sattar and El-Guindy 1988
Phthorimaea operculella	Reddy and Urs 1988; Hamdy and Salem 1988
Sitotroga cerealella	Babu and Panwar 1976; Stockel and Edwards 1981
Lepidoptera: Geometridae	
Calospilos suspecta	Jiang *et al.* 1996
Lambdina fiscellaria	Retnakaran *et al.* 1974
Lepidoptera: Lasiocampidae	
Malacosoma disstria	Retnakaran and Smith 1976
Lepidoptera: Lymantriidae	
Lymantria dispar	Sehnal *et al.* 1976
L. monacha	
Lepidoptera: Noctuidae	
Achaea janata	Shaheen and Osmani 1980; John and Muraleedharan 1993
Agrotis ipsilon	Sehnal *et al.* 1976
Autographa gamma	Sehnal *et al.* 1976
Earias vitella	Mandal and Choudhuri 1984
Heliothis armigera	El-Guindy *et al.* 1980a
Mamestra brassicae	Sehnal *et al.* 1976
Pseudoplusia includens[3]	Mohamed *et al.* 1984
Spodoptera litura	Sundaramurthy 1976
S. littoralis	Sehnal *et al.* 1976; Radwan *et al.* 1978; El-Guindy *et al.* 1983a
Trichoplusia ni	Campero and Haynes 1990
Lepidoptera: Plutellidae	

Plutella xylostella	Hong 1981; Fahmy *et al.* 1991
Lepidoptera: Pyralidae	
Corcyra cephalonica	Ambika and Abraham 1982; Chakravorty *et al.* 1986
Diatraea grandiosella	Chippendale and Yin 1976
Ephestia cautella	Tan 1975; Schwartz and Gonen 1977; Vick *et al.* 1985
E. elutella	Manzelli 1982
E. kuehniella	Tan 1975; Tan and Tan 1978
Galleria mellonella	Verenini 1984
Plodia interpunctella	McGregor and Kramer 1975
Scirpophaga incertulas	Roychoudhury and Chakravorty 1987
Lepidoptera: Tortricidae	
Choristoneura fumiferana	Retnakaran *et al.* 1977
C. occidentalis	Robertson and Kimball 1979
Cydia pomonella	MacFarlane and Jameson 1974; Brown and Brown 1982
C. molesta	MacFarlane and Jameson 1974
Rhyacionia buoliana	Burzynski *et al.* 1981
Neuroptera: Chrysopidae	
Chrysoperla carnea	Romanchenko *et al.* 1987
Orthoptera: Acrididae	
Schistocerca gregaria	El-Guindy *et al.* 1981
Orthoptera: Gryllidae	
Gryllus bimaculatus (eggs)	Crochard 1975
Phthiraptera: Pediculidae	
Pediculus humanus	Takahashi and Ohtaki 1975
Psocoptera: Lipscelididae	
Liposcelis bostrychophila	Buchi 1994
Siphonaptera: Ceratophyllidae	
Oropsylla fotus	Lang and Chamberlain 1986
Siphonaptera: Pulicidae	
Ctenocephalides felis	Osbrink *et al.* 1986
Xenopsylla cheopis	Chamberlain and Becker 1977; 1978; Chamberlain *et al.* 1988
Acari: Argasidae	
Argas walkerae[3]	Gothe and Morawietz 1979
Acari: Phytoseiidae	
Amblyseius brazilli[3]	El-Banhawy 1977; 1980
Phytoseiulus persimilis	Madanlar and Kismali 1994
Acari: Psoroptidae	
Psoroptes cuniculi	Il'-yashchenko 1981
Acari: Pyroglyphidae	
Dermatophagoides farinae	Saleh *et al.* 1976; Downing *et al.* 1990; Stepanova and Kostina 1994
D. pteronyssinus	Stepanova and Kostina 1994
Acari: Tetranychidae	
Tetranychus arabicus	El-Halawany *et al.* 1981
T. desertorum	El-Banhawy 1980
T. urticae[2]	Hamlen 1977

Acarina: Ixodidae	
Amblyomma hebraeum	Solomon and Evans 1977
Boophilus decoloratus	Solomon and Evans 1977
B. microplus	Solomon and Evans 1977

[1] Used in conjunction with chlorpyrifos-methyl
[2] Used in conjunction with cyclopropane miticide, and resmethrin formulation
[3] Low toxicity
[4] Not directly toxic

5.2. Comparative toxicity in the laboratory

Methoprene is more toxic to dipterans than other orders, except fleas, although it has relatively low LC_{50} or EC_{50}s against a number of non-mosquito hosts. Generally, lower doses are required against young larvae or nymphs than older stages, pupae or adults. Studies on the same host under different experimental conditions result in vastly different measures of toxicity of methoprene.

The extensive list of insects susceptible to methoprene (Table 4) needs to be evaluated in the context of comparative toxicity. Unlike some of the more specific agents, methoprene is not active against only one group of invertebrates. Susceptibility is a sliding scale of effects dependent upon dose level and host stage, including sublethal effects. A common measure of comparative susceptibility is represented by the LC_{50} (lethal concentration required to kill 50% of the treated individuals), LC_{90} and also the EI_{50}, EC_{50} or IC_{50} (concentration required to reduce emergence of adults to 50%). Estimates of these values vary due to formulation, application method and environmental parameters, but remain a useful measure of comparative toxicity.

Comparative toxicity, as measured by EI_{50} or LC_{50}, demonstrates a wide range of effects of methoprene (Table5), including varying susceptibility for the same species when measured by different researchers using different application methods and products/formulations. Generally, methoprene was potent against mosquitoes, especially *Aedes* spp. LC_{50} for *Aedes* spp. were most frequently around 0.0001 ppm or less while, on average, the LC_{50} for *Culex* was slightly higher. However, the dose required for effective control of the mosquito *Armigeres subalbatus* was high, 0.15-14 ppm (Table5). Interestingly, strains of *Cx. quinquefasciatus* from Cuba and France varied in their susceptibility in a single study, with fourth instar larvae from Cuba showing less susceptibility than the French strain (Navarro-Ortega *et al.* 1991).

It is obvious from studies on susceptibility of developmental stages of the same host that older larvae were more susceptible than younger larvae, but pupae were less susceptible (Table 5). For example, Noguchi and Ohtaki (1974) showed decreasing resistance of *Cx. p. pallens* to methoprene with increasing age of larvae and pharate pupae, however pupae were relatively resistant.

In comparison to mosquitoes, other orders of insects are generally less susceptible. Pridantseva *et al.* (1978) found that methoprene against *Ae. aegypti* was highly active in laboratory tests with larvae: the LC_{50} was 0.0008-0.015 mg/litre. However, fifth instar nymphs of *Rhodnius prolixus* were less sensitive to these compounds, with doses of 3-33 µg/insect producing a moderate effect. These authors thought that the effect of methoprene was therefore relatively specific. Altosid (methoprene-based product) was found to have an ED_{50} of 0.026 µg/g for the coleopteran *Tenebrio molitor* and 5.0 µg/g for the hemipteran *Oncopeltus fasciatus* (Solomon and Metcalf 1974). For the lepidopteran *S. litturalis*, the EC_{50} was over 60 ppm (Mane and Subrahmanyam 1996).

TABLE 5: Published reports of concentrations of methoprene (ppm) required to inhibit 50% of adult emergence (IC_{50}) or cause 50% mortality (LC_{50}).

Target	Stage[1]	Mortality measure (ppm)		Formulation	Reference
		LC_{50}	$IC/EI/EC_{50}$ [2]		
Diptera: Culicidae					
Aedes aegypti		0.0221		Altosid	Zebitz 1986
Ae. aegypti		0.000077			Spencer and Olson 1979
Ae. aegypti	4th	0.0008-0.015			Pridantseva *et al.* 1978
Ae. aegypti	4th	0.013		Altosid SR-10	Pridantseva and Volkova 1976
Ae. aegypti	4th		0.00038		Buei *et al.* 1975
Ae. aegypti	3rd	0.000397		Altosid ALL	Ritchie *et al.* 1997
Ae. albopictus		0.0009			Baruah and Das 1996
Ae. albopictus	4th		0.00062		Buei *et al.* 1975
Ae. albopictus	1st		0.0120	Altosid 10F	Toma *et al.* 1990
	4th		0.0009		
Ae. albopictus	4th		0.0017	Poultex 5E	Farghal *et al.* 1988
Ae. daitensis	1st		0.0743	Altosid 10F	Toma *et al.* 1990
Ae. detritus		0.0009		Altosid SR-10	Majori *et al.* 1977
Ae. epactius		0.000002			Spencer and Olson 1979
Ae. funereus	3rd	0.000072		Altosid ALL	Ritchie *et al.* 1997
Ae. notoscriptus	3rd	0.000359		Altosid ALL	Ritchie *et al.* 1997
Ae. riversi	1st		0.0176	Altosid 10F	Toma *et al.* 1990
Ae. iriomotensis	1st		0.0017	Altosid 10F	Toma *et al.* 1990
	4th		0.00006		
Ae. sollicitans	4th	0.000005		95.4% a.i	Khoo and Sutherland 1985
Ae. sollicitans		0.00015			Spencer and Olson 1979
Ae. togoi		0.0024		Altosid	Zebitz 1986
Ae. togoi	4th		0.00085		Buei *et al.* 1975
Ae. triseriatus	4th	0.000135		Altosid SR-10	Wells *et al.* 1975
		0.000093		Altosid 10-F	
Ae. triseriatus	4th	0.000176		technical 95.4% a.i	Khoo and Sutherland 1985
Ae. vigilax		0.000022		Altosid ALL	Ritchie *et al.* 1997
Armigeres subalbatus	4th		0.15		Buei *et al.* 1975
Ar. subalbatus	1st		14.9352	Altosid 10F	Toma *et al.* 1990
Ar. subalbatus	4th		1.2819		
Anopheles dirus	4th	0.00010-0.00017		Altosid sustained-release	Sithiprasasna *et al.* 1996
An. farauti	3rd	0.000057		Altosid ALL	Ritchie *et al.* 1997
An. sundaicus	4th	0.00009			Imai *et al.* 1987
Culex annulirostris	3rd	0.000089		Altosid ALL	Ritchie *et al.* 1997
Cx. fuscanus	1st		0.0976	Altosid 10F	Toma *et al.* 1990
	4th		0.0009		
Cx. infantulus	4th		0.00073		Buei *et al.* 1975
Cx. orientalis	4th		0.0010		Buei *et al.* 1975
Cx. pipiens pallens	3rd		0.03		Noguchi and Ohtaki 1974
	4th		0.02		
	pharate pup.		0.0006		
	pupae		1.0		
Cx. pipiens pallens	3rd		0.028		Buei *et al.* 1975
	4th		0.00037		
Cx. quinquefasciatus		0.0011			Baruah and Das 1996
Cx. quinquefasciatus	1st		0.0374	Altosid 10F	Toma *et al.* 1990
	4th		0.0013		

Cx. quinquefasciatus (Cuba)	4th	0.005			Navarro-Ortega *et al.* 1991
Cx. quinquefasciatus (France)		0.0006			
Cx. quinquefasciatus	4th		0.00076	Poultex 5E	Farghal *et al.* 1988
Cx. sitiens	3rd	0.001124		Altosid ALL	Ritchie *et al.* 1997
Cx. tritaeniorhynchus	1st		0.0466	Altosid 10F	Toma *et al.* 1990
Cx. tritaeniorhynchus	4th		0.0012	Altosid 10F	Toma *et al.* 1990
Cx. tritaeniorhynchus summorosus	4th		0.00065		Buei *et al.* 1975
Cx. univittatus	eggs	1.1276		Altosid	Abdel-Aal 1995
Psorophora columbiae		0.000052			Spencer and Olson 1979
Diptera: Ceratopogonidae					
Culicoides circumscriptus			0.0094	slow release	Takahashi *et al.* 1985
Diptera: Chironomidae					
Chironomus yoshimatsui	last instar		0.0025 0.00065	Altosid 10 F/ slow release	Kamei *et al.* 1982
C. yoshimatsui[3]	field		0.0044	Altosid 10 F	Kamei *et al.* 1982
Diptera: Muscidae					
Musca domestica			50.3 0.4 -15		Das and Vasuki 1992 Danish Pest Infestation Laboratory 1974
Diptera: Psychodidae					
Psychoda alternata			0.0014 0.0023	Altosid 10F/ slow release	Kamei *et al.* 1993
Diptera: Tephritidae					
Ceratitis capitata	eggs larvae prepupae pupae		1028 350 0.63 1.80	Altosid SR10	Farghal *et al.* 1983
Coleoptera: Chrysomelidae					
Dicladispa armigera	larvae pupae	1.26 1.13			Hazarika and Baishya 1997
D. armigera	eggs adults	0.92			Hazarika and Baishya 1996
Coleoptera: Coccinellidae					
Epilachna chrysomelina	eggs	6.4	1.6		Kinawy and Hussein 1987
Coleoptera: Tenebrionidae					
Tenebrio molitor			0.026	Altosid	Solomon and Metcalf 1974
Hemiptera: Lygaeidae					
Oncopeltus fasciatus			5.0	Altosid	Solomon and Metcalf 1974
Lepidoptera: Nocutidae					
Spodoptera litura	last-instar		68.077		Mane and Subrahmanyam 1996
Lepidoptera: Tortricidae					
Cydia molesta	eggs	0.000055			MacFarlane and Jameson 1974
Siphonaptera: Pulicidae					
Xenopsylla cheopis		0.00011			Chamberlain *et al.* 1988
Ctenocephalides felis	cocoon formation: larval-adult:		0.014 0.00032		Kobayashi *et al.* 1994
Acari: Pyroglyphidae					
Dermatophagoides farinae	tritonymphs		0.0028		Saleh *et al.* 1976

[1] No. of instar

[2] IC_{50}, EI_{50} and EC_{50} = 50% inhibition of emergence

[3] In the field, 2 h exposure.

6. Use of methoprene in the field

Methoprene has been used extensively against a number of pest species. Mosquitoes are one of the main targets and methoprene has generally been a successful control product in field situations. *Culex* species may not be as susceptible as other mosquitoes, although adequate results have been obtained even against this genus. Methoprene has also been used against simuliids and chironomids, dipteran pests of livestock and mushrooms, ants, fleas and stored product pests.

6.1 Use of methoprene against insects

The insect growth regulating properties of methoprene were first described in 1973 (Crosby and Minyard 1991). Since then, methoprene has been used against a number of different pest species, but has been particularly successful against Diptera. Use against mosquitoes is discussed below, but methoprene has also been used extensively against mushroom flies, *Lycoriella mali,* in compost (eg. Keil and Othman 1988), horn flies (Miller *et al.* 1977; Barker and Butler 1977) and other dipteran pests of livestock (eg. Wright 1974; Campbell and Wright 1976). Other major applications have been to control infestations of insects within closed premises like dwellings and hospitals, where use of more toxic chemicals is undesirable. Pests, such as ants (Edwards and Clarke 1978) and fleas (Corpus and Corpus 1991) have been controlled by methoprene in hospitals and education facilities. Methoprene has also been used extensively in flea control on domestic pets (eg. Maskiell 1995) and several companies are currently marketing flea treatments based on methoprene (Table 2).

Insect pests of stored grains are another common target of methoprene application. Use of methoprene for control of pests of stored agricultural products has been reviewed by Mian *et al.* (1990). Methoprene is registered in Australia for use in cereal grains, excluding malting barley, to control strains of *Rhyzopertha dominica* which are resistant to synthetic pyrethroids (Collins *et al.* 1993; Daglish *et al.* 1995). Methoprene has been particularly useful in combinations with chemical pesticides and often in situations where resistance has developed in pest species.

Use in the control of simuliids and chironomids, nuisance flies, has been less frequently reported. Mulla *et al.* (1976) used sprays of a microencapsulated formulation of methoprene applied to two residential-recreational lakes in southern California in 1973-75 and obtained excellent control of most nuisance species of chironomids by inhibiting adult emergence. Larval numbers appeared unaffected. In 1978, the microencapsulated Altosid PS10 applied at 25 or 50 µg a.i./litre for 30 min at fortnightly intervals in 3 streams in Canada caused 93.8-99.1% mortality of *Simulium* spp. and *Prosimulium mixtum* (Thompson and Adams 1979). Experiments were conducted in British Columbia on the effects of a slow release formulation (Altosid SR-10) of methoprene on simuliids (Dove and McKague 1975). It was shown that adult emergence of *Simulium verecundum* was reduced by 75-100% by application of 0.001-0.1 ppm methoprene.

The IC_{50} (dose to inhibit the emergence of 50% of adults) of a slow-release formulation (Altosid 10 F), which contains 10% methoprene, for 2 h on the chironomid *Chironomus yoshimatsui* in flowing water in drains in Japan was 0.0044 ppm. Adult emergence of *C. yoshimatsui* and *Chironomus* sp. was inhibited completely for more than 30 days when Altosid 10 F was added to a drain at 1 ppm (Kamei *et al.* 1982).

The effects of methoprene and its slow-release formulation, Altosid 10F containing 10% methoprene, were evaluated against *Psychoda alternata* (Diptera: Psychodidae) in a 10-person septic tank in Naruto City, Tokushima, Japan (Kamei *et al.* 1993). After the introduction of 2.5 g of this slow-release formulation into the tank, the adults of *P. alternata* disappeared 1 week after the treatment for a period of over 2 months. However, application of Altosid for control of *P. alternata* in turf was ineffective (Ali *et al.* 1990).

6.2. Use for mosquito control

Of direct relevance to the purpose of this report is the efficacy of methoprene in mosquito control. Clearly, Cowley *et al.* (1998) and many others have recommended methoprene for use in mosquito control, but is it an effective agent? A brief review of early published field use attempts can be found in Mian and Mulla (1982b). Below, we summarise studies reporting efficacy against mosquito species.

6.1.1. *Aedes* spp.

Methoprene has been extensively tested against *Aedes* mosquitoes and shown to be highly effective, both in fresh and salt water. In swamps and marshes in the eastern USA, the main pest species, *Aedes taeniorhynchus* and *Ae. sollicitans,* were controlled under diverse conditions by applications of 0.025 lb/acre (0.028 kg/ha) methoprene, either as high-volume or as ultra-low-volume sprays from fixed-wing aircraft or from helicopters (Turrentine and Palmer 1975). Field studies were conducted in New Jersey in 1974 in which methoprene (Altosid SR-10) was applied at 4 oz/acre to control larvae of *Ae. sollicitans* (Vorgetts and Slavin 1976). None of the pupae collected from one of the plots gave rise to adults, and in two other plots, the percentage adult emergence was 1.3-5.5; the percentage emergence in untreated plots was 42-94. The timing of the application was critical; it appeared that methoprene was rapidly absorbed and was effective only when it had been applied to larvae in the late prepupal stages.

The same formulation was used for the control of *Ae. nigromaculis* and *Ae. melanimon* in irrigated pastures in south-central California in 1974 (Norland and DeWitt 1975). Applied at 0.025 lb active ingredient/acre by aeroplane and from the ground (up to 20 applications) against third or fourth instar larvae, complete control could be achieved. Methoprene applied from the air was slightly more expensive than oil but more effective. Applied from the ground, methoprene was much less expensive than oil.

Methoprene (Altosid SR-10) was also effective in controlling *Ae. detritus* larvae in a salt marsh in Italy (Majori *et al.* 1977). Applied at 30g active ingredient/ha to natural salt-marsh pools, it gave complete inhibition of adult emergence for up to 4 days, and used at double this rate of application, inhibition was complete for the duration of the test (8 days). Treatment of a salt marsh (4550 m^2 in area) at the rate of 40 g active ingredient/ha afforded complete control of *Ae. detritus* for 4 days, and the inhibition of adult emergence did not drop below 50% until the 16th day after treatment. Dame *et al.* (1976) reported that *Ae. taeniorhynchus* was completely controlled by 0.05 lb methoprene applied by helicopter in 5-10 US gal of aqueous formulation/acre to salt-marsh mangrove habitats.

Several field studies have confirmed laboratory observations that later instar larvae are more susceptible than early instar larvae. In field trials at Guelph, Ontario, in 1975, methoprene at 0.028 kg/ha effectively controlled spring species of *Aedes* following treatment of third and fourth instar larvae (Rodrigues and Wright 1978). However, when larvae were treated in the first to third instar, only partial control was obtained. Methoprene was also effective against early summer *Ae. vexans* when applied against fourth instar larvae. Altosid SR-10 (4 fl oz/acre) used against third and fourth instar larvae of *Ae. communis* and *Ae. stimulans* in ponds in Ontario resulted in only 2% adult emergence (Baldwin and Chant 1976). In the pond treated at 8 fl oz/acre, no adults emerged. However, first and second instar larvae that were present in some tests developed to the adult stage.

