A Review and Update of the Report "Environmental and health impacts of the insect juvenile hormone analogue, S-methoprene" 1999 by Travis R. Glare and Maureen O'Callaghan

May 2005

Report for
New Zealand Ministry of Health
P O Box 5013
WELLINGTON

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Abbreviations

IGR – insect growth regulator, methoprene – S-methoprene
Bt – *Bacillus thuringiensis*, Bti – *Bacillus thuringiensis israelensis*
ppm – parts per million, ppb – parts per billion, muM – micromolar, nM – nanonmolar,
JH – juvenile hormone, JHA – juvenile hormone analogue

1. Summary

A report was developed by Glare and O'Callaghan (1999) for the Ministry of Health on the environmental and health impacts associated with the use of S-methoprene for control and eradication of mosquitoes in New Zealand. This report along with a companion report on *Bacillus thuringiensis israelensis* (Glare and O'Callaghan 1998), were developed because of recent introductions of non-indigenous mosquito species that can serve as vectors for disease in humans. These pesticides were not registered for use as mosquito control agents in New Zealand and were thought at the time to be the best choices for control and/or eradication programmes in the country. Both assessments were extremely well-done and covered the known literature of the time. The general conclusions of this report were, although methoprene is toxic to 12 orders of insects and may have effects on other nontarget organisms, particularly other nontarget arthropods, methoprene is one of the least environmentally damaging mosquito control agents and poses little risk to human and animal health. In fact, the concentrations of methoprene necessary to control mosquites (1 part per billion) are often much lower than the concentrations necessary to cause damage to populations of many nontarget organisms. Methoprene has a short half-life in the environment making it unlikely to accumulate in various environmental compartments. Although new literature has been published showing declines in insect biomass due to long-term use of methoprene and Bti in freshwater wetlands in Minnosota, USA, no evidence for permanent damage to ecosystem function has been found. Additionally, a concern discussed in the original assessment was the possibility that methoprene may be the cause of limb malformations being detected in frogs in the USA. Even though it has been six years since the last assessment, the causal agent(s) of frog deformities in the USA has still not been clearly elucidated. Some scientists believe that these deformities are caused primarily by a parasitic trematode, not methoprene. Others believe that a combination of several factors, such as trematodes, UV radiation and chemicals may be working synergistically to cause the observed malformations. It is my opinion that the conclusions reached by Glare and O'Callaghan in 1999 are still valid today and I would recommend that methoprene be the first choice for control and eradication of introduced mosquito species in New Zealand. The justification for this recommendation is that the alternative control agents, other than *Bacillus sphaericus* and Bti are organophorous insecticides like temephos that are very broad-spectrum neurotoxins that pose a much more serious risk to the environment, human and animal health than methoprene. New pesticides, such as spinosad (a natural product) and fipronil (a synthetic neurotoxin) have shown promise as controls for mosquitoes, but the potential impacts of these products on human health and nontarget organisms is not presently known because they haven't been used in wide-scale mosquito control/eradication programmes to date.

2. Introduction

It is the intention of this report to review the report of Glare and O'Callaghan 1999 and to update the literature on methoprene. This report should be read in conjunction with the original Glare and O'Callaghan report which is available at www.moh.govt.nz. **For ease of comparison to the original document (Glare and O'Callaghan 1999), each section will be numbered exactly as the original document. However, new additional literature was not necessarily found for each of the sections of the original report and therefore some sections will only contain a statement that new literature has not been published since the original report.**

A search of the Biological Abstracts database and the Agricola database with the key word methoprene from 1998 to the present produced 188 new references since the 1999 report by Glare and O'Callaghan. Of these 188 documents, only 72 were pertinent to the original Glare and O'Callaghan report and used in this update. The reason for this low number of pertinent papers is that methoprene is often used as a surrogate for juvenile hormone in insect physiology, behavioural and molecular biology studies. Many of the new papers dealt with the use of methoprene as a tool for these basic research studies and had no bearing on the use of methoprene for control of mosquitoes or the potential for nontarget effects. An additional 10 references dealing with limb malformations occurring in frogs in the USA and not necessarily having to do with methoprene were also used in this report. Several other papers indirectly related to the subject of mosquito control, nontarget effects and/or methoprene were also used in this report.

As stated in the original document by Glare and O'Callaghan (1999), methoprene is an insect growth regulator that acts as a juvenile hormone mimic to disrupt normal development of insects. It has been used widely in many countries either as a control agent to reduce mosquito populations in turn reducing the incidence of disease transmission or for full-scale eradication programmes (Hershey et al. 1998, Lawler et al. 1999, Niemi et al. 1999).

2.1 Background

The establishment of non-indigenous mosquitoes in New Zealand poses a threat to human health and the environment (Frampton 2004). The discovery of the southern saltmarsh mosquito, *Ochlerotatus camptorhynchus* (Thomson) in 1998 near Napier in the North Island and subsequent detections in other areas of the North Island and in the Wairau estuarine area in the northern part of the South Island has led to an eradication campaign for this species (Frampton 2004). Based on environmental and health impact assessments of S-methoprene and *Bacillus thuringiensis israelensis* (Bti) by Glare and O'Callaghan (1999, 1998), methoprene and to a more limited extent, Bti, have been used as the control agents in the eradication campaign.

2.2 Insect growth regulators

In this section in the original report by Glare and O'Callaghan (1999) the authors present

a thorough discussion of insect growth regulators in general. This section has not been updated because it is still valid.

2.3 Methoprene

The physicochemical properties and other facts about methoprene are presented in this section of the orginal document. This section was not updated because it is still valid.

3. Methoprene-based products

The vaious formulations of methoprene are discussed in this section of the original document. This section was not updated because it is still valid.

3.1 Application rates

This section was not updated and is still valid.

4. Activity of methoprene

There is still debate about the exact mode of action of methoprene in the literature. Methoprene disrupts the normal development of the insects and can cause death or reproductive failure depending on the timing of exposure at a specific time in the life-cycle (Glare and O'Callaghan 1999). Methoprene is known to act like juvenile hormone in insects and may therefore cause a series of disruptions to the normal timing of events in the insect life cycle. Several new studies about methoprene mode of action have been published since the original report and are discussed below.

4.1 Mode of action and effect of methoprene treatment

Studies published since the report by Glare and O'Callaghan (1999) show that methoprene can disrupt adult insect diapause (Zdarek et al. 2000) and break larval diapause (Singtripop et al. 2000), a hibernation-like state in insects.

