Environmental and Health Impacts of the Mosquito Control Agent Agnique, a Monomolecular Surface Film

June 2005,
Revised October 2005

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

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Abbreviations:

Agnique = Agnique^{TM} = Agnique® MMF
Arosurf = ISA-20E = Arosurf 66-E2 = Arosurf® MSF
Bti = *Bacillus thuringiensis israelensis*
MMF = monomolecular surface film

1. Summary

- The potential introduction of mosquitoes that vector human and animal disease poses a threat to New Zealand. Effective agents for control and/or eradication of these pest species are a necessity.

- Certain types of monomolecular surface films (MMF) are used for control of mosquitoes and midges. Agnique and Arosurf are the only commercially produced MMFs for mosquito and midge control today. Each product has the same active ingredient.

- MMFs kill by a physical mode of action. The activity of MMFs is similar to petroleum oils. Organisms that must make contact with the air-water interface to breath, such as mosquitoes, midges, and other aquatic insects may be susceptible. For example, mosquito larvae and pupae must attach their siphon tubes to the water surface to breath. Wetting of tracheal structures may also occur causing anoxia. The result is suffocation of the immature stages of mosquitoes. Adult mosquitoes that try to emerge from the pupal stage at the air-water interface treated with Agnique may drown. Adult mosquitoes that land on the surface of water to lay eggs may also drown. Thus, MMFs unlike many other mosquito control agents, have the potential to affect all mosquito life stages.

- Larvae of the following mosquitoes are not very susceptible to monomolecular surface films because they have little or no surface contact; *Coquillettidia*, *Culex pilosis*, *Culex erraticus* and *Manosonia*. However, adults of these species may be susceptible when laying eggs or emerging to the adult stage. *Coquillettidia* spp. have been shown to transmit Eastern Equine Encephalitis and West Nile Virus, while *Cx. erraticus* and *Mansonia tittilans* have been shown to transmit West Nile Virus.

- MMFs are most effective when a large proportion of the immature mosquito population has reached the fourth instar and/or pupal stages.

- MMFs can persist in the field for up to 22 days under certain conditions, but results of most studies indicate that these products break down relatively quickly in the environment and are often undetectable within 48 hours after application. Cognis Corportion lists the persistence of Agnique at 5-22 days on its Specimen Label and 5-14 days on its Material Safety Data Sheet. MMFs have been shown to be most effective when applied to small, sheltered bodies of water such as ditches, puddles, and temporary pools.

- Because Agnique is a physical pesticide, development of resistance to this product is highly unlikely if not impossible.

- Agnique is not a mutagen and is virtually non-toxic to mammals, birds, and fish. The only effect in mammals is mild eye irritation, which is reversible.

- Although MMFs were developed in the 1980s, they have not been as widely used for mosquito control in the United States and other parts of the world as other mosquito control agents. However, Agnique has been used in the United States since 1998 and is gaining

registrations in other countries.

- The low usage of MMFs is attributed to inconsistent control that can occur when wind (>16 km/hour) blows consistently in one direction for long periods of time moving the MMF to one section of a water body. Additionally, treatment of water bodies with surface and emergent vegetation, algal mats and/or debris results in areas that are essentially untreated and serve as protected sanctuaries for mosquitoes. Furthermore, MMFs work better in stagnant water because flowing water can reduce the efficacy. Highly oxygenated water makes MMFs less effective because larvae can absorb oxygen through the cuticle from the water and thus reduce contact with the surface of the water. Also, other effective controls with little environmental impact such as *Bacillus thuringiensis israelensis* (Bti) and S-methoprene are available for control of mosquitoes.

- Because Agnique is not a chemical toxicant, and floats on the surface of water, it has little effect on nontarget organisms that do not come into contact with the product. However, organisms that live on the surface of water such as water striders (Gerridae), or must make contact with the air-water interface to breathe such as water boatman (Corixidae), other hemipterans, and diving beetles (Dytiscidae) may be negatively affected by MMFs. The fish species that have been evaluated for susceptibility to MMFs are not negatively affected. One species of shrimp has been shown to be susceptible to MMFs, but other shrimp species appear unaffected after exposure. Results of a few studies have shown that *Daphnia*, copepods and frogs are not negatively affected by MMFs. The potential effects of long-term use of MMFs on many of the organisms that inhabit aquatic ecosystems such as waterfowl, reptiles, rotifers, spiders, algae, annelids, nematodes etc. are not known at this time because studies of this type have not been published. The lack of research on the potential effects of long-term use of MMFs on waterfowl including wading birds is troublesome. This issue should be addressed prior to any long-term, widespread use of these products where waterfowl reside.