Exposure of spring *Aedes* larvae (e.g. *Ae. canadensis*, *Ae. stimulans*, *Ae. fitchii*, *Ae. provocans*, *Ae. implicatus* and/or *Ae. intrudens*) to methoprene (as Altosid Liquid Larvicide formulated on granular carrier Biodac), was evaluated under field conditions in Bay County, Missouri, USA (McCarry 1996). Both aerial and hand-applied treatments in spring 1995 were monitored. Mosquitoes collected from aerially treated sites (9 kg/ha) showed an average 80% mortality. In hand-treated sites, excellent control was achieved at label rates of 11.3-14.7 kg/ha (10-13 lb/acre; 11.2-14.5 kg/ha); at dosages less than recommended rates, control was unsatisfactory.

Sustained-release pellets gave extended control of *Ae. dorsalis* in a tidal saltwater marsh in California (Kramer *et al.* 1993). Applied at a rate of 3.4 kg/ha prior to marsh inundation, methoprene provided >99% control through the July and August high tide series (up to 42 days post-treatment), 86.4% control during the November high tide series (131 days post-treatment) and 66.6% control during the February high tide series (240 days post-treatment). There was also some evidence that exposure to low concentrations of methoprene impaired the ability of the completely emergent mosquito to fly. Potholes containing larvae of *Ae. dorsalis* were treated with a 1% granular formulation of methoprene (Altosid) at a rate of 5 lb/acre (5.6 kg/ha)(Wagstaff and Minson 1975). All resulting pupae died. Methoprene (Altosid SR-10) was applied to a 60-acre irrigated pasture infested with *Ae. nigromaculis* and *A. dorsalis* from the air at 0.2 lb active ingredient/acre; no adult emergence was detected (Wagstaff and Minson 1975).

Methoprene (Altosid) and diflubenzuron (Dimilin) formulated on sand granules were applied by aircraft against larvae of *Ae. taeniorhynchus* in Florida in 1975 (Rogers *et al.* 1976). In 5 tests of each chemical, control was very effective at dose rates approximately half those recommended by the manufacturers. Gross application rates of the granular formulation ranged from 5.5 to 8.5 lb/acre (6.2-9.5 kg/ha) for methoprene and 6.1 to 11.7 lb/acre (6.8-13.1 kg/ha) for diflubenzuron; the corresponding rates of toxicant were 0.011-0.0169 and 0.010-0.0029 lb/acre (0.012-0.019 kg/ha and 0.011-0.00325 kg/ha).

6.1.2. *Culex* spp.

It has been stated that *Culex* is less susceptible to methoprene than other mosquitoes (Norland and DeWitt 1975). There is some evidence of this in reports of field use, although in many cases, methoprene has worked well in controlling *Culex* spp. The differences possibly relate to differences between the genera in feeding position: *Culex* spp. feed on the surface while other genera feed throughout the water profile or on the bottom. Similar results were found with *Bti* and the reduced sensitivity of *Culex* spp. (Glare and O'Callaghan 1998). *Bti* has been shown to settle quickly in water columns and as *Culex* spp. feed on the surface, they may

ingest less of the bacterial toxin. Altosid ALL and all the solid formulations have a specific gravity greater than one and therefore sink to the bottom of the water column. Methoprene's specific gravity is 0.9261 g/ml at 20 degrees and therefore moves towards the surface when released from the formulation. There only have been difficulties in controlling *Culex* spp. with Altosid ALL, which typically remains effective for about seven days. All other formulations have sufficient methoprene for a longer residual period and they control *Culex* spp. without a problem (D. Sullivan, pers. comm.). Non-synchronised populations of *Culex* spp. mature in more than seven days and therefore may require either a higher dose of methoprene or an additional treatment.

One of the main targets of methoprene applications has been *Cx. p. pallens.* In Japan, when methoprene was sprayed on the water surface of highly polluted ditches to give a concentration of 0.86-2.27 ppm or 0.34-0.71 g/m^2 of water surface, the effects on pupae and fourth instar larvae were noticeable 0.5 h later (Ishii *et al.* 1987). Inhibition of adult emergence reached 96-100% in pupae collected after 24 h and remained at 82-100% for 1 week after spraying in most ditches. When a second spray application was made one week after the first, the emergence remained at similar levels for 9-16 days. In Korea, methoprene (Altosid SR-10 and Altosid 10-F) at 1000 g/ha used against *Cx. p. pallens* breeding in flooded parsley fields caused high mortality during the pupal-adult moult. Altosid SR-10 remained effective for 30 days, giving an average mortality of 96.6% and Altosid 10-F was effective for 16 days, giving average mortality of 97.3%. Altosid 10-F at 200 g/ha remained 70% effective for 6 days in one field.

Charcoal briquettes containing methoprene were tested in 1977 for the control of *Cx. pipiens* in catch basins in 2 large residential areas in San Mateo County, California (Schoeppner 1978). The results showed that treatment at the rate of 1 briquette/catch basin was effective for 5-13 weeks and had to be repeated 3-4 times in order for sufficient methoprene to accumulate in the water to afford a lethal dose to the larvae. Even so, this type of treatment proved more effective and more economical than treatment with larvicidal oil.

Two formulations of methoprene were applied at several dosages in 1 or 2 applications a week to synchronous broods of *Cx. nigripalpus* in replicated small-plot field tests in Florida (Rathburn *et al.* 1980). Two applications per week of a sand granular formulation at 0.022 kg a.i./ha or of an aqueous formulation at 0.030 kg a.i./ha were required for effective control. In small field plots, methoprene was effective at rates of 0.025 lb/acre (0.028 kg/ha) against natural populations of *Cx. nigripalpus* and *Cx. salinarius* (Dame *et al.* 1976).

To achieve control of some species, relatively high doses have been required. Methoprene (4%) pellets were effective against *Cx. tarsalis* for 7 days at the rates of 0.28 kg/ha (0.25 lb a.i./acre) whereas 0.56 kg/ha (0.5 lb a.i./acre) was required to obtain similar results against *Cx. peus* larvae (Mulla *et al.* 1989). Tests with 0.1 lb methoprene/acre (0.11 kg/ha) were conducted against *Culex* spp. by Wagstaff and Minson (1975) and all pupae from initial collections died. Several other pools were treated against species of *Culex* and *Culiseta* at 0.5 lb methoprene/acre (0.56 kg/ha), but complete control was not achieved in any of them; 85% control was obtained with the granular formulation. It was concluded that methoprene showed great promise for control of these mosquitoes, but its cost at the higher dosages was very high (Wagstaff and Minson 1975). In the Sacramento Valley, California, micro-encapsulated and charcoal formulations of methoprene sprayed from the air (0.1 lb a.i/acre or 0.11 kg/ha) to rice-fields for the control of *Cx. tarsalis* reduced adult emergence by 50%

immediately and to 10-30% after four days (Case and Washino 1978). By the seventh day there was no significant difference in the rate of emergence from that before treatment.

In waste water lagoons, control of *Culex* has been disappointing. Mulla and Darwazeh (1988) found 4% methoprene slow release pellets at up to 1.12 kg/ha gave little or no control of *Culex* in the dairy wastewater lagoons. Altosid SL-10 was tested in 1973 for the control of *Cx. pipiens* breeding in vast numbers in a complex of waste lagoons in central Indiana (Lee and Siverly 1973). The first lagoon was sprayed weekly at 12 oz/acre, and it was assumed that the second lagoon would be treated by overflow. However, control was not effected in either lagoon. Methoprene applied to anaerobic pig waste lagoons in North Carolina against *Cx. quinquefasciatus* at 0.4 lb/acre (0.45 kg/ha) did not give satisfactory control (Axtell *et al.* 1980). Methoprene briquettes did not adequately inhibit the emergence of adults of *Cx. molestus* in septic tanks and underground pools in Japan, although methoprene did work against this host in other situations (Itoh 1979).

Tests were carried out in Japan, on the feasibility of controlling *Cx. p. pallens* in drainage ditches with methoprene (Buei *et al.* 1978). When a sand granule formulation of methoprene was applied at 1 ppm to drainage ditches during the rainy season (June), when there was a continuous flow of water, adult emergence was completely inhibited for the first 5 days after the application, and partial inhibition (more than 50% inhibition) was recorded for the next 53 days. In August, after the rainy season was over, a similar application remained effective for up to 72 days. A single treatment with methoprene in a briquette formulation completely inhibited adult emergence for 30 days (Buei *et al.* 1978).

Methoprene applied at a target dosage of 1 ppm to larval habitats of *Cx. p. fatigans* in a crowded area of about 1 km^2 in Jakarta, Indonesia, was highly effective in preventing successful adult emergence for 5 weeks, after one application (Self *et al.* 1978).

6.1.3. *Mansonia* spp.

In India, methoprene (Altosid) was evaluated in field trials against *Mansonia* spp. (presumed to be *M. annulifera, M. uniformis* and *M. indiana*), in coconut husk retting ponds (Krishnamoorthy *et al.* 1993). Decreased larval density resulted from dosages of 0.5, 1 and 2 ppm. The percentage reduction in larval density was greatest in ponds treated at 2 ppm (69.36%), which is a relatively high dose. Adult emergence was delayed by 14, 21 and 28 days, respectively, with the increase in dosage, but none of the larvae treated at 2 ppm emerged into adults and only 1.45 and 1.52% of those treated at 0.5 and 1 ppm, respectively, successfully emerged. The sudden decrease in larval density in treated ponds and the high mortality of treated larvae indicated the larvicidal effectiveness of this compound (Krishnamoorthy *et al.* 1993).

6.1.4. *Psorophora* spp.

A slow-release, briquette formulation of 1.8% methoprene (Altosid XR Briquets) produced long-term residual activity against *Psorophora columbiae* larvae in 37.2 m^2 rice plots in Arkansas (Weathersbee and Meisch 1991). An application rate of 1 briquette/9.3 m^2 provided significantly ($P<0.05$) greater reduction of adult mosquito emergence (98.2%) than did a rate of 1 briquette/18.6 m^2 (89.6%) during 5 insecticidal activity assessments conducted over a period of 58 days. No significant ($P>0.05$) differences in activity were detected between

treated plots which were continuously flooded and those periodically drained and reflooded. Methoprene (as Altosid SR-10) gave complete control of *P. confinnis* at 0.025 lb toxicant/acre (0.028 kg/ha), with most of the mortality occurring in the pupal stage (Mulla and Darwazeh 1975).

7. Comparison of efficacy of methoprene with other agents

When considering the environmental safety of methoprene use, it is important to compare efficacy with other agents. An ineffective, but safe product would be of little use. The efficacy of methoprene in comparison to other mosquitocidal agents has been examined by several researchers in both laboratory and field situations. In general, agrochemical controls had greater efficacy than methoprene but in certain environments, methoprene proved more effective than the best chemical pesticides. Methoprene has consistently proved to be one of the most effective IGRs against mosquitoes and is usually more efficacious than biological control agents.

7.1. Mosquitoes

7.1.1. Laboratory

Methoprene appears to be moderately effective against mosquitoes when compared to other common agents.

Methoprene has proved more effective than *Bti* in most tests. Ibrahim (1990) listed the relative *Cx. pipiens* larval toxicity in the order chlorpyrifos-methyl > deltamethrin > methoprene > teflubenzuron > *B.t. israelensis*. In some tests methoprene is less effective than other IGRs such as pyriproxyfen against specific species (Farghal *et al.* 1988), although Kelada *et al.* (1980) tested the juvenilising effects of diflubenzuron (Dimilin), JH-25, methoprene (Altosid), hydroprene (Altozar), kinoprene (ZR-777) and triprene (ZR-619) on *Cx. pipiens* of Egyptian origin. Using IC_{50} values, the order of decreasing activity was methoprene, diflubenzuron, hydroprene, kinoprene, JH-25 and triprene. Madder and Lockhart (1980) found that diflubenzuron had longer residual efficacy against *Ae. aegpyti* than methoprene in laboratory studies, with diflubenzuron toxic to mosquito larvae for up to 16 days while methoprene fell below GLC detection within 2 days, although biological activity persisted for about a week after treatment. Of 65 juvenoids tested by Dame *et al.* (1976) against *Anopheles quadrimaculatus,* methoprene was one of the most effective.

The biological activities of the insect growth regulators, Poultex 5E (methoprene) and S-71639 (pyriproxyfen), both alone and in combination with *Bti* were studied under laboratory conditions (27^{o}C and RH 60%) against early 4th-instar larvae of *Cx. quinquefasciatus* and *Ae. albopictus*. Pyriproxyfen was more toxic to both species of mosquito than methoprene. The EI_{50} of pyriproxyfen was 0.11 ppb for *Cx. quinquefasciatus* and 0.22 ppb for *Ae. albopictus*. For methoprene the EI_{50}s were 0.76 and 1.7 ppb, respectively. Both pyriproxyfen and methoprene were more active when used in combination with *B. thuringiensis* (Farghal *et al.* 1988). Little difference was found by Baruah and Das (1996) between diflubenzuron and methoprene. LC_{50} values of both insect growth regulators were almost the same for both *Ae. albopictus* and *Cx. quinquefasciatus* (between 0.0009 and 0.0011 ppm). Field trials were conducted in cemented drains, small ponds and ditches. At 0.2 ppm (0.020 kg/ha), both diflubenzuron and methoprene were found to eliminate 92-96% of *Culex* (*Cx. quinquefasciatus, Cx. vishnui* group and *Cx. gelidus*) and *Anopheles* (*An. vagus, An. crawfordi* and *An. annularis*) larvae (Baruah and Das 1996).

Ali *et al.* (1995) tested five organophosphates (OPs) (chlorpyrifos, chlorpyrifos-methyl, fenthion, malathion and temephos), 3 pyrethroids (bifenthrin, cypermethrin and permethrin), 2 microbial pesticides (*Bti* and *B. sphaericus*) and 3 IGRs (diflubenzuron, methoprene and pyriproxyfen) against larvae of *Ae. albopictus*. All OPs, except for malathion, were highly effective as indicated by low LC_{90}s ranging from 0.0069 ppm (chlorpyrifos) to 0.026 ppm (fenthion); the larvae were considered tolerant to malathion (LC_{90} = 1.043 ppm). LC_{90} values of pyrethroids were: 0.0175 ppm (bifenthrin), 0.0079 ppm (cypermethrin) and 0.0031 ppm (permethrin). Commercial products of *Bti* (Vectobac and Bactimos) were considered economically effective against *Ae. albopictus* larvae, but products of *B. sphaericus* were ineffective (LC_{90}s > 28 ppm). The IGRs showed exceptional activity. Pyriproxyfen (LC_{90} = 0.000376 ppm), was 2.23 and 21.5 times more toxic than diflubenzuron and methoprene, respectively. In general, toxicity ranking of chemicals and microbials tested was: IGRs > pyrethroids > OPs > microbials (Ali *et al.* 1995).

In Indonesia, fourth-instar *An. sundaicus* larvae were highly susceptible to methoprene (LC_{50} = 0.00009 ppm), and susceptible to temephos (LC_{50} = 0.0032 ppm) and chlorpyrifos-methyl (LC_{50} = 0.0037 ppm). There appeared to be some tolerance to fenitrothion (LC_{50} = 0.015 ppm) and fenthion (LC_{50} = 0.025 ppm) (Imai *et al.* 1987).

7.1.2. Field

The susceptibility of fourth-instar larvae of *Ae. aegypti*, *Ae. polynesiensis* and *Cx. quinquefasciatus* from Tahiti to a range of organophosphorus insecticides and the IGRs diflubenzuron and methoprene was tested according to WHO protocols by Failloux *et al.* (1990). The organophosphorus (OP) compounds were more effective than the IGRs, with temephos (Abate) and chlorpyrifos being the most effective of all.

Conversely, Itoh (1979) found methoprene and diflubenzuron more effective than diazinon, fenitrothion, temephos (Abate) and DDT against larvae of *Cx. tritaeniorhynchus*, *Cx. molestus* and *Ae. albopictus* in Japan. Two formulations of methoprene (Altosid SF-10F and briquettes) almost completely inhibited adult emergence of *Cx. tritaeniorhynchus* in experimental rice-fields immediately after treatment at 0.1 ppm. Diflubenzuron at 0.1 ppm completely inhibited adult emergence for 4 days after treatment. Methoprene briquettes did not adequately inhibit the emergence of adults of *Cx. molestus* in septic tanks and underground pools, but sand granules and briquettes with methoprene, as well as diflubenzuron, were very effective against larvae of *Ae. albopictus* in small containers. In Malaysia, field experiments in outdoor contains found that Altosid briquettes would give up to 10 weeks control of *Ae. albopictus* (Sulaiman *et al.* 1994).

A mosquito problem in a large urban cemetery in Los Angeles County, California, was studied in 1975-76 (Mulla *et al.* 1977). *Culex* spp. bred throughout the year in stagnant water in the metal flower vases. The standard method of control by applying temephos from a boom sprayer or mist-blower was found to be ineffective, and increasing the application rate gave good control for only 2 weeks. Chlorpyrifos at 0.2 lb (0.091 kg) gave excellent control for 1-2 months, but the frequency of spray application necessary would be impracticable in large cemeteries. Slow-release charcoal briquettes containing 4% methoprene placed within the vases resulted in excellent inhibition of adult emergence for over 5 months.

Methoprene (Altosid) and diflubenzuron (Dimilin) were formulated on sand granules and applied by aircraft against larvae of *Ae. taeniorhynchus* in Florida in 1975 (Rogers *et al.*

1976). In five tests of each chemical, control was very effective at dosage rates approximately half that recommended by the manufacturers. Gross application rates of the granular formulation ranged from 5.5 to 8.5 lb/acre (6.2-9.5 kg/ha) for methoprene and 6.1 to 11.7 lb/acre (6.8-13.1 kg/ha) for diflubenzuron; the corresponding rates of toxicant were 0.011-0.0169 and 0.010-0.0029 lb/acre (0.012-0.019 and 0.011-0.0033 kg/ha).

The lesser effectiveness of methoprene against *Culex* mosquitoes was reflected in several studies comparing IGRs and chemical controls. The use of several IGRs against *Culex* in dairy wastewater lagoons showed that a granular formulation of pyriproxyfen applied at 0.056 kg/ha gave mediocre reduction whereas fenoxycarb EC 1 at up to 0.28 kg/ha and methoprene 4% slow release pellets at up to 1.12 kg/ha produced little or no control of *Culex* in dairy wastewater lagoons (Mulla and Darwazeh 1988). The authors reported that these compounds need to be applied at higher rates or suitable formulations will have to be developed to achieve satisfactory control.

In another trial, four insecticides and two IGRs were applied to anaerobic pig waste lagoons in North Carolina against *C. quinquefasciatus* (Axtell *et al.* 1980). Chlorpyrifos at 0.4 lb/acre (0.45 kg/ha)afforded excellent control for 3-5 weeks. Malathion at 1.0 lb/acre (1.12 kg/ha) did not give satisfactory control. Temephos at 0.5 lb/acre (0.56 kg/ha) gave control for only 3-4 days. Flit MLO at 7 US gal/acre (54.5 l/ha) gave satisfactory control for 3-4 days and was ineffective at lower rates. Diflubenzuron at 0.1 lb/acre (0.11 kg/ha) gave satisfactory control for 1-2 weeks, but methoprene at 0.4 lb/acre (0.45 kg/ha) did not give satisfactory control (Axtell *et al.* 1980).

When considering environmentally safer alternatives for mosquito control, the effectiveness of methoprene will be compared with the bacterium *Bti*, as both are considered to have few non-target effects and low mammalian toxicity. In direct field level comparisons, methoprene has generally given better duration of control. For example, Bactimos (*Bti*) briquettes provided complete control of *Ae. aegypti* adult emergence in Malaysia in plastic containers of water for up to 75 days post-treatment, while methoprene (Altosid) briquettes gave 100% mortality for up to 122 days (Sulaiman *et al.* 1991). Becnel *et al.* (1996) evaluated the effect of three larvicides on the production of adult *Ae. albopictus*. The fungal pathogen *Lagenidium giganteum* was ineffective. A liquid formulation of *Bti* (Acrobe) provided significant control for 47 days, whereas a slow-release pellet formulation of methoprene (Altosid) provided almost complete control for 116 days.