Another study indicates that methoprene also interferes with the development of the midgut in mosquitoes (Nishiura et al. 2003). Exposure of the larvae of *Stegomyia aegypti* (formerly *Aedes aegypti*), *St. albopicta* (formerly *Aedes albopictus*), and *Culex quinquefasciatus* to methoprene resulted in pupation, but the pupal midgut remained morphologically similar to larval midgut tissue. An examination of DNA indicated that high methoprene concentrations interfere with diploid cell division and programmed death of polytene cells.

Results of a study by Wilson (2004) showed that JH mimics, including methoprene, work by interfering with the expression or action of certain genes, in particular the Broad-Complex (BR-C) transcription factor gene. This gene complex directs metamorphic change and if it is misexpressed, abnormal developmental and physiological changes occur that disrupt metamorphosis. Therefore, even though JH is a necessary molecule at certain times in insect development, it becomes a toxicant when present during metamorphosis.

4.2 Sublethal effects

Methoprene is known to cause sublethal effects in many species. Methoprene reduced the fecundity of *Rhyzopertha dominica* adults by 50% after feeding on wheat treated with 2 mg/kg but caused no mortality in adults (Daglish and Pulvirenti 1998).

4.2.1 Sublethal effects in mosquitoes

No new studies on sublethal effects in mosquitoes were found in the literature search. This section is still valid.

4.2.2 Effect on morphology/development

No new studies on the effects of methoprene on morphology and development were found in the literature search. This section is still valid.

4.2.3 Effect on behaviour

Several new studies on the effects of methoprene on behaviour have been published since the original report.

Exposure to methoprene may result in changes in behaviour. Hancock and Foster (2000) investigated the role of juvenile hormone in the behavioural shift from nectar seeking to blood-host odour preference by *Culex nigrapalpus* by topically applying methoprene to unfed females. Methoprene application caused the behavioural shift indicating that juvenile hormones mimic the effects of a sugar meal by causing both follicular growth and the shift to preference for a host.

A field study was conducted near Cairns, Australia to determine whether addition of methoprene (Altosid® pellets) to ovitraps would affect oviposition by *Stegomyia aegypti* or *Ochlerotatus notoscriptus* (Ritchie and Long 2003). No significant differences in the number of eggs laid by either species were detected between ovitraps with or without the addition of methoprene pellets.

Topical application of methoprene acid to grubs of the turf grass pest, the masked chafer, *Cyclocephala* sp. resulted in movement of the larvae deeper into the soil (Rogers et al. 2003). Similar downward movement was observed when the grubs were parasitised by a wasp, indicating that the presence of the parasitic wasp changed the host's hormone titres.

The lace bug, *Gargaphia solani* exhibits either egg guarding or egg dumping behaviour. Tallamy et al. (2002) tested the hypothesis that high JH titres promote egg production and egg dumping behaviour, while low titres terminate egg production and initiate maternal care. Exposure of groups of *G. solani* that were egg dumpers to the hormone mimic, precocene II changed their behaviour to egg guarders while egg guarders exposed to methoprene became egg dumpers. These results suggest that hormones can trigger the expression of both egg dumping and egg guarding in *G. solani*.

4.2.4 Effect on phermones

No new studies on the effects of methoprene on phermones were published since the original document. This section is still valid.

4.2.5 Effect on reproduction and sex ratios

No new studies on the effects of methoprene on reproduction and sex ratios were published since the original document. This section is still valid.

4.3 Developmental stage affected

This section is still valid.

4.3.1 Ovicidal activity

One new study on ovicidal activity was published since the original report. Methoprene, methoprene acid and precocene were topically applied to 0, 1 and 2 day-old eggs of the bug, *Dysdercus cingulatus* (Gayathri-Elayidam and Muraleedharen 2001). Egg hatch was greatly reduced in methoprene-treated eggs, while methoprene acid and precocene completely stopped egg development. Results of this study indicate that JH based products can negatively affect egg development in insects.

4.3.2 Larvicidal and pupicidal activity

One new study dealing with methoprene effects on development was published since the original report. The effects of methoprene and 20-hydroxyecdysone on development and hemagglutination activity (HA) were studied in both sexes of two members of the *Culex pipiens* complex-anautogenous; *Cx. p. quinquefasciatus* and autogenous *Cx. p. molestus* (Gelbic et al. 2002). Both chemicals caused changes in development. Increased larval mortality, a prolongation of the intermoult period in each larval instar and pupal stage as well as morphological changes in the larval-pupal and pupal-adult transformations were observed. Methoprene caused significant decreases of HA in the gut of adults of both sexes while 20-hydroxyecdysone only decreased HA in the gut of females.

4.3.3 Adults

This section is still valid.

5. Susceptible insect and mite species

Methoprene is toxic to many groups of insects and mites, particularly to Diptera (Glare and O'Callaghan 1999).

5.1 Records of susceptible insects and mites

Several new studies have been published since the original report about the susceptibility of various species to methoprene.

Ali et al. (1999b) successfully used a predaceous mite species in conjunction with methoprene to control the mushroom fly, *Lycoriella solani* in an integrated pest management programme.

The susceptibility of susceptible and Actellic-resistant strains of *Tribolium castaneum* and susceptible strains of *Rhyzopertha dominica* and *Sitophilus oryz*ae to methoprene, pyriproxyfen, RH-5849 and tebufenozide was evaluated (Kostyukovsky et al. 2000). Insects were exposed to food medium containing concentrations ranging from 0.1 to 20 ppm of these insecticides. Results showed that all these compounds could affect the development of these species but had no effect on the mortality of adults. Methoprene and pyriproxyfen greatly prolonged the life span of *T. castaneum* leading to production of giant larvae that failed to pupate. The Actellic-resistant strain of *T. castaneum* was slightly cross-resistant to methoprene and pyriproxyfen, but not to RH-5849 and tebufenozide. Pyriproxyfen was the most toxic product evaluated; a concentration of 0.1 ppm completely inhibited adult emergence of both S- and R-strains of *T. castaneum.* Methoprene was very toxic to *R. dominica*, but less toxic to *S. oryzae*.

The effects of methoprene and permethrin on larvae of two cecidomyiid species, *Heteropeza pygmaea* and *Mycophila speyeri*, that are pests of mushrooms were determined by White and Czajkowska (2000). Concentrations ranging from 0.1-100 ug/g were evaluated. Methoprene caused an increase in *H. pygmaea* generation time and a reduction in hemipupal width and fecundity in a dose-dependent manner. Effects on *M. speyeri* were even more severe. The two highest concentrations of permethrin caused complete mortality of *M. speyeri* but only low mortality of *H. pygmaea*. Permethrin also caused a reduction in fecundity.