7.2. Flies

Comparative studies against several fly species have indicated that methoprene is one of the more promising control insecticides, even though the dose required for mortality is high compared to some other Diptera (Table 5). The LC_{50} against *Haematobia irritans* was lower for methoprene and avermectin MK-933 than for deltamethrin, diflubenzuron, coumaphos and tetrachlorvinphos (Schmidt and Kunz 1980). Dimilin (diflubenzuron), hexaflumuron, methoprene and chlorfluazuron were assessed for their effectiveness in controlling *M. domestica.* Methoprene was the most promising (EI_{50} 0.0503 mg/ml), followed by hexaflumuron (EI_{50} 0.7552 mg/ml) (Das and Vasuki 1992).

The effects of triflumuron (SIR-8514) and methoprene (Altosid SR10) on the immature stages of *Ceratitis capitata* were investigated in the laboratory in Egypt (Farghal *et al.* 1983). The EC_{50}s for eggs dipped in aqueous solutions was <250 ppm for triflumuron, and more than 1028 ppm methoprene. The EC_{50}s when the compounds were incorporated into an artificial larval diet were 75 ppm triflumuron and 350 ppm methoprene. The EC_{50}s for prepupae kept in treated soil were 4.5 ppm triflumuron and 0.63 ppm methoprene, and those for pupae placed in treated soil when 24, 48 or 144 h old were more than 65 ppm triflumuron (pupae of all ages) and 0.83, 0.62 and 1.80 ppm methoprene, respectively. The results suggest that triflumuron would give the best results against the eggs and larvae, while methoprene could be incorporated into the soil beneath fruit trees to kill the pupae.

In another type of study, two herds of experimental cattle in Texas were allowed free access to mineral blocks containing 0.01, 0.12 or 0.94% methoprene in the spring and summer of 1973 (Harris *et al.* 1974). Samples of the faeces were collected 3 times weekly and seeded in the laboratory with eggs of *H. irritans* and *Stomoxys calcitrans* in order to ascertain whether the development of larvae and pupae would be inhibited by residues of methoprene in the faeces. Also, emergence traps were placed over manure pats from treated and untreated cattle in the field, and the numbers of adults of *H. irritans* that emerged were counted. The average daily intake of mineral block was 68 ga/animal. Emergence of *H. irritans* was reduced by 87% in the field tests and 97% in the laboratory tests of faeces from the cattle with access to blocks containing 0.94% methoprene, and emergence of *S. calcitrans* was reduced by about 90%. The results for blocks with lower concentrations of methoprene were less good.

7.3. Chironomids

Organochlorines, OPs, pyrethroids and IGRs have been evaluated against aquatic chironomid midge larvae in the laboratory and/or used in the field (Ali 1996). Most effective control was achieved with the OP insecticides (chlorpyrifos and temephos) and IGRs (diflubenzuron, methoprene and pyriproxyfen). The OPs have generally provided larval field control for 2-5 weeks at rates <0.56 kg a.i./ha, resulting in insecticidal concentrations of <1-5 ppm, but increased tolerance by midge larvae to some materials has been reported. IGRs (especially pyriproxyfen) have provided >90% suppression of midge emergence for several weeks at <0.25 kg a.i./ha.

7.4. Fleas

The inhibitory effects of pyriproxyfen and methoprene on embryonic and larval development of *C. felis* were determined in the laboratory. The IC_{50} values of pyriproxyfen were 0.00056 ppm for cocoon formation and 0.00017 ppm for larval-adult development, while the IC_{50} values of methoprene were 0.014 and 0.00032 ppm, respectively (Kobayashi *et al.* 1994).

7.5. Lepidoptera

Methoprene was among four juvenoids evaluated against last-instar larvae of *Spodoptera litura* by Mane and Subrahmanyam (1996). Methoprene was applied topically, resulting in an ID_{50} for inhibition of adult emergence of 0.027, 68.077 and 273.698 µg /g for cypermethrin, methoprene and hydroprene, respectively. The oral ID_{50}s for fenoxycarb and Prodron R were 4.578 and 796.159 µg /g, respectively. Cypermethrin at sublethal concentrations caused juvenomimetic effects.

7.6. Chrysomelids

Topical application of aqueous suspensions of methoprene and diflubenzuron to eggs and adults of *Dicladispa armigera* revealed that with increased concentration, the rate of egg hatch decreased, the LC_{50}s values being 0.92 and 746.13 ppm for methoprene and diflubenzuron, respectively (Hazarik and Baishya 1996). These compounds were not lethal to adults but enhanced their fecundity at low doses. Hatching of eggs from these adults was drastically reduced.

7.7. Mites

The effect of methoprene (Altosid) and hydroprene (Altozar) on tritonymphs of *Dermatophagoides farinae* was investigated in the laboratory in Alexandria, Egypt. Methoprene incorporated into the rearing medium at the rates of 0.000161, 0.00028, 0.0028, 0.0161 and 0.0322 ppm resulted in 26.7, 43, 67, 70 and 75.5% inhibition of adult emergence, respectively (Saleh *et al.* 1976). Hydroprene in the medium at the concentrations of 0.000163, 0.000326, 0.00326, 0.0163 and 0.0326 ppm likewise caused 28.3, 53.3, 56.7, 61.7 and 71.7% inhibition, respectively. Treated mites that reached the adult stage took longer to do so, the median times (DT_{50}) needed for development to the adult stage being 6.5, 9.9, 10.5, 9.3 and 10.8 days, respectively, for the tested concentrations of methoprene and 6.1, 7.7, 8.6, 11.1 and 11.5 days for those of hydroprene, as compared with 2.2 days for no treatment.

8. Use of methoprene in eradication campaigns

The use of methoprene in New Zealand could be as part of a localised eradication campaign against incursions of mosquitoes. Methoprene has previously been used in localised eradication of ants and fleas. Mosquito emergence has been reduced to 0% after application of methoprene, however these are usually in areas where re-infestation is possible and eradication not feasible.

Methoprene may be used against mosquitoes in New Zealand as part of an eradication campaign. Large-scale eradication of mosquitoes using methoprene has not been reported, but several studies have reported successful small scale eradication of other pest insects using methoprene.

Methoprene was used in an eradication campaign against Pharaoh's ants, *Monomorium pharaonis,* in a large hospital in Liverpool, England, covering an area of about 15 000 m2. Application of sugar-and-protein baits (a mixture of honey, sponge cake and liver powder) treated with 0.5% of methoprene, to the greater part of the hospital, including some uninfested areas, for 2 weeks resulted in eradication of the ants from most of the building within 18 weeks. A 98% decline in the numbers of worker ants during the first 12 weeks after treatment appeared to be due to natural mortality and lack of effective reproduction (Edwards and Clarke 1978). Similar results had been previously obtained with *M. pharaonis* (Edwards 1977; Hrdy *et al.* 1977).

During a study carried out in a large town in northern Moravia, Czechoslovakia, in 1980-81, the application of baits comprising dried egg yolks impregnated with 0.5% methoprene twice within 8-12 days at a rate of 1 g bait/3-6 m^2 floor area resulted in the complete eradication of populations of *M. pharaonis* in 2 medium-size health establishments and in an apartment house. In another health establishment where the ants had been controlled by insecticides but not eliminated, the use of methoprene-impregnated baits twice at a rate of 1 g/46 m^2 floor area resulted in complete eradication. Providing that all colonies of the ant in the premises to be treated were affected by the bait, complete eradication using these baits could be expected within 100 days of the first application (Rupes *et al.* 1983). The use of methoprene for ant control in sensitive sites such as hospital indicates the health impacts assessed for the use of methoprene were considered low. Other examples of use to control or eradicate ants were at the Berlin zoo and in the children's clinic in Olomouc, Czechoslovakia (Klunker *et al.* 1984).

Another pest which has been the target of localised eradication using, among a number of pesticides, methoprene was the cat flea, *Ctenocephalides felis.* A Mississippi child care facility was inundated with fleas, including children and personnel. Fleas were eradicated by eliminating entry of cats and using insecticides including chlorpyrifos, methoprene (Precor), propetamphos (Safrotin) and diazinon throughout the facility (Corpus and Corpus 1991).

9. Effects on non-target organisms

Review of methoprene effects on non-target organisms suggests it is one of the safer mosquitocidal agents available. In direct applications, methoprene has shown few negative impacts on non-target organisms with the exception of some aquatic invertebrates and low toxicity to some fish species has been observed. The effects on non-target organisms have generally been at dose levels considerably higher than label rates and/or methoprene was tested with solvents to increase the solubility of methoprene. Aquatic communities generally recovered after methoprene application. Mammalian toxicity is extremely low. Slight phytotoxicity was found in only one study. Methoprene is usually not toxic to other mosquito biocontrol agents such as *Bti*, nematodes or fungi. Some toxic effect has been noted with non-target insects such as parasitoids and sublethal effects found with bees. One unresolved issue is the possible role of methoprene in development of deformities in some frog species in North America. At present, methoprene and similar compounds are one of three possible causes of the deformities and research is ongoing to discover the cause(s).

9.1. Phytotoxicity

Phytotoxicity, or the toxicity of methoprene towards plants, has been the subject of several studies. Parrella and Robb (1982) screened several bedding plants for the effect of methoprene and other pesticidical compounds. Methoprene showed little phytotoxicity to the tested plants which included Antirrhinum, Impatiens, Petunia, Verbena, Zinnia, broccoli, courgettes, peas and tomatoes. There was also no effect on chrysanthemums (Parrella 1983).

In another study, methoprene (Altosid) had no effect on *Kalanchoe gastonisbonnieri* (flowering plant) grown under short day (8 h) conditions but slightly delayed the appearance of female flowers in *Cucurbita pepo* (spaghetti squash) (Felippe 1980) and delayed the appearance of plageotropic buds of *Coffea arabica* (coffee)(Felippe 1979).

9.2. Microorganisms

There are few studies which have directly examined the effect of methoprene on microorganisms. Mostly, reports involve evaluation of methoprene when used in conjunction with a microbial biocontrol agent, such as *Bti*.

9.2.1. *Bacillus thuringiensis*

Bacillus thuringiensis is one of the most widely used insect pathogenic microorganisms in insect control; the dipteran active subspecies; *Bt israelensis* (*Bti*), has been used extensively for mosquito and biting fly control. The compatibility of these two agents must therefore be considered carefully. Altosid and *Bti* have been used together for many years and this combination is usually referred to as a Duplex mixture. Duplex has been shown to control all species of mosquitoes (D. Sullivan, pers. comm.). The two control agents are usually applied in ratios of 12:1 to 6:1 of *Bti*:Altosid. When a 6:1 ratio is applied at 1 pint/acre, effective control can be maintained for about 10 days.

Several studies have found no negative effects of the use of methoprene and *Bti* together or on each other (Sokolova and Ganushkina 1982; Farghal *et al.* 1988) and in some cases the two control agents may work in synergy. Farghal *et al.* (1988) found that *Bti* was more effective against 4th-instar larvae of *Cx. quinquefasciatus* and *Ae. albopictus* when used with methoprene (Poultex 5E) than when used alone. Similarly, Mohamed *et al.* (1983) found that use of methoprene with Dipel (*B. thuringiensis kurstaki*) against 3rd-instar larvae of *Helicoverpa virescens* was synergistic.

Conversely, Ibrahim (1990) did not find any synergism between *Bti* and methoprene against fourth instar *Cx. pipens,* but treatment effects varied between antagonistic and additive effects depending on the time of mortality assessment post-treatment. In Egypt, Farghal (1982) found that methoprene treatment increased the tolerance of 2nd- and fourth instar larvae of *Cx. molestus* to *Bti* (Bactimos). After exposure for 24 h, the highest tolerance occurred in fourth instar larvae exposed to the highest doses. The LT_{50} (time taken to kill 50%) was significantly lower in larvae treated with *Bti* only than in larvae treated with both methoprene and *Bti*. In the field, exposure of larvae of *Culiseta longiareolata* to methoprene briquettes (at the rate of 2 briquettes /4 m^2) also significantly increased their tolerance of all tested concentrations of *Bti*. The authors attributed this effect either to a direct toxic effect of methoprene on the bacterium or to physiological changes caused by methoprene in the larvae that rendered them more tolerant to the bacterium. Interestingly, the authors concluded that methoprene and *Bti* cannot be combined in a programme of integrated control against mosquitoes (Farghal 1982). This report is not consistent with other published accounts presented above

9.2.2. Protozoa

Methoprene has been shown to be lethal to termites (Table 4). For some termite species (eg. *Reticulitermes flavipes*), the toxic effect of methoprene is thought to be the result of starvation induced by the elimination of the termite's symbiotic protozoa (Haverty and Howard 1979). For *Stophilus oryzae*, methoprene had no direct effect on its bacterial symbionts or mycetomes, but removal of the bacterial symbionts by antibiotic treatment considerably reduced prepupal and pupal mortality caused by methoprene (Baker and Lum 1976). A study of the effect of methoprene on internal flagellates of the termite *Reticulitermes santonensis* was conducted by Lelis (1992), who found no significant reduction in the number of flagellates from four genera. Lelis (1992) considered that any reduction in the number of flagellates is the result of the increased number of moults in treated groups of termites, rather than a specific effect of methoprene on the symbionts.

The protozoan infections of *An. stephensi* by *Plasmodium berghei* and *Ae. aegypti* by *P. gallinaceum* were examined for the effect of treatment with an analog of methoprene, Juvemon (at 0.001 to 0.05 mg/litre) (Ganushkina *et al.* 1991). Juvemon at low concentrations reduced the infection rate of *Ae. aegypti* by 2 to 30% but the differences from controls were significant in 3 tests only, at concentrations of 0.001-0.002 mg/litre that caused a larval mortality of 35%. Higher concentrations tended to cause rises in the sporozoite index to above control levels. There were no significant differences from controls in infection intensity, and no significant effect on the physiological condition of the vectors was noted.

Spencer and Olson (1982) also examined the combination of sporocysts and methoprene on mosquitoes. Mortality rates for test populations of *Ae. aegypti* were significantly increased with increases in concentrations of methoprene in the larval rearing medium from 1.0 ppb

(28% average mortality) to 10 ppb (84%). Mortality rates were not significantly changed when *Ascogregarina culicis* sporocysts (15/larva) were combined with either concentration of methoprene. In contrast, mortality rates for *Ae. epactius* were not only significantly increased with increasing methoprene concentrations from 0.001 ppb (13%) to 0.01 ppb (53%) but the mortality rates at each concentration were significantly higher when larvae were first exposed to *Ascogregarina* sporocysts (73 to 82%), apparently due to the additive effect of these two agents. Exposure to 5 ppm methoprene for up to 72 h had no significant effect on the infectivity of *Ae. culicis* sporocysts or on the level of parasitism in exposed mosquitoes.

9.2.3. Fungi

The effect of methoprene has been examined for two mosquitocidal fungi, *Metarhizium anisopliae* and *Lagenidium giganteum.* Methoprene was not inhibitory to the various developmental stages of *Metarhizium anisopliae* (Mohamed *et al.* 1987). The relative toxicity of methoprene, along with a number of other pesticides and fungicides, to the vegetative growth and zoospore production of *L. giganteum* showed that zoospore production was less tolerant than mycelial growth (Merriam and Axtell 1983). The most toxic pesticides were captan, lindane (gamma-BHC), DDT, camphechlor (toxaphene), chlorpyrifos and fenthion. The least toxic pesticides, diflubenzuron, permethrin, temephos and propoxur, caused 10% inhibition of growth or failed to inhibit zoospore production at concentrations greater than 50 ppm. Methoprene was of intermediate toxicity, along with malathion, carbaryl, alachlor and atrazine. Merriam and Axtell (1983) found that at their recommended rates of application for the control of mosquito larvae, methoprene would probably be compatible with *L. giganteum*.

9.2.4. Virus

There are no viral diseases of mosquitoes currently in use commercially. However, in Lepidoptera, the effect of methoprene use on viral insect pathogens has been examined, often to determine if the use of a growth regulator during viral infection can lead to increased virus production in the insect due to induced supernumary moults. Boucias and Nordin (1980) found that incorporation of methoprene into larval diet induced supernumary moults in larvae of *Hyphantria cunea* over 2 weeks, reduced virus-induced mortality and an increase in the LT_{50} for the virus. However, it was concluded that treatment with methoprene would not be antagonistic to the natural development of nuclear polyhedrosis virus in populations of *H. cunea* and might be useful in the laboratory to increase larval biomass for greater production of pathogenic material for biological control purposes. Methoprene treatment of the larvae of *Helicoverpa* (*Heliothis*) *virescens* resulted in significant increases in both larval size and production of virus, however there was no significant effect on the virulence of the infection (Nordin 1981). Mohamed *et al.* (1983) found that use of methoprene with nuclear polyhedral virus for first and third instar *H. virescens* was synergistic.

Treatment of the *Spodoptera litura* larvae with methoprene prolonged their larval period, gave larger larvae, and increased virus yields by about 15% (Im *et al.* 1989). In studies in the United Kingdom, the granulosis virus of the apple pest *Cydia pomonella* was produced in larvae reared on artificial diet. The average yield of virus (9×10^9 capsules/larva) was increased by raising the larvae on diet containing the methoprene (Glen and Payne 1984).

9.3. Invertebrates

9.3.1. Benthic and aquatic communities

Of importance when considering mosquito control agents is the safety of organisms living in marine, estuarine or freshwater benthic communities. Individual species which make up the communities are discussed in more detail in the sections below, however in general terms the reports by Yasuno and Satake (1990), Hershey *et al.* (1995) and Retnakaran *et al.* (1974) found no evidence of lasting effects on benthic invertebrates after methoprene application. Yasuno and Satake (1990) also reported no induced drift of macrobenthos at the time of application.

Breaud *et al.* (1977) examined the long term effect on aquatic communities of six aerial applications in Louisiana. Methoprene caused significant reductions in natural populations of 14 aquatic organisms, but no species was eliminated. Populations of 5 species increased following the treatment, and no significant differences in population numbers could be detected in the case of 28 other aquatic organisms. Breaud *et al.* (1977) concluded from these results that though applications of methoprene to specific breeding sites in the marsh for mosquito control would reduce populations of some organisms, no species would be eliminated; that the control of predator species would cause corresponding increases in certain aquatic prey populations; and that re-population of the treated area would occur from adjacent untreated marsh, as was shown by the recovery of the aquatic organism populations following the drought of 1974 (Breaud *et al.* 1977).

Methoprene (Altosid) applied from a helicopter in sprays at concentrations of 0.25-3 oz/gal in July 1973 against *Lambdina fiscellaria fiscellaria* on balsam fir in Anticosti Island, Quebec had no adverse effects on aquatic fauna (Retnakaran *et al.* 1974). Similarly, in California, Case and Washino (1978) found no statistically significant effects of treatment on various non-target invertebrates of methoprene (Altosid).

Methoprene (Altosid) applied for control of *Psorophora columbiae* in rice-fields in Louisiana at 0.025/acre caused significant reductions in certain non-target aquatic insect populations (adults of *Tropisternus* spp. and nymphs of libellulids). A significant increase in immature baetids and chironomids followed the reduction in populations of these predators. There were no significant reductions in adult and immature *Notonecta* spp. and corixids and adults of *Thermonectus* spp. at application rates of 0.25 lb/acre (0.28 kg/ha)(Steelman *et al.* 1975).

9.3.2. Nematoda

The only nematode reported as exposed deliberately with methoprene is the mosquito pathogenic mermithid, *Romanomermis culicivorax.* Generally these studies have focussed on the dual application of methoprene with *R. culicivorax,* which have shown compatibility (eg. Gordon *et al.* 1976; Levy and Miller 1977; Nickle 1979; Finney *et al.* 1977). Specifically, Gordon *et al.* (1976) showed no effect on infectivity to *Ae. aegypti* of the pre-parasites or on parasitic or post-parasitic development of the mermithid. Levy and Miller (1977) found no effect of methoprene viability or infectivity (to 2nd-instar *Cx. p. quinquefasciatus*) and no effect on the viability of the resulting post-parasites; Altosid 5E, applied at doses ranging from 5 to 50 ppb did not interfere with the preparasitic, parasitic or postparasitic development of *R. culicivorax*, and host mortality was considerably increased when the mermithid and methoprene were used concurrently against mosquitoes (Finney *et al.* 1977). In contrast,

Winner and Steeleman (1978) found a 50% loss of swimming ability of the preparasitic (infective) stage of *R. culicivorax* exposed to 2.95 ppm methoprene (a relatively high dose- see Table 5) and impaired the ability of the nematode to locate and infect mosquito larvae. Winner and Steeleman (1978) reported that methoprene was significantly less toxic to *R. culicivorax* than mosquitoes.

Methoprene had no effect on the pine wood nematode, *Bursaphelenchus xylophilus* (Shuto *et al.* 1989).

9.3.3. Insects

Despite the extensive list in Table 4, most studies have reported little or no effect when methoprene has been applied to non-target insects at recommended rates. Among those species listed in Table 4 where methoprene was lethal, very high doses were often required to achieve mortality. Reports where methoprene had no effect on insects are shown in Table 6. This list includes a number of predatory insects and parasitoids. One problem in determining the effect of methoprene on non-target insects is that effects are often not seen until the emergence of adults (eg. Steeleman *et al.* (1975) and many studies examine direct effects during a relatively short timeframe, such as 48 to 72 h (Breaud *et al.* 1977).

In mosquito breeding areas, several studies have reported on effects on non-target insects. Farghal and Temerak (1981) found in Egypt that methoprene had no effect on the beneficial aquatic beetle *Dytiscus* sp. A Japanese study by Kikuchi *et al.* (1992) found no effect on several non-target aquatic organisms of treatment of urban drains with 1 ppm. Syrphidae, Chironomidae, the isopod *Asellus hilgendorfii*, the mayfly *Cloeon dipterum* (Ephemeroptera) and the stratiomyid *Hermetia illucens* survived in urban drains after treatment with 1 ppm methoprene.

An extensive study which found no effect on several non-target insects involved feeding methoprene to cows in Maryland in amounts sufficient to control larvae of *Musca autumnalis* in their faeces (Pickens and Miller 1975). Methoprene was not active against non-target insects among Diptera (Stratiomyidae) and Coleoptera (Staphylinidae, Curculionidae and Scarabaeidae)(Table 6). Another study on methoprene use in dung for the horn fly, *Haematobia irritans*, control showed no apparent effect on the reproduction of dung beetles (Fincher 1991).

Creekmur *et al.* (1982, abstract only available) reported no significant effects of methoprene on non-target insects (3 families of Coleoptera, 2 of Hemiptera) after Altosid application to ponds to control Chironomidae in California.

In some cases, methoprene has impacted on non-target insects. Constant exposure to 0.002-0.1 ppm methoprene caused disorders in metamorphosis in final-instar larvae of the aquatic naucorid *Ilyocoris cimicoides* (Heteroptera, Naucoridae)(Gelbic *et al.* 1994). In experimental ponds in California, methoprene application reduced the abundance of several arthropod prey species such as *Culiseta inornata,* Chironomid larvae and induced mortality in early- and late-instar nymphs of the mayfly *Callibaetis pacificus* (Norland and Mulla 1975). During colder winter months, mayflies were eliminated from ponds with repeated treatment. The ostracod *Cyprinotus* sp. was a major prey component and was not affected by treatment.

Blume *et al.* (1974) reported methoprene mixed in bovine faeces at 100, 10, 5 and 1 ppm inhibited the hatch of eggs of the dung beetle *Onthophagus gazellus* by 100, 56, 33.3 and 8.7%, respectively. When exposed to dung from a steer treated daily with methoprene at a rate of 1 mg/kg body weight daily egg hatch was inhibited by up to 32.6%.

Methoprene applied at 1 and 10 mg/litre to outdoor experimental streams resulted in Chironomidae and caddisflies disappearing (Yasuno and Satake 1990). Methoprene treatment appeared to increase the emergence of the mayfly, *Baetis sahoensis* in this study.

TABLE 6: Insects recorded as not susceptible to methoprene

Order/family	Species	Reference
Coleoptera: Curculionidae	*Hister abbreviatus*	Pickens and Miller 1975
Coleoptera: Dytiscidae	*Dytiscus* sp.	Farghal and Temerak 1981
	Dytiscid larvae	Floore *et al.* 1988
Coleoptera: Hydrophilidae	*Sphaeridium* spp.	Pickens and Miller 1975
Coleoptera: Scarabaeidae	*Aphodius fimetorius*	Pickens and Miller 1975
	Onthophagus gazella	Fincher 1991
	Sisyphus rubrus	Fincher 1991
Coleoptera: Staphylinidae	*Philonthus* spp	Pickens and Miller 1975; Fincher 1991
Diptera	[Syrphidae, Chironomidae]	Kikuchi *et al.* 1992
Diptera: Sarcophagidae	*Sarcophaga* spp.	Pickens and Miller 1975
Diptera: Stratiomyidae	*Sargus cuprarius*	Pickens and Miller 1975
	Hermetia illucens	Kikuchi *et al.* 1992
Ephemeroptera	*Cloeon dipterum*	Kikuchi *et al.* 1992
Hemiptera: Notonectidae	*Notonecta unifasciata*	Miura *et al.* 1978
	Buenoa scimitra	Miura *et al.* 1978
Hymenoptera: Aphelinidae	*Aphytis holoxanthus*	Peleg and Gothilf 1980
Hymenoptera: Eulophidae	*Tetrastichus ceroplastae*	Peleg and Gothilf 1980
(Hymenoptera: Pteromelidae)	*Muscidifurax raptor*	Wright and Smalley 1977
	Spalangia endius	Wright and Smalley 1977
Isoptera: Rhinotermitidae	*Coptotermes formosanus*	Haverty and Howard 1979
Lepidoptera: Gelechiidae	*Sitotroga cerealella*	Stockel 1976
Odonata: Coenagrionidae	*Enallagma* sp.	Floore *et al.* 1988

9.3.3.1. Insect predators

Predators are often an important natural control agent of pest insects such as mosquitoes, and use of methoprene should be evaluated for effects against such biological agents. Methoprene was reported by Miura *et al.* (1978) to have no deleterious effect on the predators *Notonecta unifasciata* and *Buenoa scimitra* when used to control *C. tarsalis* in California, and the effect of methoprene and these Notonectidae was additive in suppressing mosquito populations. Also in California, repeated application of 0.1 ppm of methoprene (Altosid EC4) to experimental ponds eliminated larva of the Dytiscid predator beetle *Laccophilus* sp. This represented a loss of 84% of the predator biomass during one period. *Odonata* nymphs formed the second major group of predators during the study; these preyed heavily on mosquitoes and ostracods and were not affected by Altosid (Norland and Mulla 1975). Two larval predators, damselfly naiads (*Enallagma* sp.) and dytiscid larvae, present in the plots during this test, appeared not to be affected by the Altosid applications against *Cx. quinquefasciatus* in Florida (Floore *et al.* 1988).

First instars of the predator of scale insects in Israel, *Chilocorus bipustulatus*, was exposed to scales and plants dipped in 0.025% concentrations of methoprene, diflubenzuron and fenoxycarb. All the larvae on squashes dipped in diflubenzuron died in the 1st instar. Methoprene and fenoxycarb did not arrest larval development but inhibited pupation. The fecundity of females of *C. bipustulatus* was not affected, but none of the eggs hatched. Egg viability was regained when females exposed to the growth regulators were transferred to an uncontaminated environment (Peleg 1983).

When eggs of the aphid predacious neuropteran *Chrysopa carnea* were treated with methoprene as third instar larvae, treatment at 100 µg/larva inhibited metamorphosis and adult development, and prolonged larval development (Romanchenko *et al.* 1987).

9.3.3.2. Parasitoids

The activity of methoprene against beneficial non-target organisms is of significant interest in any environmental safety evaluation. Parasitoids perform natural control of many potential pest species and, in addition, have been introduced to countries for the control of pest populations. Therefore, there are many studies reporting the direct and indirect effects (ie via the host) of methoprene on parasitoids. There are no parasitoids which attack mosquitoes, although, depending on the area of methoprene application, it is conceivable that methoprene could be used in areas where parasitoids were part of an integrated management system for pests, either naturally or introduced (such as orchards). Methoprene has been found to disrupt parasitoid success at high doses and to alter sex ratios. However overall, the effect of indirect exposure to methoprene would be unlikely to negatively impact on parasitoid control of a non-target insect.

Methoprene treatment of the host of *Parasierola nephantidis* and *Bracon brevicornis* larvae of the coconut pest *Opisina arenosella* in India, showed treatment significantly affected the number and sex ratio of adults of both parasites emerging (Sundaramurthy *et al.* 1985; Jayaraj 1989). In Israel, Peleg and Gothilf (1980) demonstrated that sprays of 0.1% methoprene (Altosid) applied to *Chrysomphalus aonidum* parasitised by *Aphytis holoxanthus*, *Saissetia oleae* parasitised by *Coccophagus pulvinariae* or *Ceroplastes floridensis* parasitised by *Tetrastichus ceroplastae* had no adverse effect on the developmental stages of the parasites. There was some mortality among pupae of *C. pulvinariae*.

Methoprene (ZR-515 4E) at 0.1-0.8 µl/insect on final-instar larvae of *Galleria mellonella*, subsequently parasitised by *Gonia cinerascens* only caused indirect effects on the parasite related to changes in the quality of the host (Verenini 1984).

Fenoxycarb, hydroprene, kinoprene and methoprene were applied in sprays at 0.01, 0.1 and 0.5% in pear orchards in Hungary when larvae of the parasitoid *Aphytis mytilaspidis* were abundant on overwintered females of the scale pest *Epidiaspis leperii* (El-Kareim *et al.* 1988). The rate of parasitism was unaffected by the lower concentrations used, but at 0.5%, hydroprene, methoprene and kinoprene disrupted development of the parasitoid.

Direct application of methoprene to the pupal stage of *Nasonia vitripennis*, a hymenopteran parasite particularly of Diptera, caused up to 100% mortality while methoprene inoculation of a host with the parasite, *Sarcophaga bullata,* had no effect on *N. vitripennis* (Fashing and Sagan 1979; Loof *et al.* 1979). Topical application of methoprene (ZR-515) to newly formed puparia of *Chrysomya albiceps*, at the rate of 10 µg/5 µl acetone per puparium, had no effect

on the emergence, longevity, oviposition behaviour, fecundity or sex ratio of the parasitic pteromalid *N. vitripennis* (Omar 1987).

Topical application of methoprene to parasitised larvae of *Manduca sexta* inhibited subsequent emergence of the endoparasite *Apanteles congregatus* in a dose-dependent manner, causing either a delay or total inhibition of emergence (Beckage and Riddiford 1982). It was also observed that parasites emerging from hosts treated with a low dose of methoprene later pupated normally but then formed non-viable pupal-adult intermediates. Beckage and Riddiford (1982) suggested the use of IGRs must be undertaken carefully to prevent possible adverse effects on natural parasite populations.

Methoprene, applied as a drench, also reduced *Liriomyza trifolii* emergence and percentage parasitism by *Oenonogastra microrhopalae* when fully-grown leaf-miner larvae were placed on potting media from 2 days before to 6 days after application (Oetting 1985). Surface sprays of methoprene to clay loam did not affect leaf-miner or parasite survival.

Wright and Smalley (1977) reviewed published laboratory and field work by the authors and others on the effectiveness of the 4 juvenile hormone analogues, including methoprene, against the immature stages of *Stomoxys calcitrans* in the USA. It was shown that the analogues effectively prevented the emergence of adults of *S. calcitrans* from breeding sites in cattle feeding compounds in Nebraska and from marine vegetation in Florida without interfering with the oviposition activities and development of the endoparasites *Muscidifurax raptor* and *Spalangia endius*.

9.3.3.3. Bees

There are some contradictions in the reported effects of methoprene on honeybees. A study by Barker and Waller (1978) concluded that methoprene was relatively safe for honeybees. In their study, small colonies of honeybees in outdoor flight cages were fed insecticide-treated syrup and water for 31 days. Consumption and utilization of food, and production of sugar honey, wax and bees were measured. Even at 1000 ppm, methoprene had no observed hormonal effects, but one formulation, a 65.5% methoprene emulsion concentrate, eliminated brood production. In the most recent study reported in the literature, Deng-GuiYun *et al.* (1997) found that methoprene had no effect on preferences for pollen or nectar and did not influence nectar foraging rate, nectar load size, and foraging span.

In another study with honeybees, Redfern and Knox (1974) compared the effect of methoprene (ZR-512 and ZR-515) with the carbamate insecticide, carbaryl. Methoprene was applied in 1 ml drops to individual honeybees topically to the dorsum of the thorax of bees or in 50% sucrose solution orally. Mortality after 48 h did not exceed 10% at any dose tested (up to 1000 μg per insect). By contrast, carbaryl caused 100% mortality at a dosage of 1 μg/bee orally, or 10 μg/bee applied topically.

Other work suggests that methoprene may be harmful as it has been shown to significantly reduce wax secretion. This suggests that methoprene, applied pharmacologically as is done routinely in polyethism studies, may be sublethal and poisonous to worker honey bees (Muller and Hepburn 1994). A study carried out in Egypt examined the effect of methoprene (ZR 515) on *Apis mellifera* (Hussein and Abdel 1978). Methoprene was topically applied to 3 and 5 day old worker larvae in the hive. One day after treatment, 55-70% of the treated larvae were removed from their brood cells. Abnormal formation of abdomen, wings and

wax glands was observed. The degree of deformity was dependent on the concentration of methoprene applied and was highest in the 3-day old larvae.

Methoprene may also affect the behaviour of bees. For example, worker honeybees treated with 250 μg of methoprene moved from the broodnest to the food storage area prematurely and displayed precocious foraging behaviour (Robinson 1985). Treatments with 25 and 2.5 μg caused slight but non-significant effects. Methoprene did not affect individual foraging performance but induced premature production of two alarm pheromones. The results suggest that methoprene was disrupting normal hormonal regulation of the temporal division of labour in the honeybee colony. In another study, topical or oral administration of methoprene to worker honeybees at one day of age caused them to begin foraging activity during the second week after emergence compared with the third week after emergence for untreated bees (Robinson and Ratnieks 1987). In workers treated with methoprene (doses of 25-250 μg/insect), the normal stages of behavioural development (cell cleaning, brood and queen care, food storage, foraging) were compressed into a shorter time period (Robinson 1987). At low doses of methoprene, bees passed through all four 'age-castes' but their time in the second and third was shortened. Bees treated with high doses tended to miss out the second and/or third age-castes. Methoprene had only a weak or no effect on social interactions, self-grooming and other non-task behaviours which were not age-dependent.

9.4. Rotifers and marine worms

Rotifers constitute as large group of aquatic or semi-aquatic species which may be free living or sessile, predatory or prey. They are often an important part of the foodchain and so of interest in any non-target considerations of methoprene. However, few studies have examined methoprene effects on rotifers. Schaefer *et al.* (1974) reported methoprene at 224 g ai/ha did not adversely affect the rotifer, *Asplanchna* sp. in experimental ponds. Mian and Mulla (1982b) considered that in general IGRs were innocuous to rotifers. Altosid was not toxic to the amphipod *Elasmopus bampo* and the polychaete *Neanthes arenaceodentata*, at 100 mg/litre (Reish *et al.* 1985).

9.5. Mollusca

Non-target impacts on mollusca have rarely been documented. Schaefer *et al.* (1974a) reported methoprene applied to experimental ponds had no adverse effect on the snails *Physa* or *Lymnaca*. Kikuchi *et al.* (1992) also reported that the toxicity of methoprene and some other insecticides against *Physa fontinalis* and the isopod *Asellus hilgendorfii* was lower than that of other insecticides. Significant differences in the toxicity against *P. fontinalis* were found between methoprene and the other insecticides tested: 48 h LC_{50} values were 1.9 ppm for fenitrothion and dichlorvos, 2.5 ppm for diazinon, but 10.6 ppm for methoprene. The 48 h LC_{50} values of fenitrothion, dichlorvos, diazinon, methoprene and fenthion to the juveniles of *A. hilgendorfii* were 0.018, 0.035, 0.25, 0.3 and 0.65 ppm, respectively. Creekmur *et al.* (1982) found no significant effect of methoprene on snails. In a review of IGRs, Mian and Mulla (1982b) concluded that IGRs generally had "good safety margin" for use around non-target snails.

9.6. Crustaceans

9.6.1. Microcrustaceans

A large portion of the aquatic fauna are crustaceans, making the group important in assessing the non-target effects of a mosquitocidal agent, such as methoprene. Overall, application of methoprene can have an effect on some microcrustaceans, but does not appear to cause long term disruption (Mian and Mulla 1982b).

Ostracods (*Cyprinotus* sp.) exposed to methoprene eight times at 5 day intervals did not show major changes (Norland and Mulla 1975). The acute and chronic effects of methoprene on the survival and reproduction of the freshwater cladoceran (water fleas) *Moina macrocopa* was studied by Chu *et al.* (1997). In laboratory toxicity tests, the 24- and 48-h LC_{50}s were 0.51 and 0.34 mg litre^{-1}, respectively. Survival, longevity and fecundity were reduced at 0.05 mg litre^{-1} and higher concentrations. At 0.005 and 0.01 mg litre^{-1}, longevity and fecundity increased slightly compared to untreated controls. It was concluded by Chu *et al.* (1997) that if environmental concentrations of methoprene do not exceed 0.05 mg litre^{-1}, as is generally the case, application of this insecticide is unlikely to induce detrimental effects on natural cladoceran populations. The stimulatory effect of very low concentrations of methoprene on reproductive performance was consistent with the hypothesis of a regulatory role of juvenile hormone-like compounds in crustacean reproduction.

Copepods are important components of the food chain in aquatic systems and some species are predatory on mosquitoes. In addition, a number are an obligatory secondary host of mosquito pathogenic fungi in the genus *Coelomomyces*. *Coelomomcyes psorophorae* var. *halophilus* is an mosquitocidal fungus which requires an obligate secondary copepod host. Gettman and Rupp (1993) showed that methoprene did not affect the secondary host, *Nitokra sewelli*. In an early study, Miura and Takahashi (1973) reported that methoprene adversely affected copepods and cladocerans. However, subsequent studies by Schaefer *et al.* (1974a) found no effect of methoprene (Altosid) on Cladocera (*Daphnia* and *Moina* spp.), Eucopepoda (*Cyclops* and *Diaptoms*), Conchostraca (*Euliminadia* sp.) and Podocopa (*Cypricerus* sp.).

In salt marshes in Italy, methoprene (Altosid SR-10) was applied against larvae of *Ae. detritus* (Majori *et al.* 1977). Populations of copepods and 4 species of dytiscids that were present showed only slight reductions, which were not permanent. Similarly, the toxicity of methoprene to the salt marsh copepod *A. spartinus* (from Massachusetts) was evaluated and compared with sensitivity of mosquito larvae (*Aedes sollicitans*, plus freshwater *Aedes* spp.) (Bircher and Ruber 1988). All stages of the life cycle of the copepod were tested at concentrations ranging from 0.1 to 10.0 ppm. Eggs and the earliest hatched stages, nauplius I-III, were most sensitive to methoprene, with little mortality seen in the later stages. Toxic effects were manifested as death, or failure of eggs to hatch; however, the life cycles were not prolonged. In general, the copepods were resistant at concentrations of methoprene used to control mosquitoes. Early nauplii, however, did show some mortalities to methoprene concentrations near the lower margins of mosquito susceptibility. This might lead to transient decreases in copepod population growth rates, but not necessarily to decreases in their standing populations.

Larvivorous copepods (*Macrocyclops albidus*, *Mesocyclops longisetus*, *M. ruttneri* and *Acanthocyclops vernalis*) were tested for their sensitivities to commonly used mosquito

larvicides and adulticides. LD_{50}s for temephos and methoprene were nearly the same and 130 times the LD_{50} required for larvae of the mosquito *Ae. albopictus*. (Marten *et al.* 1993).

The copepods, *Mesocyclops* sp. were not greatly affected by methoprene application in the laboratory in India (MAnonmani 1989). Methoprene effect on *M. longisetus* was also assessed in the laboratory using concentrations 10 times the maximum labelled or suggested rate and based on a water depth of 7.6 cm and exposing newly hatched copepods (i.e. nauplius larvae) and monitoring their development to maturity (Tietze *et al.* 1994). Methoprene was not deleterious to copepods at concentrations exceeding those expected in the field. Copepods exposed to methoprene matured normally, and when mated 50% developed egg sacs.