A study on the susceptibility of a pest psocopteran species, *Liposcelis entomophila* to methoprene and pyriproxifen indicated that both products were lethal to nymphs but did not cause mortality in adults (Ding et al. 2002). Pyriproxifen was more toxic to nymphs than methoprene, but methoprene was five times more toxic in terms of inhibiting fecundity than was pyriproxifen.

5.2 Comparative toxicity in the laboratory

Several new studies on the toxicity of various species to methoprene have been published since the original document by Glare and O'Callaghan (1999). These studies usually include LC50 data and are discussed in sections 6, 7, 9, and 13.

6. Use of methoprene in the field

Methoprene has been used extensively in the field for control and eradication of mosquitoes in various areas of the world. Methoprene is also routinely used to control chironomidae, simuliidae, fleas, ants and other pest species.

6.1 Use of methoprene against insects

Several new studies have been published on the use of methoprene against insects since the publication of the original document. Methoprene has been used in a series of programmes to control pest ant species usually as the toxic component of a bait. For example, Pharaoh ant colonies have been controlled with bait stations containing methoprene (Varjas and Bajomi 2001). Pereira (2003) reported on an integrated pest management programme for the control of red imported fire ant, *Solenopsis invicta* in Gainesville, Florida, USA. The chemical control portion of this programme consisted of

bait stations containing bait laced with a 1:1 mixture of hydramethylnon and methoprene applied at a rate of 1.7 kg per ha. The programme has resulted in suppression of fire ant colonies. Harris et al. (2003) have had good success in controlling red imported fire ants in pecan groves in Texas with methoprene baits. Methoprene used as a bait for fire ant control in peanut fields in the USA provided effective control, reducing the number of foraging ants by 85-98% (Mitchell and Knutson 2004).

Methoprene-based baits have also been developed for control of the migratory locust, *Locusta migratoria migratorioides* (Nemec 2003).

6.2 Use for mosquito control

Methoprene has been extensively used for the control and eradication of various mosquito species in many different parts of the world.

Carlson et al. (1999) discussed some of Florida's saltmarsh management issues in the 1990s with particular regard to the politics of running mosquito control programmes in the USA. They state that "larvicides remain as an important salt-marsh integrated pest management tool with the greatest acreage being treated with temephos, followed by *Bacillus thuringiensis israelensis* and methoprene. However, over the past 14 years, use of biorational larvicides has increased greatly."

Claborn et al. (2002) conducted a cost-comparison of two methods for the control of malaria in the Republic of Korea. They determined that the cost of larviciding with methoprene granules was estimated at $93.48 USD/hectare and that the annual cost of providing chemoprophylaxis was estimated to be $37.53 USD/person.

Sticky ovitraps were used to sample female *Stegomyia aegypti i*n Cairns, Queensland, Australia before and after applications of methoprene and lambda-cyhalothrin (Ritchie et al. 2004). High densities (3.5 females per trap per week) of *St. aegypti* were captured prior to insecticide applications. After treatment with methoprene or lambda-cyhalothrin trap catches dropped to <0.5 per trap.

6.1.1 *Aedes spp.*

Recently the taxonomic nomenclature of some mosquito groups has changed. For example, the subgenera, *Ochlerotatus* and *Stegomyia* have been elevated to genus level (Reinert et al. 2004). The yellow fever mosquito, *Aedes aegypti* is now *Stegomyia aegypti* and the Asian tiger mosquito, *Aedes albopictus* is now called *Stegomyia albopicta*.

The toxicity of the organophosphate insecticides, temephos and pirimiphos-methyl, methoprene, and Bti to *Ochlerotatus vigilax*, an Australian saltmarsh mosquito vector of Ross River virus was evaluated in the laboratory followed by field studies in southeastern Queensland, Australia (Brown et al. 1999). A nontarget study with the shrimp species *Leander tenuicornis* was also conducted. Methoprene and Bti were the most selective pesticides for *Oc. vigilax*, with selectivity ratios (LC95 nontarget/LC95 target) of 255,000 and 38,000, respectively. Selectivity ratios greater than 1 indicate that the pesticide is

more toxic to the pest species than to the beneficial species. Selectivity ratios for temephos and pirimiphos-methyl, were 13 and 0.01, respectively. Field applications of methoprene and Bti were highly effective against *Oc. vigilax*, and had no effect on the survival of *L. tenuicornis*. Temephos and pirimiphos-methyl were also effective controls for *Oc. vigilax*, but killed all of the caged *L. tenuicornis*. Additionally, methoprene and Bti did not affect water quality, while temephos and pirimiphos-methyl significantly changed pH and turbidity.

Ryan and Kay (2000) detailed a study where emergence traps with sticky adhesive were used to monitor adult mosquitoes that emerge from brackish water pools in swamp oak and tea-tree forests in Maroochy Shire, southeastern Queensland, Australia. During the study, a total of 825 adult mosquitoes were collected, with *Ochlerotatus procax* (55%) being the most abundant species followed by *Verrallina funerea* (16%) and *Culex halifaxii* (9%). The authors stated that aerial applications of persistent methoprene formulations are the most appropriate option for control.

Studies on the effectiveness of Bti and methoprene (for control of the Asian tiger mosquito, *Stegomyia albopicta*) in non-circulating hydroponic tanks of lettuce in Hawaii showed that these products reduced mosquito larval and pupal populations for the duration of the lettuce crop (4-5 weeks) compared to an untreated control (Furutani and Arita-Tsutsumi 2002).

6.1.2 *Culex* spp.

This section is still valid. The only study on methoprene effect on *Culex* species was discussed above in section 4.3.2.

6.1.3 *Mansonia* spp.

This section is still valid. No new studies have been published on *Mansonia* spp. and methoprene since the publication of the original document.

6.1.2 *Psorophora* spp.

This section is still valid. No new studies have been published on *Psorophora* spp. and methoprene since the publication of the original document.

7. Comparison of efficacy of methoprene with other agents

Methoprene has been proven to be an effective and relatively safe product for control of mosquitoes. Certain organophosphate insecticides may be more effective at controlling mosquito species, but these products are almost always more damaging to ecosystems than methoprene.