The crustacean *Gammarus aequicauda*, which is considered an important food constituent of fish, shares its breeding places with *Ae. detritus* a major pest mosquito in Central Italy coastal marshes (Gradoni *et al.* 1976). A high margin of safety appears to exist between the lethal doses for *G. aequicauda* and those for *Ae. detritus*, thus allowing the use of local mosquito antilarval measures employing methoprene.

9.6.2. Macrocrustaceans

Macrocrustacean, such as shrimps and crabs, have been tested against methoprene as they commonly occur in environments treated with the IGR. Generally, methoprene can be toxic to macrocrustaceans such as shrimps and crayfish (Table 9). The toxicological studies reported by Wright (1976) listed several shrimp and crayfish species with LD_{50} =100 ppm.

In studies on non-target effects of methoprene when used against mosquitoes, an experimental long-duration (150 days) controlled-release formulation was applied to breeding sites of *Ae. vexans* in Minnesota in 1983-85 to maintain a level of methoprene of 1.5 ppb (Batzer and Sjogren 1986). No significant differences in the presence, population density and size of the shrimp *Eubranchipus bundyi* were found between treated and untreated sites.

The influence of methoprene, used in mosquito control, on larval development of the estuarine grass shrimp *Palaemonetes pugio* was examined in the laboratory (McKenney and Matthews 1990). No crustacean larvae successfully completed metamorphosis when continuously exposed to 1000 μg methoprene/litre. Completion of larval metamorphosis was significantly reduced by exposure to 100 μg/litre of the isomeric mixture (R,S)-methoprene but not the single isomer formulation (S)-methoprene. No statistically significant difference was revealed, however, in ability to inhibit metamorphosis between these 2 isomeric types across the broad range of exposure concentrations from 0.1 to 1000.0 μg/litre. The first 2 larval stages and the final premetamorphic larval stage were more sensitive to methoprene toxicity than intermediate larval stages. Methoprene exposure did not alter either the duration of total larval development or the total number of larval stages prior to metamorphosis. McKenney and Celestial (1993) also found that methoprene inhibited successful completion of metamorphosis of *P. pugio*. Methoprene exposure retarded growth in early larval stages and postlarvae, but enhanced growth in premetamorphic larvae. In field tests in Delaware in 1974-75 to determine the toxicity of methoprene to non-target saltmarsh organisms McAlonan *et al.* (1976) found one formulation of methoprene (10-F) killed more than 60% of *P. pugio* at rates of 0.048-0.12 lb/acre (0.054-0.13 kg/ha). Four fortnightly applications of a second formulation (SR10) at rates to give 0.024-0.384 lb/acre (0.028-0.43 kg/ha) caused no significant mortality among *P. pugio* or alteration in the frequency of ecdysis, and in a further

test with SR-10, 3 fortnightly applications at the same rates caused no significant mortality or alteration of ecdysis frequency.

Conversely, use of 10 times the recommended dose of 0.02 ppm active ingredient of Altosid SR-10 against *P. pugio* and the crab *Uca pugilator* in the laboratory had no adverse effects on the moulting cycle for either species, and no increase in mortality was found (Barber *et al.* 1978).

Moulting in third instar larvae of the brine shrimp, *Artemia* (Artemiidae) was interrupted, or even accelerated, when populations were exposed to various concentrations of methoprene in artificial sea-water (Ahl and Brown 1990). The effects were believed to be salt-dependent, because exposure to these compounds in sea-water that is isotonic to larval haemolymph had no effect. This suggests that the juvenoids may target the ion transporting epithelia (Ahl and Brown 1990).

Acute toxicity studies on the shrimp *L. tenuicornis* to temephos, *Bti*, methoprene and pyriproxyfen was tested in 96-h laboratory trials for registration purposes in Australia. Temephos was the most toxic compound, with a LC_{50} of 0.01 ppm (0.33 times the estimated field concentration (EFC) for a 15-cm-deep pool). Methoprene was the least toxic compound, with an LC_{50} of 14.32 ppm (1790 times the EFC). *Bti* and pyriproxyfen produced LC_{50} values of 60.9 x 10^6 ITU (176 times the EFC) and 0.098 ppm (12.25 times the EFC), respectively (Brown *et al.* 1996).

The mud crab, *Rhithropanopeus harrisii,* susceptibility to methoprene was study by Christiansen *et al.* (1977a) in the laboratory. Using 0.01, 0.1 and 1 ppm of methoprene with various salinity (5-35 ppm) and temperature (20-35^{o}C), these authors found a significant reduction in the survival of zoeal larvae with increasing methoprene concentrations at almost all temperature/salinity combinations. One ppm completely arrested further development. At under 0.1 ppm little effect on metamorphosis was noted. Forward and Costlow (1978) did not find any effect of sublethal concentrations of methoprene against this mud crab.

9.7. Fish and amphibians

The EPA (1991) summarised available fish studies concluding that methoprene is moderately toxic to warmwater, freshwater fish and slightly toxic to coldwater, freshwater fish. Exposure of fish to methoprene has produced LC_{50} values ranging from 3.3 mg/l for trout (species not specified) to >100 mg/l for channel catfish (*Ictalurus punctatus*) (Anon 1973). Acute fish toxicity would not be expected during control programmes as the concentration of methoprene in water at any one time is unlikely to exceed 2 ppb (Ross *et al.* 1994b). It should also be noted that some of the experimental work examining methoprene toxicity to fish used special solvents to increase the solubility in water, for example, use of dimethyl-formamide by Ross *et al*. (1994b). Solvents are not used in Altosid formulations and the solubility is 1.39 ppm (D. Sullivan, pers. comm.).

In field tests in Delaware in 1974-75 to determine the toxicity of methoprene to non-target saltmarsh organisms (McAlonan *et al.* 1976). One formulation of methoprene (10-F) applied to give 0.012-0.12 lb/active ingredient acre (0.013-0.13 kg/ha) caused no mortality of *Fundulus heteroclitus* or *Uca* spp. at rates of 0.048-0.12 lb/acre (0.054-0.13 kg/ha). Four fortnightly applications of a second formulation (SR10) at rates to give 0.024-0.384 lb/acre

(0.027-0.43 kg/ha) caused no significant mortality or alteration of ecdysis frequency among *Uca* spp.

Toxicity of mosquito adulticides and larvicides to 12- to 16-day-old inland Silverside, *Menidia beryllina* was determined using static bioassays in the laboratory by Tietze *et al.* (1992)(Table 7). They determined the 48-h LC_{50} for the inland silverside to be 2.781 mg/l (278x label rate). Methoprene was more toxic than some compounds, such as temephos however only resmethrin was highly toxic to the fish at recommended field application rates. Conversely, Lee and Scott (1989) found methoprene far less toxic to mummichog *F. heteroclitus* than most other mosquito larvicides (Table 7).

TABLE 7: Acute toxicity of mosquito larvicides to mummichog, *Fundulus heteroclitus*, (from Lee and Scott 1989) and Inland Silverside, *Menidia beryllina* (from Tietze *et al*. 1992).

Insecticide	*F. heteroclitus* Mean 96h LC_{50} (ppm) (95% confidence limits)	*M. beryllina* Mean 24h LC_{50} (ppm)
Temephos	0.04 (0.02-0.05)	6.256
Fenoxycarb	2.14 (2.01-2.27)	0.885
Diflubenzuron	32.99 (29.01-37.52)	
Methoprene	124.95 (90.01-171.64)	2.781*
Fenthion		1.474
Naled		3.544
Resmethrin		0.00387
Petroleum distillates		137.542
VectoBac (*Bti*)	980.00 (730-1330)	
Fenoxycarb/VectoBac	1.55 (1.40-1.72)	

* after 48h

McKague and Pridmore (1978) determined the methoprene 96-h LC_{50} for rainbow trout and coho salmon (*Oncorhynchus kisutch*) to be 106 and 876 mg/l, respectively (10,600 and 8,600x the maximum label rate). In another study, Brown *et al.* (1998) found no mortality of the Pacific blue-eye, *Pseudomugil signifer*, to s-methoprene at 500 times the estimated field concentration. No solvent was used to increase solubility in this study. *P. signifer* is a small larvivorous fish abundant in shallow estuarine mosquito habitats.

Newly spawned fathead minnow, *Pimephales promelas,* continually exposed to methoprene concentrations of 13, 23, 48, 84 and 160 µg/litre for 37 days in a 2-litre proportional diluter system showed no significant reductions ($P>0.05$) for hatchability, fry survival or total survival when compared to controls (Ross *et al.* 1994a). Significant reductions ($P<0.05$) in length and weight were detected at the two highest mean measured test concentrations compared to controls. The maximum acceptable toxicant concentration (MATC) limits, the no-observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC), based on analyses of fry length and weight, were 48 and 84 µg/litre. While these concentrations appear to be rather low, they are 196-466 times higher than average levels of methoprene present when formulations containing methoprene are applied for mosquito control (Ross *et al.* 1994b).

In the absence of published literature describing sublethal effects, several blood serum parameters commonly used to study stress responses in fish were measured subsequent to treatment of rainbow trout (*Salmo gairdneri*) (Madder and Lockhart 1978). Trout were

exposed to methoprene at levels ranging from 0.625 to 10.0 mg/l, which were 62.5-1,000 x the maximum label rate (10 μg/l). During exposure to the high levels of methoprene, the rainbow trout in treated aquaria became visibly lethargic in comparison with the control fish. Analyses of sera from fish showed a dose dependent decrease in blood serum glucose concentrations. Blood sugar measurements are regarded as indicators of stress in fish (Silbergeld 1974).

In another study, Ellgaard *et al.* (1979) found that the locomotor activities the goldfish, *Carassius auratus* did not significantly alter after exposure to 0.2 ppm methoprene.

9.7.1. Mosquito predatory fish, *Gambusia affinis*

The predatory fish, *Gambusia affinis*, has been used for mosquito control in several countries. Therefore a number of studies have evaluated the effect of methoprene on adult *G. affinis.* Twenty-four hour exposure methoprene (Altosid) at concentrations of 5, 10, 25, 50, 100 and 200 ppb of *G. affinis* adults significantly lowered the thermal tolerance in male fish at concentrations of 50 ppb, whereas the corresponding concentrations in the case of female fish was 200 ppb (Johnson 1977). No mortality occurred at the concentrations tested, and loss of orientation was not observed. Miura and Takahashi (1974a) found no adverse effect on *G. affinis* when the SR-10 formulation of Altosid was applied

Populations of *G. affinis* were exposed to methoprene (Altosid) or diflubenzuron (Dimilin) in experimental ponds in California to determine the effects on them of multiple treatments as used for mosquito control over a period of 5 months (Takahashi and Miura 1974a). No visible adverse effect on the fish resulted from 5 applications of methoprene at 0.03 lb active ingredient/acre (0.034 kg/ha) or diflubenzuron at 0.05 lb active ingredient/acre (0.056 kg/ha) at monthly intervals. Fluctuations in population were similar to those in untreated ponds. Ellgaard *et al.* (1979) used 10 times the generally recommended dose of methoprene (0.2 ppm) against *G. affinis* and found no significant effect on locomotor activity.

9.8. Deformed frog controversy

In 1995, high school students discovered deformed frogs in Minnesota and began a controversy (partly conducted on the internet) involving the widespread use of methoprene and other chemical pesticides. Since then, deformities have been reported from many North American locations (Figure 1) and a website has been established to collect information and reports of deformities (Northern Prairie Wildlife Research Center 1997). Species that have been reported with malformations include northern leopard frogs, wood frogs, bullfrogs, green frogs, mink frogs, gray treefrogs, Pacific treefrogs, spring peepers, American toads, long-toed salamanders, tiger salamanders, and spotted salamanders (Northern Prairie Wildlife Research Center 1997). Some scientists suspected environmental contamination. However, the finding of deformed frogs has not resulted in a clear indication of a cause, but three major theories have emerged 1) a parasite of frogs; 2) ultra-violet radiation; 3) chemical contamination (Kaiser 1997), or some combination of the three (Manuel 1997). Each theory has supporters and critics and the debate has not been resolved (Sessions 1998). Whether or not the deformities are a large or small scale problem has been questioned, with Sessions (1998) pointing out that most recent reports are from a single study by Sessions and others, mainly in Minnesota.

The first two hypotheses for the cause of frog deformities in Minnesota are discussed in the literature and of little relevance to this report, if they prove true. Manuel (1997) largely discounts the involvement of parasites, pointing out that even where parasites were not present, deformities were observed. The chemical contamination theory is relevant, however, as some reports have implicated methoprene as a likely contaminant. One theory suggested that an unidentified teratogen in water, possibly a retinoid (which are involved in controlling growth in embyros) is inducing these deformities. Methoprene has been targeted as a possible retinoid, as it has been widely sprayed in Minnesota for pest control.

NIEHS and other US agencies began examining water quality. Testing of water showed differences in frog deformities in samples from Minnesota and another region (Hawkins *et al.* 1997). Two USA scientists, Drs James LaClair and Jack Bantle added to the debate by implicating methoprene directly in deformities induced in the laboratory in the African Clawed frog (*Xenopus laevis*). Drs LaClair and Bantle knew from earlier work that fresh methoprene did not cause frog deformities, however when they exposed methoprene to sunlight and then added the sunlight-treated methoprene to rearing water containing tadpoles, emerging frogs had (mainly hindleg) deformities (Froehlig 1997).

Bantle and LaClair (in LaClair 1997) stated that " S-methoprene is reported to be about as toxic as common sugar in rats, however it rapidly reacts with normal sunlight to produce materials which we find induce high levels of deformed African Claw Toed Frogs (*Xenopus laevis*) when added during their early development. Unlike their parent these photoisomers persist in aquatic environments for considerably longer periods of time and further present even more dramatic deformation when metabolized either by microorganisms or by 'host' organisms". Bantle and LaClair concluded that "the outcome of this study illustrates the need for stricter regulation to build guidelines which require full examination of all products of a material's metabolism and natural degradation in order to minimise impact on human and environmental health" (LaClair 1997).

However other scientists have not been able to reproduce these results and questioned the conclusions drawn. LeClair's study used high levels of synthetically manufactured methoprene acid to produce malformations that were different from those found in nature (D. Sullivan pers. comm.) In addition methoprene acid does not appear to be a normal breakdown product of sunlight exposed methoprene. Occasional occurrences of malformations appear to be normal, since reports of malformed frogs exist from as long ago as 1740 (Northern Prairie Wildlife Research Center 1997).

Stanley and Sessions (in Sessions 1997) found several problems with attributing deformities to methoprene:

1) There was no geographic connection between methoprene and the deformities. Deformities were found at many locations and methoprene had not been used at all these sites. Altosid is used in only three counties in Minnesota, one of which has reported frog deformities. Most of the counties that are reporting frog deformities have never been treated with methoprene. There are also many counties which have been using methoprene for as long as twenty years with no reports of frog deformities.

2) The level of exogenous retinoids needed to produce a duplicated limb in a frog was extremely high; they estimated the amount to be approximately $13M worth of retinoids in a pond the size of a backyard pool to replicate even the most basic effect. (Le Clair's application rate was greater than 15000 times the normal application rate for methoprene).

3) The type of deformities should be also found in other amphibians, which is not the case. In addition, the deformities found by Le Clair and Bantle were in the gut and craniofacial area, different from the deformities found in nature.
4) Also, why just amphibians, since the effect is attributed to substances common in all animal development?

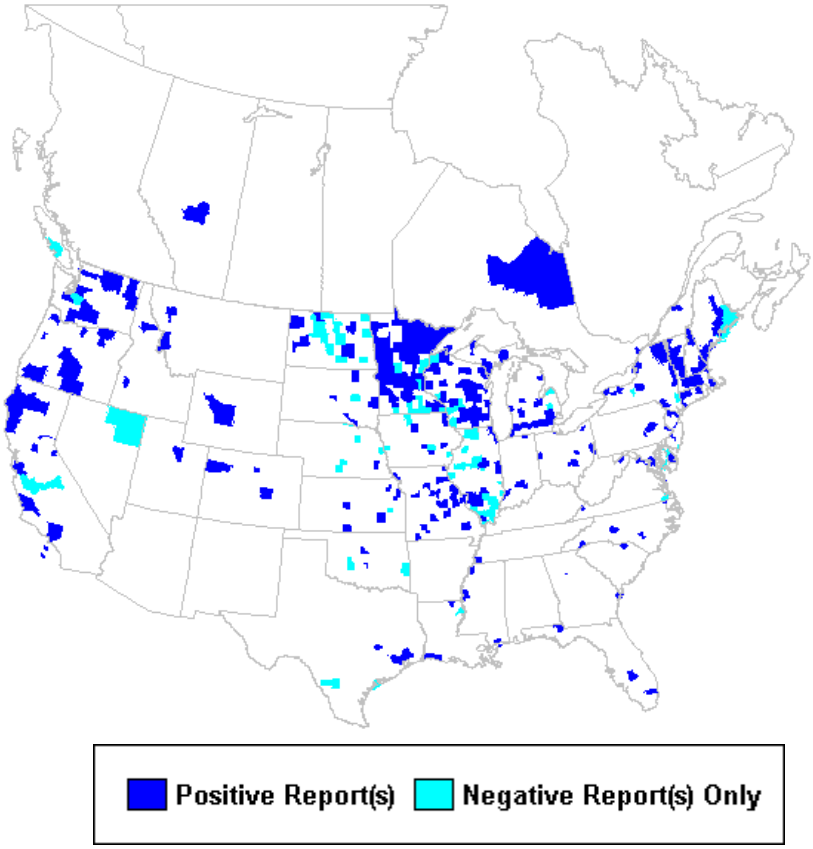
More recently, Sullivan (1998) presented data from more than 17 methoprene/amphibian studies, which included six species of frogs, from egg to larva to tadpole to adult. There was no evidence of frog deformities, even where methoprene was used at 500 times the field application rate. Of importance, Sullivan (1998) states "La Clair initiated his studies using 30 ml of ALL in 10 liters of pond water in nalgene dishes in partial shade. The high rate of Altosid® ALL is 300ml/hectare and provides initially 10 ppb. La Clair's study used approximately 15,000 times the label rate (ca 150 ppm). At this rate, he exposed *Xenopus* embryos to methoprene and found little deformities or mortality. He found frog embryos with deformities only when he exposed them to **methoprene acid.** His embryo exposure to methoprene acid was 7.5ppm in solution (changed daily), which is at least 5250 times the rate for Altosid® ALL (300ml/hectare)". Sullivan (1998) also mentioned the lack of link between methoprene treated areas and distribution of frog deformities and that a recent study of methoprene by the United States Environmental Protection Agency concluded that methoprene does not cause frog deformities

Kaiser (1997) recorded other concerns with the results. *X. laevis* does not grow in plain water, but requires certain salts. When the experiment was repeated with salts added, embryos developed normally.

Sessions (1997) and others have called for the possible link between methoprene and deformities to be researched immediately. However, he stated "the good news of the situation is that the methoprene/retinoid mimic scenario seems unlikely. This is very good news because the alternative has horrendous implications for all organisms that share the environment with frogs, including people. The bad news is that the simple possibility that this could be correct is so terrible that we just cannot afford to ignore it completely. If methoprene or a [retinoid] mimic is causing these problems, then the deformed amphibians are telling us something of extreme importance, and we need to do something to counteract the situation".

In a separate study, Sparling and Lowe (1997) examined the effect of commercial formulations of methoprene and temephos on frogs. They tested Abate (44.6% temephos) and Altosid (5% methoprene) on tadpoles. They found that temephos was more toxic than methoprene to Gray Treefrog (*Hyla versicolor*) tadpoles. Abate depressed growth of tadpoles and was also more toxic in laboratory tests. Although they were not able to calculate dose-response curves for methoprene, the median lethal dose for Altosid was at least 10 times greater than that for temephos. The authors concluded that of the two mosquito abatement chemicals, Abate clearly presents the greater risk to amphibian larvae.

FIGURE 1: Records of deformed frogs from North America (from Northern Prairie Wildlife Research Center, 1997 [http://www.npwrc.usgs.gov/narcam/index.htm]). The data posted on the Web site are all confirmed by biologists. Reports of malformed amphibians that are received at the Reporting Center, but not verified by a biologist, are not included.


Positive Report(s)
Negative Report(s) Only

9.9. Mammalian toxicity

In their 1991 review of methoprene, the EPA summarised data indicating methoprene had low toxicity and posed low risk to humans and other non-target species, with the exception of estuarine invertebrates (EPA, 1991). Garg and Donahue (1989) reviewed the activity and safety of methoprene used to control insect pests of cattle, dogs and cats. They reported that methoprene could be considered safe by insecticide standards. The World Health Organisation has approved its use in drinking water for control of mosquitoes because it was regarded as posing minimal or no risk to humans, animals or the environment (Kiess 1981). Toxicological evaluations of methoprene in swine, sheep, cattle, dogs, rats, rabbits, hamsters and guinea pigs have revealed no clinical signs of toxicosis (Wright 1976).

Following the article by Garg and Donahue (1989), in which it is stated that there were no reports of teratogenicity or other undesirable side effects of methoprene, a letter was published in the same journal by Socha and Marec (1990), citing references describing abnormal development in several small mammals. They drew attention to the work of Paulov (1976), who found toxic and inhibitory effects of methoprene on the development and metamorphosis of toad tadpoles (*Bufo bufo*). In another study, Unsworth *et al.* (1974) reported that methoprene was teratogenic when administered to mice as a single intraperitoneal injection of 1mg/g on days 9 and 10 of pregnancy. Socha *et al.* (1990) also cited the work of Kensler *et al.* (1978), who showed that juvenile hormones inhibit mitogenesis of bovine lymphocytes and suggested that similar effects might be expected in the case of treatment with methoprene.

In a reply to this letter, Garg and Donahue (1990) reiterated the view that methoprene provided a safe alternative for insect pest control. Methoprene is used as an insecticide at very low concentrations. Teratogenic effects were not observed in rats and rabbits given methoprene at dosages up to 1000 mg/kg of body weight. Similarly, administration of methoprene (10,000 ppm in water) to sheep for 12 weeks also failed to cause adverse effects (Wright 1976). Possible teratogenic effects reported by Unsworth *et al.* (1974) may have been caused by impurities in the methoprene sample tested, which were revealed to the researchers by the supplier after the study was completed. The EPA (1991) R.E.D. (Registration Eligibility Decision) fact sheet noted that, in sub-chronic studies on animals, there was some evidence of increased live weight in test animals at high doses, however no chronic effect were observed. Methoprene metabolises rapidly and completely in animals (EPA, 1991).

9.9.1. Humans

The EPA described methoprene as "showing no significant adverse toxicological effects in any human health effects screening studies" (EPA 1991, pg 2). It has been placed in the “least toxic” category for eye and skin irritants and is not a human skin sensitiser. Recently, the United States EPA have lowered the Restricted Entry Intervals (REI) of methoprene and several other agricultural compounds (EPA, June 1996). Methoprene was reviewed for toxicity and found to pose little or no risk to workers and the REI was reduced to 4 hours after spraying. In the opinion of the EPA, the carcinogenic risk posed by methoprene has been adequately tested; they consider that methoprene does not demonstrate mutagenic or

carcinogenic properties and has been found to be extensively metabolized via beta oxidation, becoming almost totally incorporated into components of the tricarboxylic acid cycle.

Sidhu and Collisi (1989) reported a case of accidental exposure to a veterinary insecticide containing methoprene (0.15%) and organophosphates. A veterinary technician was accidentally exposed to a commercial veterinary insecticide canned product formulation while opening the package. The contents from the pressurised can were released to the indoor air and got splashed on face, bare parts of the body and clothes of the technician. None of the symptoms reported in the patient were attributed to methoprene, but were thought to be caused by exposure to the organophosphates and solvents in the formulation.

Humans may be exposed to small amounts of methoprene through food supply. However, the amount of methoprene in the consumers's diet is well below the level at which any adverse health effects could occur (EPA 1991). Humans can also be exposed while mixing, loading or applying the pesticide and while working among treated crops. However, the EPA is satisfied that methoprene poses no risk to people who are occupationally exposed to the pesticide.

Whereas some studies in mice and rats found an increased incidence of birth defects with methoprene eg (Unsworth *et al.* (1974), no human studies or case reports have been published which examine pre-natal methoprene exposure. Therefore, because positive findings in animal studies are not always predictive of human response, it is not presently possible to determine whether methoprene exposure in pregnancy poses an increased risk (Pergament and Stein Rissman 1992).

9.9.2. Residue tolerances in animal products

The tolerances established under the Federal Food, Drug and Cosmetic Act for residues of methoprene (Table 8) have been increased gradually. For example, the tolerance level for methoprene in fat of cattle has increased from 0.1 ppm in 1975 to 1 ppm in 1994 with the level in milk increasing from 0.01 ppm to 0.1 ppm in the same period.

9.9.3. Cattle

R.L. Harris, USDA (in Wright 1976) reported that over 800 cattle were treated with methoprene for three months for hornfly control without the development of any adverse effects. In another study, cattle were treated topically with juvenile hormones, including methoprene for control of *Hypoderma* larvae (Younger *et al.* 1975). There were no observed changes in the biochemical or haematological values that were measured. Acute toxic effects were not observed in the cattle.

In another study, the effect of methoprene on rumen function in Jersey cows was examined by incubating rumen inoculum with cattle feed samples containing methoprene at 100 and 200 mg/kg (Barker and Newton 1976). There were no significant differences between the molar percentages of acetic, propionic, butyric and isovaleric acid relative to the control, although there was a trend towards reduced acetate:propionate ratios and total volatile fatty acid production for all treatments. The authors considered that as the changes were slight, there were no contra-indications against use of methoprene for fly control in cattle.

TABLE 8: Pesticide tolerances for methoprene: (from Chem-News June 1994 http://pmep. cce.cornell.edu/chemnews/1994/jun-94.html)

	Commodity (ppm)
Barley.	5.0
Buckwheat	5.0
Cattle, fat	1.0
Cattle, meat	0.1
Cattle, meat byproducts.	0.1
Corn (except popcorn and sweet corn).	5.0
Eggs	0.1
Goats, fat	1.0
Goats, meat	0.1
Goats, meat byproducts	0.1
Hogs, fat	1.0
Hogs, meat	0.1
Hogs, meat byproducts	0.1
Horses, fat	1.0
Horses, meat	0.1
Horses, meat byproducts	0.1
Milk	0.1
Millet	5.0
Mushrooms	1.0
Oats	5.0
Peanuts	2.0
hulls	40.0
Poultry, fat	1.0
Poultry, meat	0.1
Poultry, meat byproducts	0.1
Rice	5.0
Rye	5.0
Sheep, fat	1.0
Sheep, meat	0.1
Sheep, meat byproducts	0.1
Sorghum (milo)	5.0
Wheat	5.0

9.9.4. Sheep

In tests in Kentucky during 1972-73, lambs treated nasally with methoprene (0.1, 0.5, 0.75, 1 and 1.5 mg/lamb) displayed no adverse reaction to treatment (Prasert *et al.* 1975). In toxicity tests on sheep, pigs and hamsters, a methoprene sample of 96.2% purity showed no toxic effects, but a sample of 74% purity showed some toxicity to these animals (Wright and Smalley 1977). No adverse effects were found on hematologic or biochemical values or on serum proteins in sheep given methoprene (up to 10,000 ppm in water) for 12 weeks (study reported in Wright 1976). No evidence of toxicosis occurred in any of the sheep throughout the test period and weight changes were normal. Perturition was normal and no teratogenic effects were observed. No visible histopathological lesions attributable to methoprene were found in any of the sacrificed animals.

9.9.5. Small mammals and birds

Exposure and reproduction studies on chickens, mallard ducks and quails indicated very high tolerance to methoprene (Table 9). Methoprene incorporated into the food of laying hens at concentrations of 0.005 and 0.01% did not cause any weight loss in the fowls (Morgan *et al.* 1975). Methoprene was selected for ant control on the island of Fregate in the Seychelles, where decline in numbers of the magpie robin (*Copsychus seychellarum*), one of the world's rarest birds, was thought to have been caused by the widespread use of insecticides (Edwards 1992).

The oral administration of methoprene at doses up to 34,600 mg/kg of body weight has failed to induce clinical signs of acute toxicosis in rats. Acute oral LD_{50} in dogs was 5000 to 10000 mg/kg (Siddall 1976). In rats and rabbits, oral and dermal LD_{50} doses of methoprene, respectively, were the highest among 17 pesticides registered for indoor flea control in the United States, indicating a high safety ranking for methoprene (Bledsoe *et al.* 1982). Nagano *et al.* (1977) found that the maximum intake of methoprene (Altosid) that is non-toxic to rats is 400 ppm in their food or 20 mg/kg body weight/day.

Domestic shorthaired cats infested with fleas were treated with two environmental applications of methoprene/pyrethrins. None of the cats showed side effects (Harvey *et al.* 1997).

Populations of song birds, small mammals and aquatic fauna were monitored before and after application of juvenile hormone analogue (Altosid or ZR-515) to *Lambdina fiscellaria* infested forest. No effects on the fauna were reported (Buckner *et al.* 1975). Methoprene (Altosid) was also applied against the same pest on balsam fir (*Abies balsamea*) in Anticosti Island, Quebec (Retnakaran *et al.* 1974). Applications were made by helicopter at concentrations of 0.25-3 oz/gal in July 1973. While there was a significant reduction in pupae and adults of the target pest, no adverse effects on small mammals, birds or the aquatic fauna were detected.

TABLE 9: Toxicological properties of methoprene (from Wright 1976).

Property	Dose for effect
Acute oral toxicity - rat	34,600 mg/kg
Acute oral toxicity - dog	LD_{50}=5000-10000 mg/kg
Subacute oral studies (90 days, rate and dog)	No effects with 5000 ppm
Primary skin and eye irritation	Non irritating
Acute dermal toxicity (rabbit)	Dermal LD_{50}=3000-10000 mg/kg
Acute aerosol inhalation (rat)	No effects at 2000 ppm
Three generation reproduction study (rat)	No effects at 2500 ppm
Teratology studies (rat, rabbit)	No effects at 1000 mg/kg
Dominant lethal mutagenicity	No effects at 2000 mg/kg
Static fish toxicity studies	
Bluegill	LD_{50} =4.62 ppm
Channel catfish	LD_{50} >100 ppm
Coho salmon	LD_{50} =32 ppm
Trout	LD_{50} =106 ppm
Crustacean toxicity studies	
Crayfish	LD_{50} =100 ppm
Fresh water shrimp	LD_{50} =100 ppm
White shrimp	LD_{50} =100 ppm
Pink shrimp	LD_{50} =100 ppm
Subacute oral feeding studies	
Mallard duck	LD_{50} >10,000 ppm
Bobwaite quail	LD_{50} >10,000 ppm
Chickens	LD_{50} >4640 ppm
Reproduction studies (bobwhite quail and mallard duck)	No effects at 30 ppm
Mammalian hormone bioassay (mouse and rat)	No estrogenic, androgenic, anabolic or glucocorticoid activity

10. Persistence and activity in the environment

Half-life of methoprene under controlled conditions in soil and water is around 1-2 days. However, under field conditions and protected from UV, activity against a number of pests including mosquitoes has continued for 100 days or more. Formulation can extend activity beyond one year in some circumstances and a number of sustained-release formulations (briquettes, pellets, boluses) have shown such prolonged activity. In animals unformulated methoprene is rapidly metabolised and degraded. Water quality can profoundly influence persistence, with pollution and salt water having negative impacts. Extremes of temperature can influence persistence and activity, however methoprene is relatively unaffected by temperatures between 10-25^{o}C. UV rapidly degrades methoprene. Following application against mosquito larvae, methoprene is more persistent in the environment that *Bti* after application against mosquitoes.

10.1. Environmental persistence

Incomplete degradation of control products and subsequent carryover of active ingredients to the next year are operational concerns of pesticide application. In general, it is considered that methoprene degrades rapidly in sunlight, both in water and on inert surfaces. It is metabolised rapidly in soil and does not leach. The literature shows some variability in the reported persistence of methoprene in the environment, which reflects the diversity of formulations and products available and the diverse settings in which it was applied, ranging from relatively protected environments in stored products to aquatic use with full exposure to environmental factors. Duration of persistence is also determined by the initial rate of application.

TABLE 10: Environmental properties of methoprene (From Wright 1976)

Property	
Persistence in soil (1 lb/acre, 1.12 kg/ha)	Halflife < 10 days
Movement in soil	Remains in top few inches of soil
Persistence in water in field	Halflife < 2 days
Persistence in plants (1 lb/acre, 1.12 kg/ha)	
Alfalfa	Halflife < 2 days
Rice	Halflife < 1 day
Uptake by plants	Wheat did not take up residues from treated soil
Fate in food chain	Does not accumulate in food chain
Fate in animals (mice, rats, guinea pigs, steers or cows)	Rapidly metabolised and eliminated
Fate in fish (natural field conditions)	No accumulation
Effects on non-target insects	No deleterious effects on non-target species

10.2. Persistence in water

The persistence of methoprene in aquatic environments is an important consideration from the standpoint of habitat pollution and impact on non-target aquatic organisms. Methoprene is reported to remain in the upper layers of water after application (Schaefer and Dupras 1973) but the distribution will be affected by the formulation used. Laboratory data provide evidence that methoprene is relatively short-lived in water, possibly due to hydrolytic degradation. Hangartner *et al.* (1976) reported the hydrolytic stability of methoprene to be approximately one week, while Wright (1976) listed the half-life as 2 days (Table 10).

Short persistence in water was reported by Madder and Lockhart (1980) in Canada. A series of sod-lined pools was constructed and used to monitor repeated applications of methoprene. As determined from bioassays with larvae of *Ae. aegypti* and by gas-liquid chromatography (GLC), methoprene 'disappeared' rapidly from the pool water. Levels of methoprene fell below the limit of GLC detection within 2 days, although biological activity persisted for about a week after treatment. Madder and Lockhart (1980) concluded that methoprene would not cause a long-term persistence hazard when used for mosquito control in Canadian prairie waters. In field trials at Guelph, Ontario, in 1975, methoprene at 0.028 kg/ha effectively controlled spring species of *Aedes* following treatment of third and fourth-instar larvae. The formulation (Altosid SR-10) remained active in pools for 13 days at 20^{o}C. The persistence of methoprene (SR-10) in saline water was determined by bioassay with third instar larvae of *Ae. sollicitans*. At 0.024 lb/acre (0.027 kg/ha), adult emergence was prevented at 48h, but not 96h, after application; at higher rates (0.096, 0.192 and 0.384 lb/acre or 0.108, 0.215 and 0.043 kg/ha), methoprene was still active after 96h (Edwards 1992).

Five sustained-release methoprene formulations were applied to microcosm tanks at maximum label rates to measure methoprene concentrations in the water over time (Ross *et al.* 1994b). Replicate water samples (1 litre each; 4 samples/microcosm/date; n=432) were collected pre-treatment and 1, 2, 4, 7, 14, 21, 28 and 35 days post-treatment, and analysed for methoprene residues using capillary gas chromatography. The highest (methoprene residue detected in any individual sample on any date was 6 μg/litre. Eighty-five of all samples contained residues >1.0 μg/litre; 71% were below the minimum quantitation (sic) limit (MQL = 0.2 μg/litre). Neither Altosid Briquets, XR Briquets, pellets, nor experimental granules produced (S)-methoprene concentrations >10 μg/litre, the Expected Environmental Concentration produced by application of Altosid Liquid Larvicide at 4 fluid oz./acre (293 ml/ha). These data indicated that use of these solid, sustained-release methoprene formulations does not constitute any undue risk to non-target organisms, compared to the use of methoprene liquid formulation.

Methoprene formulated in briquettes can have extended life in water. Altosid XR briquettes were weighed before and after 6-18 months of exposure in temporary wetlands in studies conducted in Minnesota in 1991-93, to determine the rate of physical degradation (Boxmeyer *et al.* 1997). Degradation rate was influenced mainly by the number of days a briquette remained under water. The average briquette degraded to 19% of its weight within 150 days of immersion and was completely degraded after 1.5 years under water. The methoprene content of briquettes declined faster in those exposed to air and more slowly in those that were immersed. In California, methoprene (Altosid) in the form of briquettes placed in catch basins, remained effective for 8-10 weeks, even in water with a high organic content

(Schoeppner 1977). In Malaysia, methoprene briquettes (Altosid) completely inhibited the adult emergence of *Ae. albopictus* for 66-72 days post-treatment when applied against fourth instars. Activity began to decline to 90% emergence inhibition (EI) at 119-129 days post-treatment and by 232-240 days had fallen to 50% EI, giving adequate control for about 10 weeks (Sulaiman *et al.* 1994).

Methoprene formulated as 4% briquettes was trialed in various kinds of mosquito breeding sources (septic tank systems, catch basins, swimming pools and irrigation ditches) in California in 1976; the number of briquettes applied depended on the depth of water to be treated. The average period during which larval control of *Cx. tarsalis*, *Cx. peus* and *Cx. quinquefasciatus* persisted was 44 days (Stewart 1977).

Sustained-release pellets (Altosid) persistence have shown long persistence in water. In a tidal saltwater marsh in California, against primarily *Ae. dorsalis* pellets applied prior to marsh inundation at 3.4 kg/ha provided >99% control through the July and August high tide series (up to 42 days post-treatment), 86.4% control during the November high tide series (131 days post-treatment) and 66.6% control during the February high tide series (240 days post-treatment) (Kramer *et al.* 1993). The same rate (3.4 kg/ha) and 9.0 kg/ha were evaluated against *Aedes* mosquitoes through 7 flood cycles (126 days) in an irrigated pasture in California by Kramer and Beesley (1991). At both rates, the pellets provided >98% control through 2 flood cycles, or 20 days post-treatment, and >80% control through 5 flood cycles, or 69 days post-treatment.

10.2.1. Effect of water quality

As mosquitoes are usually targeted at larvae in water, the effect of water quality on efficacy of methoprene will be important. The level of salinity in water is commonly measured in field evaluations as many mosquitoes breed in saltwater. Salt concentration has been shown to influence methoprene activity. While methoprene effectively controls mosquitoes in salt water (eg. Floore *et al.* 1990; Floore *et al.* 1991), Pree and Stewart (1975) found a difference in half-life of methoprene at 4.5^{o}C between fresh (about 100 days) and salt water (35 days). Degradation at all temperatures and formulation was faster in salt water than in fresh water. Moulting in larvae of the brine shrimp, *Artemia,* could be affected by exposure to methoprene, however the effect was salt-dependent (Ahl and Brown 1990). When larvae were exposed to methoprene in sea-water that was isotonic to larval haemolymph, there was no effect, possibly because methoprene targets the ion transporting epithelia.

In field situations, water quality may have a profound effect on persistence in some situations. Reduced recoveries of residues in polluted waters from sewage and dairy drains were due to adsorption on organic matter and degradation by microorganisms (Schafer and Dupras 1973). In contrast, high organic content did not appear to reduce the effectiveness of methoprene in the study of Schoeppner (1977).

10.2.2. Effect of light

Methoprene will remain exposed to sunlight after application for pest control and is very susceptible to photodecomposition and photoisomerization which can result in detoxication. In a field study, sunlight was found to reduce the biological activity of methoprene against mosquitoes (Schaefer and Wilder 1972); this led to further studies to demonstrate

quantitatively the effect of light on methoprene in the laboratory. Exposure of methoprene at 0.1 ppm in tap water to direct sunlight on a clear day (38°C ambient temperature) resulted in 97, 98 and 100% decomposition at 4-, 8-, and 24-hour exposures, respectively (Mian and Mulla 1982c).

Quistad *et al.* (1975) reported on photolysis of radiolabelled methoprene. At 0.01 and 0.05 ppm concentrations in aqueous solution, exposure to sunlight resulted in a residue half-life of < 1 day. After 2 weeks, no methoprene was detectable. The predominant photolytic pathway was the oxidative scission at the C_4 double bond, resulting in 9% methoxycitronellal and 7% methoxycitonellic acid. As many as 46 other photolytic products were detected.

Further tests by the same workers examined photodecomposition of methoprene in a thin film on a glass surface exposed to sunlight. Examination of the residues at 27 hours revealed that 97% of the applied methoprene was broken down photochemically. The remaining 3% was in the form of a 50:50 mixture of 2*E* and 2*Z* isomers as against a 97.9:1.5 mixture of these isomers in the original compound. The isomerization from 2*E* to 2*Z* is a detoxification step and reduces the activity of methoprene against target insects. The 2*Z* isomer is 1000 times less active against *Aedes* larvae than the 2*E* isomer (Henrick *et al*. 1975).