7.1 Mosquitoes

7.1.1 Laboratory

A comparison of the laboratory toxicity of five organophosphate insecticides (OPs) (chlorpyrifos, chlorpyrifos methyl, fenthion, malathion and temephos), three pyrethroids (bifenthrin, cypermethrin and permethrin), one phenyl pyrazole (fipronil), two microbial pesticides (Bti and *Bacillus sphaericus*) and three insect growth regulators (IGRs) (diflubenzuron, methoprene and pyriproxyfen) to field-collected *Culex quinquefasciatus* larvae from urban Dhaka, Bangladesh was reported by Ali et al. (1999a). The ranking of toxicity from most toxic to least toxic was: fipronil> IGRs > pyrethroids > microbials > OPs.

7.1.2 Field

Kay et al. (2002) assessed the importance of treating surface containers and wells with methoprene for control of *Stegomyia aegypti* inhabiting wells in the semiarid town of Charters Towers, north Queensland, Australia. Results of this study indicated that treating a relatively small number of key wells with methoprene during winter significantly reduced populations of *St. aegypti*.

7.2 Flies

This section is still valid.

7.3 Chironomids

In a review of control methods for chironomids, Ali (1997) found that methoprene is one of the most effective products available for control of these species.

Methoprene pellets (4% ai) were evaluated for control of the chironomid midges, *Chironomus stigmaterus*, *Goeldichironomus amazonicus* and *Tanypus imperialis*, in man-made lakes in Palm Desert, California, USA (Lothrop and Mulla 1998). Applications of 4.5 kg/ha of controlled release pellets (4% methoprene) resulted in > 90% control of *C. stigmaterus* and *T. imperialis* for 3 weeks and 75% control in the 4th week. A lower rate (3.4 kg/ha) provided >90% control for 2 weeks, and the rate of 2.25 kg/ha provided control for 1 week. Less than 90% control of *G. amazonicus* was achieved for 2 weeks at the rate of 4.5 kg/ha and 1 week at the rates of 3.4 kg/ha and 2.25 kg/ha.

7.4 Fleas

Cocooned pupal cat fleas, *Ctenocephalides felis*, exposed to methoprene emerged to the adult stage earlier than controls but died at a higher rate. Fecundity of surviving adult females was not affected (Miller et al. 1999).

The residual activity of methoprene to adult cat fleas, *Ctenocephalides felis*, in topsoil contained within nursery pots was determined by Rajapakse and Meola (2002). Methoprene was found to be as as effective as fenoxycarb and pyriproxyfen against cat fleas for up to 42 days in clay, peat, and plastic pots at a concentration of 64.56 mg a.i./m^2 (6 mg a.i./ft^2), but then its activity declined significantly thereafter. Fenoxycarb and pyriproxyfen had longer residual activity than methoprene providing effective control

of the flea for 63 days. Methoprene activity declined even more rapidly over time in wooden flats. LC50 values for methoprene, fenoxycarb and pyriproxyfen applied to topsoil were estimated to be 0.643, 0.031, and 0.028 ppm, respectively.

Young et al. (2004) investigated the efficacy of fipronil (10% (w/v) solution), methoprene (9% (w/v) solution), and a combination of fipronil and methoprene (10 and 9% (w/v) solution, respectively, as controls for cat fleas, *Ctenocephalides felis* on experimentally infested dogs. The fipronil/methoprene combination provided 95% control adult fleas for 5 weeks, providing longer-term control than fipronil alone.

7.5 Lepidoptera

This section is still valid. No new literature on the effects of methoprene on Lepidoptera was published since the original document.

7.6 Chrysomelids

This section is still valid. No new literature on the effects of methoprene on Chrysomelids was published since the original document.

7.7 Mites

A new study on the effects of methoprene and a predaceous mite species was discussed above in section 5.1 (Ali et al. 1999b).

8. Use of methoprene in eradication campaigns

This section is still valid.

9. Effects on non-target organisms

Methoprene is considered one of the safest mosquito control agents available. Several new studies on the effects of methoprene on nontarget organisms have been published since the report by Glare and O'Callaghan (1999). The most important of these are three papers reporting on the effects of methoprene and Bti to nontarget organisms in 27 wetlands in Minnesota, USA (Hanowski et al. 1997, Hershey et al. 1998, Niemi et al. 1999). The reason that these papers are so important is that the studies are extremely well done in terms of experimental design and statistical robustness and these wetlands were studied over a six-year period and thus potential long-term impacts could be identified. These studies are discussed below in sections 9.3.1 and 9.9.5.

9.1 Phytotoxicity

This section is still valid.

9.2 Microorganisms

9.2.1 *Bacillus thuringiensis*

This section is still valid.

9.2.2 Protozoa

The causal agent responsible for Chaga's disease, the flagellate protozoan, *Trypanosoma cruzi* was controlled *in vitro* with methoprene (Esteva et al. 2002). However, treatment of mice at a rate of 200 ug methoprene/mouse/day for five days reduced the disease titre but did not eliminate it completely. The authors suggested that methoprene might be used to sterilise human blood for transfusions.

9.2.3 Fungi

This section is still valid.

9.2.4 Virus

This section is still valid.

9.3 Invertebrates

9.3.1 Benthic and aquatic communities

The effects of applications of methoprene and Bti for mosquito control on the benthic macroinvertebrate communities of 27 wetland ecosystems in Wright County, Minnesota, USA were evaluated from 1991-1993 (Hershey et al. 1998). The wetlands were separated into three groups of nine, the first group receiving methoprene treatment, the second Bti and the third acting as a control. During the first year of treatment (1991) only minimal effects on nontarget groups were detected. However, during 1992, significant reductions in certain insect groups were detected in methoprene and Bti treated wetlands. Predatory insects were reduced in methoprene-treated sites but not in Bti-treated sites. By 1993, insect biomass had decreased significantly in methoprene- and Bti-treated sites. Diptera were affected most strongly, especially the suborder Nematocera and in particular the Chironomidae (midges). Both insecticides had very little impact on noninsect macroinvertebrates.

The efficacy and nontarget effects of temephos, Bti and methoprene applied by helicopter to control mosquito larvae in mangrove swamps was determined on Sanibel Island, Florida, USA, in May 1997 (Lawler et al. 1999). Three sites were treated with pesticides and three sites served as controls. Application rates for temephos (Abate) were 37 ml/ha (43% a.i.), Bti granules (Vectobac GTM) 5.606 kg/ha (200 International Toxic Units/mg) and for methoprene (AltosidTM ALL), 213 ml/ha (5% a.i.). Caged *Ochlerotatus taeniorhynchus* were used to monitor efficacy while sentinel nontarget amphipods (Talitridae) were monitored for nontarget effects. Additionally, the effect on flying insects was measured using light traps to collect dead insects that fell into tarps suspended under mangroves in areas treated with either temephos or methoprene. Each pesticide worked well in controlling mosquitoes but failures did occur occasionally. No amphipod mortality or mortality of flying insects was detected in the study sites.