As discussed in section 9.8, LaClair (1997) report on a study where methoprene exposed to sunlight caused deformities in frogs not found with methoprene which was not exposed to sunlight. If these studies are replicated, there may be toxic breakdown products of methoprene. This work is continuing and is discussed in more detail in section 9.8.

10.2.3. Effect of temperature

High temperatures, especially during the summer months, may have a profound effect on the stability of methoprene in aquatic environments. Schafer and Dupras (1973) showed in laboratory studies that persistence of methoprene in water was greatly affected by increasing temperatures. At 10°C the loss in methoprene residues was slightly over 30% in five days, with a reduction of 70-80% at 24°C. At 38°C, the residue levels dropped to <5% of the original concentration. The same authors also reported that methoprene at 0.1 ppm in tap water exposed to an ambient temperature of 39°C for 8 hours on a hot summer day showed a residue loss of 98.7%. Pree and Stewart (1975) demonstrated a difference in half-life of methoprene of 134 days at 4.5°C and 49 days at 20°C.

Dove and McKague (1975) considered that temperatures in the range of 10°-25°C had little influence on methoprene effectiveness. However, Mansour and Dimetry (1978), working in Egypt, showed that the effect of methoprene (Altosid) on the metamorphosis and reproduction of *Spodoptera littoralis* was greatly affected by the temperature at which the larvae were kept following treatment as well as at the time of application. Larvae kept at 22°C had more abnormalities than those kept at 30°C.

10.2.4. Persistence in water compared with *Bacillus thuringiensis*

Methoprene generally maintains residual activity in water for longer periods than *Bt* products. Sulaiman *et al.* (1991) compared residual activities in water of briquettes (Altosid) (containing 7.9% methoprene) and Bactimos (containing 10% *Bti*), against larvae of *Ae. aegypti* in Malaysia. The Altosid briquette provided complete control of *Ae. aegypti* adult

emergence 114-122 days post-treatment and its residual effect was much longer than that of the Bactimos briquette with 100% mortality up to 64-75 days post-treatment. Similarly Becnel *et al.* (1996) evaluated the effect of larvicides on the production of adult *Ae. albopictus*. A liquid formulation of *Bti* (Acrobe) provided significant control for 47 days, whereas a slow-release pellet formulation of methoprene (Altosid) provided almost complete control for 116 days.

Methoprene compared less favourably with *Bt* products in a study by Kramer (1990). The efficacy of *B. sphaericus, Bti* (as Vectobac AS) and methoprene (as Altosid SR10) was evaluated against *Cx. incidens* in tyres exposed to full sunlight vs. shaded tyres. In shaded tyres inoculated with *B. sphaericus* (15 ppm) and *B.t. israelensis* (15 ppm), mortality exceeded 90% for 5 and 2 weeks, and 50% for 10 and 4 weeks, for the two bacteria, respectively. Larvae were adequately controlled (>75% mortality) in the sunny tyres for approximately 1 week. Methoprene (applied at 1.5 ppm) inhibited the emergence of approximately 90% of the larvae present at the time of treatment, but not of larvae subsequently introduced into either the sunny or shaded tyres.

10.3. Persistence in soil

Methoprene remains in the top layer of soil after application (Table 10). Schooley *et al.* (1975) studied the metabolic fate of methoprene (Altosid) in soils and found that it was rapidly degraded in a variety of soils under different environmental conditions. On aerobic sandy loam, radio-labelled methoprene showed an initial half-life of about 10 days at a surface treatment rate of 1 kg/ha; decomposition was much slower on autoclaved soil. Only small amounts of nonpolar metabolites were isolated, including the hydroxy ester resulting from O-demethylation (0.7% of the applied dose). Over 50% of applied dose was converted to $^{14}CO_2$. Radioactivity from labelled methoprene incorporated into humic acid, fulvic acid and humin fractions of sandy loam. These data indicated rapid and extensive breakdown of methoprene in soils.

10.4. Persistence on crops and stored products

Protected from sunlight, methoprene can be stable for extended periods. Therefore, it is not surprising to find reports of methoprene persistence for over 7 months on stored grains (Daglish *et al.* 1995). In field trials on maize, Arthur *et al.* (1990) found that methoprene was more stable than chlorpyrifos-methyl. It is reported that methoprene at 10 ppm can be effective for more than 2 years during the storage of treated tobacco (Manzelli 1979). The effectiveness of methoprene in suppressing populations of *Ephestia cautella* in unshelled groundnuts was evaluated in laboratory tests in the USA (Nickle 1979). Groundnut samples (of 1.5 kg) were sprayed with several concentrations and infested with eggs. Methoprene at 25 ppm completely suppressed adult emergence and residues of methoprene were as effective as residues of malathion (at 35 ppm) against the moth after storage for at least 8 months.

Mian and Mulla (1982c) found that the residual activity of 1-10 ppm methoprene on stored barley, maize and wheat grain against *Rhyzopertha dominica* gave effective control for over 12 months, but not at <0.5 ppm. Methoprene tested at 1, 5 and 10 ppm against *Sitophilus oryzae* was less effective, resulting in only 80-93% control at various intervals during the test period when applied at 10 ppm (Mian and Mulla 1982c).

On crops in the field, survival is reduced. Quistad *et al.* (1974) studied the metabolic fate of methoprene in alfalfa and rice as a function of time. Rapid metabolism of methoprene to biologically innocuous derivatives was found in both plants. After 30 days in alfalfa, 1% of the applied methoprene could be recovered and only 0.4% was found from rice metabolism after 15 days. In this laboratory experiment, photochemical decomposition was minimised by the relatively low light intensity used and the authors suggested that residual methoprene could be considerably less under field conditions after comparable times. In addition, the high surface treatment rate (1 lb/acre or 1.12 kg/ha) was approximately 40x the maximum field treatment rate of methoprene as a mosquito larvicide.

10.5. Effect of formulation on persistence

Formulation has a significant effect on methoprene persistence, especially in water. Briquette and other slow release formulations extend the duration of methoprene persistence. For example, Rathburn and Boike (1977) showed that a 0.4% black-sand granular formulation of methoprene remained effective against *Ae. taeniorhynchus* for 2 days when applied preflood in brackish pond water. A briquette formulation was effective for over 28 days. Briquettes applied post-flood at the same rate and tested at weekly intervals for residual activity remained effective for 31 days. Comparison in Egypt of either 2 briquettes of Altosid (methoprene) containing 8% a.i. or 50 g of Altosid wettable powder (WP) mixed with 200 g of sand against larvae of *Cx. p. molestus* and *Theobaldia longiareolata* in natural ponds showed activity for 30 days with briquettes but only 18 days for the WP formulation (Farghal 1987). Pree and Stewart (1975) showed that a flowable liquid micro-encapsulated (slow release) formulation of methoprene was more persistent than an emulsion of methoprene (ZR-515). In field trials with aerial application against *Ae. sollicitans* and *Psorophora columbiae.* Spencer *et al.* (1979) found that a powdered charcoal formulation of methoprene (Altosid SR-10F) appeared to enhance the continued presence of effective levels of active ingredient over the micro-encapsulated flowable-liquid formulation of methoprene (Altosid SR-10).

In studies in several states in the USA in 1977-78, sustained-release boluses containing methoprene provided long-term control of the development of both *Haematobia irritans* and *Musca autumnalis* in the faeces of treated cattle (Miller *et al.* 1979). A 3% methoprene bolus inhibited the development of *H. irritans* in the faeces of a treated herd for 28-32 weeks, while in other tests, 10% methoprene boluses provided 80-90% inhibition of the development of *M. autumnalis* in faeces for 10-12 weeks.

11. Metabolic fate of methoprene

Methoprene is rapidly degraded in the environment and animals. Methoprene undergoes hydrolysis, demethylation and oxidative scission in microbes, plants and insects. In animals, another pathway is used to convert into natural biochemicals. Metabolic bioproducts identified are biologically innocuous compounds. Methoprene was rapidly metabolised in fish, birds and mammals.

Methoprene has been reported to undergo ester hydrolysis, *O*-demethylation and oxidative scission at the C_4 bond in microorganisms, plants and insects. The metabolism of this compound is almost identical in both microbes and plants, but in animals, especially fish, birds and mammals, methoprene is converted by another pathway into natural biochemicals. Via the acetate pathway, methoprene passes through α and β oxidation reactions before being converted into natural products such as cholesterol, cholic acid, fatty acids, protein and CO_2. Conjugation of some of the metabolic products into natural biochemicals has been shown in microorganisms, plants and animal systems.

11.1. Microorganisms

The biodegradation of methoprene was studied in pond water containing unknown micro-organisms. A time plot of recovery of radio-labeled methoprene from pond water showed a half-life of approximately 30h at 0.001 ppm and 40h at 0.01 ppm. Incubation of labelled methoprene for 3 days at 0.42 ppm, generated three primary metabolites, the result of ester hydrolysis or *O*-demethylation or both. These metabolites and recovered methoprene were photoequilibrium mixtures of 2-ene double bond isomers. In another incubation experiment with labelled methoprene at 0.66 ppm in a pond water sample with a presumably different microflora, a completely different metabolic profile was observed, the sole identifiable metabolite resulting from oxidative scission of the 4-ene double bond. The principal metabolite in the latter experiment was 7-methoxycitronellic acid (20% of applied dose) (Schooley *et al.* 1975).

11.2. Plants

On stored crops, the major metabolic pathways identified involved ester hydrolysis, *O*-demethylation and oxidative scission of the 4-ene double bond (Quistad *et al.* 1974). These authors also studied the metabolic fate of methoprene in alfalfa and rice as a function of time. Rapid metabolism of methoprene to biologically innocuous derivatives was found in both plants and the authors noted a significant and unusual (for pesticides) conversion of the metabolites to natural products such as cellulose and possibly chlorophylls and carotenoids.

11.3. Insects

The fate of methoprene in medically important Diptera has been reported in the literature. Methoprene has been found to undergo three major metabolic reactions, namely ester hydrolysis, *O*-demethylation and oxidative scission at the C-4 double bond in insect systems. Quistad *et al.* (1975) studied the metabolic fate of methoprene in *Ae. aegypti*, *Cx.*

quinquefasciatus and *Musca domestica* using radiolabelled compound. The most abundant metabolite in mosquitoes was hydroxy ester, a product of *O*-demethylation while in the housefly, hydroxy acid was predominant. Biological isomerization (conversion of *E* to *Z*) at the C_2 double bond appeared to be an effective mode of detoxification by these insects.

Solomon and Metcalfe (1974) reported on the metabolism and pathways of methoprene in two insects, the yellow meal worm, *Tenebrio molitor* and the large milkweed bug, *Oncopeltus fasciatus*. The presumptive metabolites of Altosid, 1-methylethyl 11-hydroxy-3,7,11-trimethyl-2,4-dodecadienoate (II), 11-methoxy-3,7,11-trimethyl-2,4-dodecadienoic acid (III), and 11-hydroxy-3,7,11-trimethyl-2,4-dodecadienoic acid (IV) were tested for juvenilising activity in *O. fasciatus* and II was found to be four times as active as Altosid. Compound IV was more active than III but less active than Altosid. Uptake studies with methoprene (Altosid) labelled with ^{14}C showed that the difference in activity in the two insects was not due to differences in uptake. Forty-eight hours after treatment, 30% of the Altosid applied was lost from the cuticle by evaporation.

11.4. Fish

Fish constitute by far the largest group of vertebrates dwelling in aquatic habitats. As well as being economically important as human food, some of the carnivorous fish such as mosquitofish, *Gambusia affinis* are known to be good predators of mosquitoes and midges. Investigations on the fate of [5-^{14}C] methoprene in the bluegill sunfish, *Lepomis macrochirus*, were carried out by Quistad *et al.* (1976). Residue analysis showed that methoprene accumulated in the muscle tissue. Whole fish analyses showed that hydroxy ester was the main metabolite. Most of the nonpolar residue via ^{14}C-acetate was conjugated with natural products, eg trigycerides, digycerides, cholesterol and fatty acids. Studies showed that while methoprene can be readily accumulated, accumulated residue can be eliminated by the fish when the methoprene pressure is released. Methoprene can be degraded to hydroxy ester through *O*-demethylation and to CO_2 via acetate in the body tissues of fish. Conjugation of some of the metabolic products to natural biochemicals in the fish tissue has been observed.

11.5. Birds

Treatment of Leghorn chickens with a single oral dose of methoprene labelled with ^{14}C resulted in residual radioactivity in the tissues and eggs (Quistad *et al.* 1976). The chemical nature of the residual label in tissue (muscle, fat, liver), eggs and excrement was thoroughly examined at several doses (0.6 to 77 mg/kg body weight). Although a high initial dose (59 mg/kg) resulted in methoprene residues in muscle (0.01 ppm), fat (2.13 ppm) and egg yolk (8.03 ppm), these residues of methoprene represented only 39 and 2% of the total ^{14}C label in fat and egg yolk, respectively. Labelled natural products from extensive degradation of methoprene were by far the most important labelled residues in tissues and eggs, particularly at the lower dose of 0.6 mg/kg where labelled cholesterol and normal labelled fatty acids (as triglyceride) contributed 8 and 71% of the total label in egg yolk. Novel minor metabolites of methoprene were observed in lipid depots resulting from saturation of the dienoate system. These minor metabolites were conjugated to glycerol or cholesterol, or both.

11.6. Mammals

By tracing radioactively labelled methoprene in a treated steer, Quistad *et al.* (1975) concluded that methoprene was metabolised as a methyl-branched fatty acid in addition to being detoxified and excreted. The degradation of methoprene to natural products (eg cholesterol, fatty acids etc) is indicative of extensive biodegradability. In their study, Quistad *et al.* (1975) analysed for radioactive residues, samples of fat, muscle, liver, blood and bile from a steer which received a single dose of ^{14}C methoprene. No primary metabolites could be detected, but the majority (6-88%, depending on tissue) of the total tissue radioactivity was positively identified as ^{14}C cholesterol. Radioactivity from catabolised methoprene was also associated with protein and cholesteryl esters of fatty acids.

When the metabolic fate of methoprene labelled with ^{14}C was studied in a guinea pig, a steer and a cow, a fairly large percentage of the label was incorporated in the tissues and respired by the animals. In the urine and faeces, a small amount of label was found metabolised into primary metabolites, somewhat more was incorporated into simple glucuronides, and a considerable quantity was found in polar compounds, possibly complex conjugates or polar biochemicals. No methoprene was found in the urine, but approximately 40% of the label in the faeces was contributed by unmetabolised methoprene. The formation of conjugates and the metabolism of methoprene was more extensive in the steer than in the guinea pig (Chamberlain *et al.* 1975). All muscles of the cow had <0.1 μg of the total radioactivity/g, which was less than the tolerance limit established by the Environmental Protection Agency for cattle meat and meat by-products (Table 9).

In studies in Texas, residue levels of the methoprene were determined in fat taken by biopsy from cattle 30, 60, 90 and 180 days after either 1 or 2 boluses containing 1% of methoprene had been placed in the reticulum. The concentrations ranged from 0.02 to 0.159 ppm Only 2 cattle contained residues above the lower limit of detection (0.02 ppm) 90 days after treatment, and no residues were detected 180 days after treatment (Ivey *et al.* 1982).

12. Detection methods

Methoprene can be detected in environmental samples at very low levels, using a number of assay techniques. Methods based on high performance liquid chromatography and/or immunoassay using polyclonal antibodies have been developed with maximum sensitivity at the parts per billion level. A hexane extraction and Se-pak cleanup method has also been used to test residues in rice.

In safety evaluations of methoprene, methods to detect and quantify the analog are necessary. Any further work tracing methoprene in animals or the environment in New Zealand will require such methods. There are several studies in the literature which detail methods for quantification of methoprene from environmental and food samples. Yang (1992) described a rapid sample preparation procedure combined with a short reversed-phase HPLC separation for the quantification of methoprene in tobacco. Methoprene is used in the tobacco industry to control the stored products pests *Lasioderma serricorne* and *Ephestia elutella*. The detection limit for methoprene in tobacco samples was 1 ppm. The concentration of methoprene in water samples can be reliably detected at concentrations between 0.005 and 0.5 µg/ml using liquid chromatograph (Allen and Dickinson 1990). Ong and Frio (1993) and Tamiya *et al.* (1994) used high-performance liquid chromatography (HPLC) for methoprene detection from foods. Ong and Frio (1993) could detect to around 0.05 mg/kg in stored maize. Using a hexane extraction, distillation in a Dean-Stark apparatus and clean up with a Sep-pak Florisil cartridge, Tamiya *et al.* (1994) could recover 80-86% of methoprene when the insecticide was spiked at 0.5 ppm in rice. The detection limit was 0.02 ppm.

Mei *et al.* (1991) developed two immunoassay formats for the detection of low levels of methoprene. These depended on the production of polyclonal antibodies specific to methoprene. An indirect ELISA and a competitive inhibition enzyme immunoassay (CIEIA) were developed using the polyclonal antisera. The range of the methoprene indirect ELISA was from 5 to 300 ng/ml (ppb), while the CIEIA ranged from 1 to 10 ppb.

Detection of methoprene from foods such as stored grain has been the subject of several methods (Hill *et al.* 1991; Ferguson *et al.* 1992; Ong and Frio 1993; Tamiya *et al.* 1994). Enzyme immunoassays were described by Hill *et al.* (1991) and Ferguson *et al.*(1992). Hill *et al.* (1991) used immunoassay to determine methoprene in whole wheat grain and milling fractions. Their assay had a sensitivity of 250 pg/ml; 50% inhibition of antibody binding occurred at 3 ng/ml, corresponding to a maximum sensitivity of 60 ppb. Ferguson *et al.* (1992) described a quantitative enzyme immunoassay of pesticides in food at ppb levels. Enzyme immunoassay methods using antibody-coated plastic tubes or microwells for detecting a range of pesticides including methoprene on tobacco were described.

13. Resistance

Development of resistance is a major concern with the use of pesticides. Studies in the laboratory have demonstrated that insects, including mosquitoes, can develop resistance to methoprene rapidly, in as few as eight generations. Laboratory induced resistance to methoprene has resulted in cross-resistant to other pesticides, especially IGRs. Tolerance to methoprene has also been detected where insects are strongly resistant to another chemical pesticide. However very few examples of resistance developing after field applications have been found although a recent study of *Aedes taeniorhynchus* in Florida has shown resistance in populations exposed to sub-lethal doses of s-methoprene.

Williams (1967) first suggested that JHAs could be advantageously used to control insect pests because treated insects would be incapable of evolving resistance and at the same time continuing to regulate their endogenous juvenile hormone titre for normal development. However, insects highly resistant (100 to 1000 times) to toxic levels of methoprene have been artificially selected (Brown and Brown 1974; Georghiou *et al.* 1978). These resistant insects either retain fecundity and fertility or substantially regain these fitness parameters after a number of generations. Resistance in *Drosophila melanogaster* has been found in strains having chromosomes derived from natural populations (Wilson and Thurston 1988) and in susceptible laboratory strains following mutagenesis (Shemshedini and Wilson 1990). Although few cases of methoprene-resistant field populations resulting from direct methoprene exposure have been reported to date (probably because of limited use of methoprene), cross-resistance of insect populations resistant to other classes of insecticides have been documented (Cerf and Georghiou 1974). Moreover, populations of *Culex* mosquitoes resistant to methoprene have been selected in the laboratory (Brown and Brown 1974) and there is a single report of development of resistance in the field in *Aedes* (Dame *et al.* 1998).

13.1. Development of resistance

Brown and Brown (1974) first demonstrated laboratory selection for methoprene resistance in the mosquito, *Cx. p. fatigans*. When successive generations of larvae were reared in concentrations of methoprene that caused about 50% inhibition of adult emergence (EC_{50}), larvae of the F8 generation showed 13-fold resistance to methoprene, about 15-fold cross-resistance to its ethyl analogue, hydroprene, and slight cross-resistance to malathion, but their susceptibility to the JHA, R-20458 [6,7-epoxy-1-(p-ethylphenoxy)-3,7-dimethyl-2-octene], was not increased and that to carbaryl was unchanged. Further studies by Brown *et al.* (1978) on the induction of resistance to 7 juvenile hormone mimics and diflubenzuron, laboratory strains of *Cx. p. pipiens*, *Tribolium confusum* and *Oncopeltus fasciatus* were submitted to selection pressure at the EC_{60} level in each generation. For each insect, test methods for assessing susceptibility levels were designed to trace the development of resistance. Methoprene induced resistance in *Cx. p. pipiens* and tolerance in the other two species. The methoprene resistance developed in *Cx. p. pipiens* was at first handicapped by a greatly reduced reproductive success, but after 40 generations of pressure and the attainment of a 100-fold resistance, the reproductive success had returned to normal. This resistance showed polyfactoral inheritance and extended in cross-resistance to five other juvenile hormone mimics but not to diflubenzuron or conventional insecticides (Brown *et al.* 1978).