A six-year field study from 1988 to 1993 in 27 wetlands of central Minnesota, USA was conducted to determine the potential ecological effects of methoprene (applied as Altosid® sand granules) and Bti (applied as Vectobac G® granules), on zooplankton, insects and breeding birds (Niemi et al. 1999). Insect biomass was reduced by 57 to 83%

in the second (1992) and third (1993) years of treatment. There was no evidence that ecosystem health was impaired even though such a large decline in insect biomass occurred. No negative effects on zooplankton or breeding birds could be attributed to pesticide treatment or reductions in insect biomass. It appears that organisms that fed on aquatic insects were able to switch to other sources of food. For example, red wing black birds appeared to collect insects outside of the treated areas where insect biomass had declined. The authors concluded that it was unclear what the long-term consequences of insect reductions might mean to wetland health.

A study of the effects of a combined formulation (duplex) of Bti and liquid methoprene on nontarget insects inhabiting saltmarshes was carried out by Lawler et al. (2000). The pesticides were applied to replicated saltmarsh ponds at maximum label rates. Effects were measured by rearing immature *Ochlerotatus dorsalis* and the water boatman, *Trichocorixa reticulata* in predator exclusion cages and by monitoring uncaged populations of invertebrates using replicated sweep-net samples. Caged mosquitoes were killed by the pesticide mixture and the activity of the Altosid® pellets continued through 99 days. No negative effect on survival or maturation of *T. reticulata*, or on abundance of uncaged invertebrates caused by either pesticide was detected.

In another study, Pinkney et al (2000) investigated the effects on aquatic insects of Abate® 4E (44.6% temephos) applied at 0.054 kg of active ingredient (a.i.) per ha and of Altosid® Liquid Larvicide (5% methoprene) applied at 0.011 kg a.i./ha to 18 experimental ponds located at the Patuxent Wildlife Research Center, Laurel, Maryland, USA. Applications of these pesticides were made to the ponds at 3-week intervals. Six of the ponds received Abate sprays, six received Altosid and six received distilled water. Significant differences in diversity, equitability, numbers of individuals, number of species, and families of insects were found in the Abate treatments compared to controls. In particular, significant reductions occurred in Ephemeroptera, Odonata, Diptera, Chironomidae, and *Chaoborus* sp. However, in Altosid-treated ponds, only isolated cases with significant reductions were detected. Hester-Dendy data indicated no significant differences between Altosid-treated ponds and control (water-treated) ponds.

9.3.2 Nematoda

This section is still valid.

9.3.3 Insects

A study conducted to investigate the effects of methoprene (Altosid®), temephos (Abate®) and Bti on a biological control agent of the weed, purple loosestrife (*Lythrum alicaria*), the leaf feeding beetle, *Galerucella calmariensis*, determined that the egg and larval stages were susceptible to methoprene at concentrations likely to be encountered in the field (Lowe and Hershberger 2004).

9.3.3.1 Insect predators

This section is still valid. No new literature was found.

9.3.3.2 Parasitoids

Parasitoids are important components of ecosystems as they often keep pest populations in check. The morphogenetic effects of methoprene and hydroprene on *Bracon hebetor*, an ectoparasitic wasp, reared on juvenoid-treated ultimate instar host larvae of *Corcyra cephalonica* (Lepidoptera: Pyralidae) were evaluated (Chanda and Chakravorty 2000, 2001). Methoprene was found to be more harmful than hydroprene to this wasp and caused anatomical deformities in the reproductive systems of females and males that survived exposure as immatures.

Application of methoprene to fifth instar *Manducta sexta* parasitised by the braconid wasp, *Cotesia congregata* resulted in a delay or total suppression of wasp emergence (Beckage et al. 2002).

9.3.3.3 Bees

Bees are extremely important components of ecosystems and are especially important to agriculture due to their role in pollination and providing honey and wax. As such, a great deal of work on the effects of various pesticides to bees has been carried.

Methoprene topically applied to honey bees significantly increased sucrose responsiveness, indicating that hormones play a significant role in the regulation of colony-level and individual foraging behavior (Pankiw and Page 2003).

9.4 Rotifers and marine worms

Preston et al. (2000) developed a 96-h reproductive assay with the freshwater rotifer, *Brachionus calyciflorus* to evaluate the potential for endocrine disruption by cadmium, chlorpyrifos, naphthol, pentachlorophenol, estradiol, methoprene, precocene, nonylphenol, flutamide and testosterone. Flutamide, testosterone and nonylphenol inhibited fertilisation of sexual females at concentrations of 1, 10, and 50 ug/l, respectively. However, methoprene at a concentration of 10 ug/l had no statistically significant effect on rotifer reproduction.

9.5 Mollusca

This section is still valid. No new literature was found.

9.6 Crustaceans

9.6.1 Microcrustaceans

The water flea, *Daphnia magna* and other *Daphnia* species will produce only female offspring under optimal environmental conditions. Under stressful conditions, *Daphnia* will go through a sexual cycle and produce males. The effects of methoprene on *D. magna* were evaluated in a series of studies by Olmstead and LeBlanc (2000, 2001a and b, 2003). In the first study (2000) Olmstead and LeBlanc found that methoprene exposure (0.080 muM) altered the development of secondary sex characteristics in daphnids. In the second study (2001a), *D. magna* exposed to 160 nM methoprene along

with food deprivation and crowding produced fewer males during the early phase of sexual reproduction then produced a larger number of males during the latter stages of the sexual cycle. In the third study (Olmstead and LeBlanc 2001b) exposure of *D. magna* to methoprene resulted in a concentration-dependent reduced growth rate, reduced moult frequency, delayed reproduction, and reduced fecundity. Methoprene was shown to mimic the action of the crustacean juvenoid hormone methyl farnesoate and programme oocytes of *D. magna* to develop into males (Olmstead and LeBlanc 2003). These results are contrary to those obtained by Peterson et al. (2001) for *D. pulex* (see below). Clearly however, methoprene appears to be able to produce detrimental effects in *Daphnia* at certain concentrations.

Exposure of the water flea, *Daphnia pulex* to methoprene concentrations of 10 to 100 ug/l resulted in a decrease in the incidence of all-male broods and an increase in the incidence of all-female broods compared with controls suggesting that juvenile hormones play a role in *Daphnia* sex determination (Peterson et al. 2001).

9.6.2 Macrocrustaceans

Methoprene, at a concentration of 10 muM, was found to cause a series of changes to the cuticular epithelial cells of postmoult adult blue crabs, *Callinectes sapidus* in laboratory studies (Horst and Walker 1999). The changes included loss of secretory organelles and distention of the outer membrane of the nuclear envelope. Decreased deposition of cuticular chiton and protein and an accumulation of chitoprotein precursors were also observed. Exposure of *C. sapidus* to methoprene at environmental concentrations (2-10 muM) resulted in morbidity and mortality and a reduction in the number of successful hatchings as well as lethargic behaviour in surviving zoeae. Exposure to a concentration of 3.3 muM methoprene resulted in a delay of the moult to the first crab form and death of 80% of larvae after 10 days.

Acute toxicity (96 hour LC50) of methoprene and the insecticide endosulfan was estimated for adult grass shrimp, *Palaemonetes pugio* (Wirth et al. 2001). Results indicated that methoprene was not toxic to this species at 1 mg/l concentrations. Endosulfan was toxic with an LC50 of 0.62 ug/l. A chronic reproduction study was also performed whereby shrimp were exposed to 1 mg/l methoprene or 200 ng/l endosulfan. Methoprene exposure caused no significant reproductive damage however, endosulfan caused a significant increase in time for eggs to hatch compared to controls.

Verslycke et al. (2004) evaluated the acute toxicity of the following suspected endocrine disruptors: testosterone, flutamide, ethinylestradiol, precocene, nonylphenol, fenoxycarb and methoprene to the estuarine mysid shrimp, *Neomysis integer*. Acute LC50 estimates (96 hour) for these products ranged from 0.32 and 1.95 mg/l. The most toxic products to *N. integer* were methoprene and fenoxycarb, both synthetic insect JHAs. Additonally, sublethal exposures of methoprene (100 ug/l) significantly induced energy consumption resulting in lower cellular energy allocation. Testosterone metabolism was negatively affected after exposure to 10 ug/l methoprene. These results indicate that methoprene causes damage to *N. integer* at the concentrations tested.

9.7 Fish and amphibians

Brown et al. (2002) studied the acute toxicity of 1-hour pulse exposures of five insecticides. Two organophosphates, temephos and pirimiphos-methyl, an entomopathogenic bacterium, Bti, and two insect growth regulators, methoprene and pyriproxyfen were evaluated for toxicity to juvenile and adult crimson-spotted rainbowfish, *Melanotaenia duboulayi*, a mosquito predator in Queensland, Australia. None of these insecticides were acutely toxic to adults of this fish species. However, temephos and pirimiphos-methyl were toxic to juveniles, with 24 h LC50 values of 27 and 15 ug/litre (ppb), respectively. Methoprene, Bti and pyriproxyfen were not toxic at concentrations that were up to 10 to 12.5 times the estimated environmental concentration for a 15 cm deep body of water.

Exposure of zebrafish embryos to sunlight-induced photolytic products of the pesticide methoprene resulted in developmental defects in the head, heart, pectoral fins and somites, and in spinal motor and optic nerve axons (Smith et al. 2003). Exposed embryos were found to be phenocopies of zebrafish mutants that exhibited underexpression of the signalling protein sonic hedgehog.

The effects of the synthetic retinoids, Am80 (a retinoic acid receptor) and methoprene acid (a retinoid X receptor-selective retinoid) on the jaw development of larvae of the Japanese flounder was evaluated (Haga et al. 2003) . Methoprene acid (25 nM) induced lower jaw deformities in less than 20% of the fish but no deformities in the upper jaw. Am80 caused both upper and lower jaw deformities.

9.7.1 Mosquito predatory fish

This section is still valid. No new literature was found.

9.8 Deformed frog controversy

A concern discussed in the original methoprene assessment was the possibility that methoprene or a breakdown product(s) of methoprene may be the cause of deformities being detected in frogs in the USA. Even though it has been 6 years since the last assessment, the causal agent(s) of frog deformities in the USA has still not been clearly elucidated. However, researchers generally believe that one or more of the following are causing the deformities: 1) parasitic trematodes, (Johnson et al. 1999, Ankley et al. 2004), 2) retinoids, including methoprene and methoprene breakdown products (Gardiner and Hoppe 1999, Gardiner et al. 2003), 3) increased UV radiation due to the destruction of the ozone layer (Blaustein et al. 1997), 4) chemicals other than methoprene such as pesticides, endocrine disruptors, or mineral deficiencies (Bridges 2000), 5) agricultural runoff in general (deSolla et al. 2002), or 6) a combination of some or all of these factors (Meteyer et al. 2000, Kiesecker et al. 2001, Kiesecker 2002, Johnson et al. 2003, Johnson and Sutherland 2003). Several new studies on the potential impact of methoprene and methoprene breakdown products on frogs are discussed below.

Ankley et al. (1998) assessed the potential of methoprene and UV radiation to cause hindlimb deformities in Northern leopard frogs, *Rana pipiens*. Early embryonic stages of

R. pipiens were exposed to five concentrations of methoprene (1.95, 7.8, 31.3, 125, and 500 μg/L) in the presence or absence of UV light. Exposure to the highest concentrations of methoprene in the presence or absence of UV light caused severe developmental effects and all frogs died within 12-16 days of test initiation. However, no limb malformations were caused by methoprene. Hindlimb malformations did occur in approximately 50% of *R. pipiens* held under UV light for 24 days. However, the authors point out that although the deformities observed were similar to those found in field collected specimens, the full range of malformations seen in the field were not evident in the frogs evaluated in this study. The conclusion was that additional studies with UV light and hindlimb malformations in frogs are warranted.

Methoprene was found to produce minimal amounts of limb malformations in amphibians, but products of its reaction with sunlight, water, and microorganisms dramatically interfered with normal amphibian development (LaClair et al. 1998).

Gardiner and Hoppe (1999) speculated that retinoids, like methoprene were probably causing malformations in frogs because of the types of malformations being reported.

Of 43 froglets of the Northern leopard frog, *Rana pipiens* collected from Foggy Bottom Marsh in southern Michigan, USA in 1997, all were found to be infected with helminthes (twelve helminth taxa – six Nematoda, five Trematoda and one Cestoda). Yet no deformities were observed in any of these frogs (Gillilland and Muzzall 1999). This may indicate that trematodes are not the causal agent of limb malformations observed in frogs.