Multiresistant strains of fourth instar *Cx. quinquefasciatus* from Tanzania exposed for 6h to methoprene over 10 generations increased the LC_{50} by 3.9 times (Amin and White 1984).

High levels (>1000-fold) of resistance to methoprene was induced in a dimethoate-resistant laboratory strain of *Musca domestica* and a multi-resistant field strain by exposure of old larvae to methoprene-treated pupation medium. Resistance extended to three other juvenoids. However, cross-resistance to dimethoate, fenthion, parathion and isolan originally present in the dimethoate-R strain declined during the course of selection for methoprene resistance (Georghiou *et al.* 1978). In Czechoslovakia, an organophosphate-resistant strain of *M. domestica* from the field in had an eightfold resistance to methoprene at LD_{50} and sixfold resistance at the LD_{90} (Rupes *et al.* 1976).

Methoprene is toxic to late-instar larvae of *D. melanogaster*. High- and low-level resistant mutants were selected following chemical mutagenesis of male parents. One of the high-level mutants (termed *Met*) was partially characterised; it was nearly 100-fold more resistant to either methoprene or juvenile hormone III than susceptible wild-type strains. The locus responsible for resistance mapped to the X chromosome. Flies homozygous for *Met* have fecundity and fertility equivalent to wild type. This finding implies not only that *Met* females have retained their ability for endogenous juvenile hormone regulation but also that such a mutation would be rapidly selected under methoprene pressure in the field (Wilson *et al.* 1987). This study showed that considerable variation of resistance genes also occurs naturally in unselected populations.

Interestingly, endrin, fenitrothion, leptophos or aminocarb resistant strains of *S. littoralis,* selected with diflubenzuron for one generation resulted in enhanced development of resistance to methoprene, while selection for resistance to methoprene resulted in increased susceptibility to diflubenzuron (El-Guindy *et al.* 1980b and c).

Use of methoprene has not always resulted in development of resistance. Use of high doses of methoprene against the mushroom pest, *Lycoriella mali*, has not resulted in significant differences in dose-mortality results and resistance does not appear to be developing rapidly (Keil and Othman 1988).

13.2. Field resistance

There have been few reports of the development of resistance to methoprene after field use. In some cases, given above, resistance to other insecticides has also resulted in some level of resistance or tolerance to methoprene (ie. Rupes *et al.* 1976; El-Guindy *et al.* 1980b). Resistance to methoprene has been detected independent of other insecticides. Methoprene resistance was detected in three strains of the stored products pest *Lasioderma serricorne* among eight strains derived from tobacco stores in the south-eastern USA (Benezet and Helms 1994). Observations suggested field application of methoprene induced resistance in the anobiid.

Recently, Dame *et al.* (1998) showed that an island strain of *Ae. taeiorhynchus* in Florida was 14.9x more resistant than a strain collected from the mainland. This mosquito population had been exposed to s-methoprene briquettes for 5 years. This demonstrates that resistance can develop in natural populations from low-level exposure to methoprene. Naturally-occurring

differences in susceptibility to methoprene of the mosquito *Cx. quinquefasciatus* from Cuba and France have been found by Navarro-Ortega *et al.* (1991), with LC_{50}'s of the Cuban strain (0.005 mg/litre) higher than for the French strain (0.0006 mg/litre). As methoprene has never been used in Cuba, the authors suggested that the levels of tolerance found represent cross-resistance to other insecticides used in public health and/or agriculture. Field-collected strains of *Tribolium castaneum* sometimes show resistance to methoprene (Hoppe 1981).

Differences in the susceptibility of *M. domestica* to methoprene after two years of application were attributed to an existing cross-resistance to methoprene followed by the induction of resistance resulting from continuous exposure to the compound (Breeden *et al.* 1981).

13.3. Effect of resistance on insect fitness

The methoprene-tolerant (*Met*) mutation of *D. melanogaster* results in a high (100-fold) resistance to methoprene. Studies to evaluate the potential of such mutants to persist in wild *D. melanogaster* populations were carried out in the laboratory (Minkoff and Wilson 1992). Fitness components (survival, time of development and fecundity) of flies homozygous for each of 5 *Met* alleles were compared with those of wild-type strains. In the absence of methoprene, *Met* flies were outcompeted by a wild-type strain both in a multigeneration population cage and in single-generation competition experiments. Small but significant differences were found between the pooled *Met* alleles and wild type for pupal development time, pupal mortality and early adult fecundity, resulting in a large competitive disadvantage. Although *Met* flies were found to have reduced fitness by these measures, the phenotype was not as badly affected as might be expected from the disruption of juvenile regulation seen in *Met* flies.

13.4. Management of resistance

The most effective method for delaying development of resistance appears to be to use of a range of control agents. For example, El-Guindy *et al.* (1990) determined the effect of selection regime on the development of resistance to the insecticide monocrotophos, and the IGRs diflubenzuron and methoprene by the noctuid *Spodoptera littoralis* over 16 generations. The resistance potential to monocrotophos alone was high. However, selection using a monocrotophos-methoprene mixture was the most effective in delaying resistance to either compound, followed by alternative selection for one generation with monocrotophos followed by selection with diflubenzuron. Selection with a monocrotophos-diflubenzuron mixture or selection with monocrotophos for one generation followed by selection with methoprene was also effective in reducing the development of resistance to either compound but to a lesser extent. Under all test regimes, a significant rise in resistance was observed in the tenth generation of selection which suggested that different selection regimes may retard, but not prevent, resistance to each compound. Mixtures containing methoprene, or methoprene used in alternation with monocrotophos produced high sterility in the progeny of treated insects. However, the level of sterility was poor or moderate following selection using the other regimes.

14. Discussion and conclusions

Methoprene is an effective mosquito larvicide. Assessment of methoprene's environmental safety (based on evaluation of over 500 published articles) indicates that it is a relatively safe agent. While some impact on non-target organisms (especially in aquatic communities) could be expected, the effects of methoprene application would be less harmful than those caused by most mosquitocidal pesticides. Methoprene has longer persistence than *Bti* after application, but also causes greater impact on non-target organisms. Despite this, there is no indication in the literature of permanent disruption to ecosystems after methoprene application. One unresolved issue is the possible involvement of methoprene and other IGRs in frog deformities in North America. At present, no clear cause for these frog deformities has been identified and research is continuing.

14.1. Is methoprene safe for use in New Zealand?

The major issues for environmental safety and health impacts of methoprene for New Zealand include: toxicity to non-target organisms, including native and beneficial species; mammalian toxicity; and its fate in the environment.

Methoprene has a broader host range than biological control agents of mosquitoes, such as *Bti*, *B. sphaericus* and *Lagenidium giganteum.* However, it is far more specific than widely used chemical controls such as temephos. While the list of susceptible insects is extensive for methoprene (Table 4), many reported susceptible organisms require doses which greatly exceed the field application rate. Several researchers have suggested that methoprene can be specific to Diptera in field situations, which would be likely to include some beneficial dipteran species. The non-target effects observed after methoprene use include some reduction in benthic communities and direct, but low toxicity to fish (section 9), however such communities appear to recover quickly (Majori *et al.* 1977; Bicher and Ruber 1988; Yasuno and Satake, 1990; Hershey *et al.* 1995; Retnakaran *et al.* 1974).

If methoprene was used against mosquitoes in New Zealand, what effects on New Zealand fauna could be expected? It is most probable that all of the 14 mosquito species presently found in New Zealand (Debenham and Hicks 1989) would be susceptible to methoprene. There would be some effect on non-target benthic organisms in the short term, and possibly exposed terrestrial insects and mites, but methoprene would be unlikely to cause extinction of any organism after limited application in specific areas. The EPA (1991) believed that methoprene use would not result in unreasonable adverse effects to the environment, with the exception of slow release formulations in estuaries, as methoprene appears acutely toxic to estuarine invertebrates. Methoprene degrades quickly in sunlight, both in water and on surfaces and is metabolised rapidly in soil. It should not, thefore, persist after application to enter ground water.

The “deformed frog controversy” is the most serious question about the environmental safety of methoprene presently. Retinoids, including methoprene, are one of three hypothesised causes of frog deformities in the USA (the others being a parasite and UV). However, the scientific community is hotly debating experiments which have shown methoprene acid can cause such deformities, in particular questioning the very high doses used to achieve the

effect (Sessions 1997; Sullivan 1998). However, the use of a JHA in the environment will continue to be debated until a cause of the frog deformities is identified.

Mammalian safety is always a major area of concern with insecticides. There appears very little direct concern over methoprene as a mammalian toxin and methoprene is metabolised rapidly in animals and birds. The World Health Organisation has approved its use in drinking water for control of mosquitoes because it was regarded as posing minimal or no risk to humans, animals or the environment (Kiess 1981).

Development of resistance is of concern with the extensive use of any pesticide. While resistance to methoprene has been demonstrated in the laboratory, resistance after extensive field applications has only been detected once (Dame *et al.* 1998), suggesting that it does not occur rapidly under normal use. However, it has been clearly demonstrated that resistance to methoprene can develop and therefore any eradication or management strategy should include management of the development of resistance in the target population. There have been some findings of cross-resistance where insects selected for resistance to another pesticide have tolerance to methoprene. Such cross-resistance could reduce the efficacy of methoprene in the event that a mosquito invasion includes pesticide resistance strains. Should methoprene be selected for use against mosquitoes in New Zealand, appropriate strategies to minimise the likelihood of resistance would need to be developed, including the use of more than one control agent.

14.2. Other potential responses to mosquito invasion

Mosquito control has relied on chemical pesticides for many years. The search for new mosquitocidal agents increased in the 1970s as control of the larvae with pesticides such as temephos (Abate) and chlorpyrifos (Dursban) became ineffective owing to incipient development of resistance (eg. Hazelrigg and Pelsue 1980). Many alternative agents have been investigated, with *Bti*, *Bacillus sphaericus* and IGRs showing the most promise and these have been widely used in the last decade. Chemical pesticides are still frequently used in many countries and, although this report does not cover environmental safety of all agents, safety of methoprene must be evaluated in relation to other available agents.

14.2.1. Chemical mosquitocides agents

Cowley *et al.* (1998) in the New Zealand draft national pest management strategy for exotic mosquitoes list a number of chemicals and compounds which are used against mosquitoes overseas. For larvae, only the petroleum oils (diesel, kerosene) are currently registered for use. The insecticides alpha-cyrpermethrin (Fendona 15 SC), bendiocarb (Ficam W), betacyfluthrin (Responsar SC 125), cyfluthrin (Solfac 50EW), deltamethrin (Cislin 10), dichorvos (Nuvan 1000EC), lambacyhalothrin (Icon 10 WP) and permethrin are listed as adulticides registered for use in New Zealand. Pirimiphos methyl (Actellic), pyriproxyfen (Sumilarv) and temephos (Abate 50 SG) are larvicides used overseas for mosquito control, but are not registered for use against mosquitoes in New Zealand, while bioresmethrin and malathion are adulticides not registered for use against mosquitoes in New Zealand.

One chemical that could be used in New Zealand is temephos, an organophosphate. Granules have good shelf life and persistence of over 150 days. Imai *et al.* (1987) found that methoprene was more toxic than temephos for *An. stephensi,* while Ali *et al.* (1995) reported higher toxicity of methoprene than temephos against *Ae. albopictus.* Temephos, as with other organophosphates, is relatively toxic to non-target organisms compared to methoprene which has raised occupational health and safety concerns, and it is also less specific than methoprene (e.g. Yap *et al.* 1982). Insects appear to develop resistance to temephos more rapidly than to methoprene (Georghiou *et al.* 1975; Hazelrigg and Pelsue 1980), another issue in choice of agent(s) for use in New Zealand. As we noted previously (Glare and O'Callaghan 1998), the long residual activity of temephos can make it attractive for specialised uses such as container treatment (which contributes to the development of resistance). This needs to be compared with methoprene, which also has long residual activity in protected (from sunlight) environments. A further problem with organophosphates is the likelihood of withdrawal from the market in the future, due to concerns over environmental safety.

14.2.2. Other insect growth regulators

Diflubenzuron (Dimilin) is a common insect growth regulator used in New Zealand for insect control. Cowley *et al.* (1998) note that it is not recommended for use with mosquitoes due to questions over mammalian and non-target safety, such as carcinogenic breakdown products (Smith 1994).

14.2.3. Biological agents

There are a number of biological agents which have been introduced in other countries for mosquito control. In Glare and O'Callaghan (1998) we briefly reviewed other control options for mosquitoes, such as other bacterial agents, nematodes, fungi, chemicals and small invertebrates such as copepods. There is no evidence that methoprene would reduce effectiveness of any of these agents.

The most promising biological agent for mosquito control use in New Zealand is *Bti.* We have previously reviewed the environmental and health impacts of *Bti* (Glare and O'Callaghan 1998). Our assessment of *Bti* was that it would be a relatively safe agent compared to most chemical alternatives. It is also host specific to Diptera.

14.2.3.1. Comparison of methoprene with *Bacillus thuringiensis israelensis*

The draft national pest management strategy (Cowley *et al.* 1998) suggested methoprene and *Bti* (as environmentally benign mosquito control agents) should be investigated for registration in New Zealand. It is therefore logical to compare these two agents in light of this report and that prepared earlier on *Bti* (Glare and O'Callaghan 1998).

Methoprene has distinct advantages over biological agents such as *Bti.* It is more stable, has long persistence in mosquito environments and high efficacy against a large range of mosquito species. As methoprene can remain in the upper surface of water longer than *Bti,* it may be more effective against surface feeding mosquitoes such as *Culex* spp.

The two agents have very different activities, toxicity and characteristics. *Bti* is a bacterium which produces toxins active against Diptera, especially mosquitoes and biting flies.

Methoprene is less specific being active against insect pests from a number of classes, including Diptera, but also pests such as ants, forestry Lepidoptera and apple pests. A direct comparison of the reported susceptible insects and mites is shown in Appendix 1, which demonstrates the differences between methoprene and *Bti*. Both methoprene and *Bti* have low mammalian toxicity and laboratory and field evaluations show both have low toxicity to non-target organisms, although methoprene has more questions over its toxicity to non-target aquatic organisms, including fish.

Both *Bti* and methoprene are the subject of some controversy over higher vertebrate safety. With *Bti,* there are a number of studies reporting the occurrence of *B. thuringiensis* in immunosuppressed patients, leading to discussion about the role of *Bti* and other *Bt*s in human disease. With methoprene, health concerns are based on the frog deformities reported in Section 9.8.

Efficacy of the two agents can be compared and several studies have shown similar levels of activity against most mosquito species. However, methoprene has a longer residual activity (Sulaiman *et al.* 1991; Becnel *et al.* 1996), which increases its efficacy and usefulness against mosquito larvae. This also has the adverse effect of increasing the chances of potential non-target problems. The registration of both mosquitocidal agents would be of great benefit, as they appear to be the most effective agents which have low environmental risk.

14.3. Further considerations of methoprene before use in New Zealand

Methoprene would be a useful addition to the agents available in New Zealand for use against mosquitoes. There are several areas which require further evaluation and/or experimentation prior to use in New Zealand. Methoprene is not currently registered in New Zealand, and suitable information needs to be collated to proceed with registration. Specifically, the effect of methoprene on New Zealand aquatic organisms which would be exposed during an eradication effort need to be assessed. While general trends can be assessed from overseas data, specific species toxicity cannot be calculated except by exposure experiments. In particular, the effect on beneficial Diptera in New Zealand needs to be quantified. There are also few studies which examine the susceptibility of egg stages and the effect on beneficial species eggs could be quantified from research in New Zealand.

In addition, evaluation of evidence presented in the frog deformity debate in the USA is required as more information is obtained. In the light of the frog deformities debate, laboratory exposure trials of New Zealand native frogs to methoprene would be prudent.

A number of formulations of methoprene are available, each with different characteristics. Briquette and pellet formulations give the longest residual activity however, this needs to be experimentally evaluated in terms of increased effect on non-target organisms under New Zealand conditions. Liquid formulations may be more suitable in many situations if rapid effect is required with no residual activity desired.

As resistance and non-target effects may occur with the use of methoprene, the use of *Bti* during any eradication/control effort would be encouraged. Data on the non-target impacts of

methoprene/*Bti* combinations are scarce and research in New Zealand in this area would be of benefit in assessing likely impacts.

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Appendix 1. Records of susceptible insect hosts comparison of methoprene and *Bti* (from Glare and O'Callaghan 1998 and Table 3, this report)

Insect class and family	Number of species recorded susceptible	
	Methoprene	*Bti*
Coleoptera: Anobiidae	1	
Coleoptera: Bostrichidae	1	
Coleoptera: Bruchidae	1	
Coleoptera: Chrysomelidae	1	
Coleoptera: Coccinellidae	3	
Coleoptera: Curculionidae	3	
Coleoptera: Dermestidae	1	
Coleoptera: Scarabaeidae	2	
Coleoptera: Scolytidae	2	
Coleoptera: Silvanidae	2	
Coleoptera: Tenebrionidae	4	
Coleoptera (total)	**21**	**0**
Dictyoptera: Blattellidae	2	
Dictyoptera (total)	**2**	**0**
Diptera: Anisopodidae		1
Diptera: Anthomyiidae	1	
Diptera: Agromyzidae	1	
Diptera: Culicidae	70	112
Diptera: Calliphoridae	1	
Diptera: Ceratopogonidae	2	
Diptera: Cecidomyiidae	1	
Diptera: Chironomidae	7	26
Diptera: Drosphilidae	1	
Diptera: Glossinidae		1
Diptera: Muscidae	4	1
Diptera: Hippoboscidae	1	
Diptera: Oestridae	3	
Diptera: Phlebotominae		3
Diptera: Phoridae	1	1
Diptera: Psychodidae	1	3
Diptera: Sarcophagidae	1	
Diptera: Sciaridae	7	3
Diptera: Simuliidae	12	34
Diptera: Syrphidae	2	
Diptera: Tabanidae		1
Diptera: Tachinidae	2	
Diptera: Tephritidae	4	1
Diptera: Tipulidae		2
Diptera (total)	**122**	**189**
Hemiptera: Aleyrodidae	2	
Hemiptera: Aphididae	2	
Hemiptera: Cimicidae	1	
Hemiptera: Coccidae	5	
Hemiptera: Diaspididae	3	
Hemiptera: Lygaeidae	1	
Hemiptera: Piesmatidae	1	
Hemiptera: Pseudococcidae	2	
Hemiptera: Psyllidae	1	
Hemiptera: Pyrrhocoridae	3	
Hemiptera: Reduviidae	1	
Hemiptera (total)	**22**	**0**
Hymenoptera: Braconidae	1	

Hymenoptera: Chalcidoidea	1	
Hymenoptera: Formicidae	8	
Hymenoptera: Pteromalidae	1	
Hymenoptera: Vespidae	2	
Hymenoptera (total)	**13**	**0**
Isoptera: Rhinotermitidae	3	
Isoptera: Termitidae	1	
Isoptera (total)	**4**	**0**
Lepidoptera: Arctiidae	2	
Lepidoptera: Bombycidae	1	
Lepidoptera: Gelechiidae	3	
Lepidoptera: Geometridae	2	
Lepidoptera: Lasiocampidae	1	
Lepidoptera: Lymantriidae	2	
Lepidoptera: Nocutidae	11	
Lepidoptera: Plutellidae	1	
Lepidoptera: Pyralidae	8	
Lepidoptera: Tortricidae	4	
Lepidoptera (total)	**39**	**0**
Neuroptera: Chrysopidae	1	
Orthoptera: Acrididae	1	
Orthoptera: Gryllidae	1	
Phthiraptera: Pediculidae	1	
Psocoptera: Lipscelididae	1	
Siphonaptera: Ceratophyllidae	1	
Siphonaptera: Pulicidae	2	
Neuroptera- Siphonaptera (total)	**8**	**0**
Acari: Argasidae	1	1
Acari: Phytoseiidae	2	
Acari: Psoroptidae	1	
Acari: Pyroglyphidae	2	1
Acari: Tetranychidae	3	
Acarina: Ixodidae	3	1
Acari/Acarina	**12**	**3**