Severe limb deformities were induced in Pacific treefrogs (*Hyla regilla*) exposed to cercariae of a trematode parasite (*Ribeiroia* sp.) in the laboratory. The induced limb deformities closely matched those found in the field (Johnson et al. 1999). These findings indicate that parasitic trematodes may be the cause of the deformities being found in frogs in the USA.

The neurotoxic insecticide, carbaryl was evaluated for its potential to cause deformities in the southern leopard frog, *Rana sphenocephala* exposed at various life stages (Bridges 2000). Carbaryl caused visceral and limb malformations in 18% of individuals exposed during developmental stages compared to only 1% deformities in controls.

A survey of limb-malformed and normal (no limb malformation) bullfrogs, *Rana catesbeiana*, and green frogs, *Rana clamitans*, was conducted in central and southern New Hampshire, USA and showed that 81% of the frogs in 13 of 16 sites sampled had limb deformities (Sowers et al. 2000). An examination of androgens and brain gonadotropin-releasing hormone (GnRh) revealed that deformed frogs has significantly lower concentrations of androgens and brain GnRh indicating that environmental factors or endocrine-disrupting chemicals may be the cause of developmental abnormalities.

Laboratory and field studies with the wood frog, *Rana sylvatica* linked limb deformities with trematode infection and pesticide exposure (Kiesecker 2002). Field studies showed that trematode exposure was necessary to cause limb deformities, but limb deformities

were more common at sites adjacent to agricultural runoff. Laboratory experiments corroborated the link between trematode infection and pesticide exposure. Exposure to pesticides resulted in a decrease in immune function making frogs more susceptible to trematode infection. Thus, these results indicate that the high incidence of deformities found in frogs in the USA is mediated by pesticides and trematodes.

Embryos of the African clawedfrog, *Xenopus laevis* were exposed to methopene and the degradation products of methopene, methoprene acid, methoprene epoxide, 7-methoxycitronellal and 7-methoxycitronellic acid in 96 h developmental toxicity tests (Degitz et al. 2003). No developmental toxicity was caused by methoprene at concentrations up to 2 mg/l, a concentration that is slightly higher than its water solubility. The minor degradation product, methoprene acid did have negative effects on development, but only when concentrations exceeded 1.25 mg/l. Methoprene epoxide and 7-methoxycitronellal caused developmental toxicity when *X. laevis* embryos were exposed to concentrations of 2.5 mg/l and higher while 7-methoxycitronellic acid was not developmentally toxic even after exposure to concentrations as high as 30 mg/l. The authors concluded that because methoprene concentrations in the field rarely exceed 0.01 mg/l, it is highly unlikely that methoprene is the cause of amphibian deformities.

A detailed analysis of malformed frogs collected in Minnesota ponds and lakes suggested that limb malformations resulted from a modification of a retinoid-sensitive developmental signaling pathway (Gardiner et al. 2003). Retinoid treatment of frog embryos at sensitive times of development produced the full spectrum of limb abnormalities observed in field specimens in the laboratory. These data indicate that some environmental contaminants cause inappropriate modulation of retinoid signaling and this is the mechanism underlying the increased incidence of frog malformations.

The potential of field-collected pond water in north-central Minnesota, USA to cause deformities in tadpoles of the native northern leopard frog, *Rana pipiens* was evaluated by Bridges et al. (2004). Pond water was extracted with semipermeable membranes to collect fat soluble contaminants at an impacted and a reference site. Tadploes were exposed to extracts from the membranes, extracts combined with two pesticides, atrazine and carbaryl and at two levels of UV radiation. The results showed that tadpoles exposed to membrane extracts from impacted sites caused hatchling deformities only in the presence of UV light indicating that UV light potentiates the teratogenicity of the compounds present there.

Ankley et al. (2004) published a review of the current knowledge of the causes of limb malformations in anuran amphibians from different regions across North America. The conclusion of the review is that there is little evidence that chemical contaminants including methoprene or UV radiation are responsible for limb malformations. Although solar radiation has been implicated in limb malformation in the laboratory, a probabilistic assessment based on dose-response exposure data indicates that levels of UV radiation sufficient to cause these malformations are unlikely to occur in most wetlands. The most likely explanation for limb malformation is infection by digenetic trematodes, a parasitic organism.

9.9 Mammalian toxicity

This section is still valid.

9.9.1 Humans

This section is still valid.

9.9.2 Residue tolerances in animal products

This section is still valid.

9.9.3 Cattle

This section is still valid.

9.9.4 Sheep

This section is still valid.

9.9.5 Small mammals and birds

The potential effect of methoprene (applied as Altosid sand granules) and Bti (applied as Vectobac-G granules) to a wetland breeding bird community was examined from 1988 to 1993 in Minnesota, USA (Hanowski et al. 1997). No effect due to pesticide treatments was found on the bird community or on 19 individual bird species.

10. Persistence and activity in the environment

This section is still valid.

10.1 Environmental persistence

This section is still valid.

10.2 Persistence in water

This section and all subsections (10.2.1 - 10.2.4) are still valid. No new studies were found.

10.3 Persistence in soil

This section is still valid. No new studies were found.

10.4 Persistence on crops and stored products

This section is still valid. No new studies were found.

10.5 Effect of formulation on persistence

This section is still valid. No new studies were found.

11. Metabolic fate of methoprene

This section and all subsections (11.1 - 11.6) are still valid. No new studies were found.

12. Detection methods

Two new papers on detections methods for methoprene have been published since the original report.

An analytical method based on reversed-phase high performance liquid chromatography was developed for the detection of methoprene, methoprene acid, the insecticide, permethrin and two of its metabolites in rat urine (Abu-Qare and Abou-Donia 2001). The limits of detection ranged from 50 to 100 ng/ml.

Wang et al. (2002) developed a rapid immumoassay technique for the detection of methoprene and carbaryl residues in grain. The test had a limit of detection of 4 ppb for methoprene and 4.5 ppb for carbaryl and took approximately 20 minutes to run the analysis.

13. Resistance

There is some evidence that certain mosquito populations are developing resistance to methoprene. Therefore, long-term use should be considered carefully. Rotation of methoprene with other products such as Bti should ensure that resistance does not occur. Several new studies on resistance have been published since publication of the original document by Glare and O'Callaghan (1999).

13.1 Development of resistance

The potential for cross-resistance to methoprene and other insect growth regulators in pyriproxyfen-resistant housefly was investigated by Li et al. (1998). They found that third instars of resistant YPPF house fly strain had medium cross-resistance to methoprene and fenoxycarb but no cross-resistance to diflubenzuron, a chitin synthesis inhibitor. The authors suggested that insecticides with no cross-resistance to each other be rotated in control programmes to avoid future development of cross-resistance.

Keiding (1999) published a review article on the development of resistance in house fly, *Musca domestica* populations. In this review, some examples of some house fly populations having developed resistance to methoprene are cited.

13.2 Field resistance

Failures of field applications of methoprene to control *Ochlerotatus nigromaculis* were detected in the summer of 1998 in the outskirts of Fresno, California, USA (Cornel et al. 2000). Methoprene had provided effective control for the previous 20 years. A field survey indicated that low levels of control were achieved with applications of Altosid® (Liquid Larvicide) and Altosid XR-G while control with Altosid Pellets ranged from 52-99%. A followup study was published in 2002. Several thousand-fold higher LC50 and LC90 values were calculated for field-collected populatons of *Oc. nigromaculis* compared to untreated populations (Cornell et al. 2002). Additional studies with the synergists, piperonyl butoxide and S,S,S-tributyl phosphorotrithioate and 3-octylthio-1,1,1 trifluoro-2-propanone showed little synergistic effect, signifying that methoprene tolerance was not mediated by P450 monooxygenase or carboxylesterase enzyme

degradation. Six consecutive applications of Bti applied together with two oil and two pyrethrum + piperonyl butoxide (PBO) applications resulted in a partial reversion back to methoprene susceptibility.

A survey of resistance to insect growth regulators in Danish field populations of the house fly, *Musca domestica* was reported by Kristensen and Jespersen (2003). Results of their study indicated that field populations were resistant to diflubenzuron and cyromazine and that some of the strains resistant to these products were also resistant to methoprene.

13.3 Effect of resistance on insect fitness

This section is still valid. No new studies were found.

13.4 Management of resistance

As mentioned in section 13.2, Cornel et al. (2000) found that applications of the combination of Bti, oil, pyrethrum, and piperonyl butoxide to methoprene-resistant *Ochlerotatus nigromaculis* resulted in a partial reversion to methoprene susceptibility.

14. Discussion and conclusions

Since the report of Glare and O'Callaghan (1999), many new studies on the effectiveness of methoprene as a control for mosquitoes have been published. Additionally several important nontarget field assessments have been published. Methoprene continues to be one of the most important tools for control and eradication of mosquitoes throughout the world. Although development of resistance continues to be a concern, few examples of full-scale resistance, whereby methoprene is no longer an effective control of mosquitoes in the field have been documented. However, important studies by Cornell et al. (2000, 2002) show the development of field resistance in *Ochlerotatus nigromaculis* after 20 years of methoprene applications. This serves as a warning that certain mosquito populations may develop resistance after repeated exposure. Several important studies on the impact of methoprene on nontarget organisms in the field have been published since the report by Glare and O'Callaghan (1999). Long-term studies indicate that continued application of methoprene to wetlands can greatly reduce the biomass of aquatic insects. This reduction however, did not result in a loss of ecosystem function. It is still unclear what the long-term consequences of large reductions in insect biomass might mean to wetland health. Methoprene has been implicated as one of the possible causes for limb malformations in frog populations in the USA. There is no definitive evidence that methoprene or breakdown products of methoprene can cause the types of malformations being observed at the environmental concentrations being detected. At one time New Zealand had seven native frog species all in the genus *Leiopelma*. However, some of these species have become extinct (www.doc.govt.nz/Conservation/001~Plants-and-Animals/001 ~Native Animals/Pekeketua-Native-Frogs.asp). According to Lindsey and Morris (2003) only three native frogs, Hochstetter's frog, *Leiopelma hochstetteri*, Archey's frog, *Leiopelma archeyi*, and Hamilton's frog, *Leiopelma hamiltoni* still exist. Presently, three introduced frog species are found in New Zealand as well. The

introduced species all belong to the genus *Litoria.* The native species are quite unique from other species worldwide because they have no external eardrum, they have round-, not slit-shaped, eyes, they don't croak regularly like most frogs, and they don't have a tadpole stage (the embryo develops inside an egg, and then hatches as an almost fully-formed frog). Furthermore the young of the species are cared for by their parents. Because of the uniqueness of the remaining frog species in New Zealand, efforts to protect them should be taken seriously. However, the three remaining native frogs have very restricted ranges (Lindsey and Morris 2003). Hochstetter's frog occurs from Northland to East Cape; Archey's frog only lives on Coromandel Peninsula; and Hamilton's frog only inhabits Stephens and Maud Islands.

14.1 Methoprene safe for use in New Zealand?

This section is still valid.

14.2 Other potential responses to mosquito invasion

This section is still valid.

14.2.1 Chemical mosquito agents

A few new chemicals are being evaluated as potential controls for mosquitoes. Fipronil and spinosad are potential candidates that have been shown to be toxic to mosquitoes. However, no long-term studies that evaluate these products in the field have been published to date. *Daphnia pulex* were found to be quite susceptible to Spinosad, but spinosad was much less toxic than diazinon to this species (Stark and Vargas 2003). Fipronil was also found to be toxic to *D. pulex* but at the concentrations used for fruit fly control programmes in the South Pacific and Australia, it poses little risk to this species (Stark and Vargas in press). Based on the original report by Glare and O'Callaghan (1999) and an evaluation of new literature published after their report, methoprene remains the primary choice for control and eradication of mosquitoes.

14.2.2 Other insect growth regulators

Novaluron a new chiton synthesis inhibitor has been shown to be an effective control for *Stegomyia aegypti* in the laboratory and the field (Mulla et al. 2003). Emergence of adults was 100% after exposure of 2nd and 4th instars to concentrations of 0.25 to 1.0 ug/l. Furthermore, Novaluron is a persistent control agent that has been shown to provide control of *S. aegypti* for 60 to 190 days depending on the concentration applied to water storage containers in Thailand.

14.2.3 Biological agents

This section is still valid.

14.2.3.1 Comparison of methoprene with *Bacillus thuringiensis*

This section is still valid.

14.3 Further considerations of methoprene before use in New Zealand

This section is still valid.

15. Acknowledgements

I appreciate the help of E. R. Frampton, Critique Limited, Christchurch, New Zealand, for help with this project. I am also grateful to the personnel of New Zealand Biosecure for taking the time to make me familiar with the field aspects of the mosquito eradication programme.

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