- MMFs can be applied with other products such as S-methoprene, *Bacillus thuringiensis israelensis* and *Bacillus sphaericus* and provide better control than either product alone.

- Because of the limitations to the use of MMFs for mosquito control, Agnique should not be considered as the first choice for control/eradication programmes in New Zealand, especially of exotic saltmarsh mosquito species. S-methoprene and Bti have proven to be very effective mosquito control agents with low impact on nontarget organisms and these products should be the first line of defence for control and eradication in New Zealand. However, MMFs have a place for control of mosquitoes in certain habitats or settings such as treatment of standing water in containers or small, stagnant, sheltered bodies of water such as ditches, puddles, and temporary pools. Additionally, Agnique may be useful for localised control of recently arrived or established mosquito species in the immediate vicinity of ports in areas where water may collect. Agnique may also be useful in settings where a significant portion of the immature mosquitoes have reached the pupal stage. The use of Agnique in combination with Bti and/or S-methoprene may also provide an effective approach to mosquito control in certain circumstances.

2. Introduction

The introduction of exotic mosquitoes into New Zealand with the potential to vector serious mammalian diseases has highlighted the need for control technologies for use in control and/or eradication programmes (Frampton 2004). Following the recommendations of Glare and O'Callaghan (1998, 1999), S-methoprene and *Bacillus thuringiensis israelensis* (Bti) have been effectively used in eradication programmes for the southern saltmarsh mosquito, *Ochlerotatus camptorhynchus* (Thomson) in the North and South Island. Another product being considered for mosquito control/eradication programmes is the monomolecular surface film (MMF), Agnique. According to the Environmental Risk Management Authority (ERMA NZ), Agnique is a notified toxic substance under the Toxic Substances Act. Agnique MMF has a transitional approval under the Hazardous Substances and New Organisms Act (HSNO Act). It is expected to be transferred to the HSNO Act by July 2006. Agnique is presently being used by certain groups/organizations, such as port companies, city and district councils. This assessment of Agnique was undertaken to evaluate the potential environmental and health impacts of this product.

2.1 Background

A search of the Biological Abstracts, Agricola, and Biosis databases with the keywords monomolecular surface film, Agnique and Arosurf resulted in 36 references. These references along with others related to this subject were used to develop this assessment. An examination of the literature revealed major data gaps in terms of the environmental fate and toxicology of Arosurf/Agnique. Many of the studies on efficacy, environmental persistence, and toxicity to nontarget organisms were published in Mosquito News. During the 1980s papers in this journal did not adhere to the rigid requirements of other peer-review journals. As such, the papers are hard to follow and quite often the methods are not clearly elucidated. In some of the toxicity studies (see Webber and Cochran 1984, and section 7 below), so few organisms were evaluated that the results would not hold up in other higher tier journals. Also, no long-term environmental studies have been conducted where MMFs have been repeatedly applied to wetlands and nontarget organisms monitored.

The first commercial MMF, Arosurf® MSF, was produced by Sherex Chemical Company, Dublin, Ohio, USA. This product was designated ISA-20E prior to registration and appears in the scientific literature as ISA-20E, Arosurf® 66-E2, and Arosurf® MSF. The active ingredient in Agnique is exactly the same as Arosurf. However, Arosurf contained significant amounts of polyethylene glycol that could result in jellification of the product when mixed with water (Nayar and Ali 2003). A new manufacturing process used to produce Agnique removed the polyethylene glycol (Nayar and Ali 2003).

The feasibility of using MMFs for control of mosquitoes was first discussed in the scientific literature by Lorenzen and Meinke (1968) and then by Garrett (1976). Early research projects with actual MMFs or related products such as lecithin monolayers were conducted by Garrett and White (1977), White and Garrett (1977), Reiter (1978, 1980) and Reiter and McMullen (1978).

A review article on the use of MMFs as control agents of mosquitoes was published by Nayar and Ali (2003). In this article, 26 references are discussed and the authors cover general safety and use of MMFs, their mode of action, laboratory and field studies, and effects on nontarget organisms. The general conclusions of this paper are that MMFs can be safely used for mosquito control in a wide variety of habitats, including freshwater and saltwater marshes, pastures, ditches, sewage treatment plants, vats, storm sewers, dairy waste ponds and tree holes. Furthermore, MMFs can be applied with other larvacides such as diflubenzuron, S-methoprene, Bti and *B. sphaericus*. However, the authors caution that MMFs should only be used in areas where these products can remain undisturbed, particularly by high winds.

3. Monomolecular surface films

Agnique is an ethoxylated alcohol non-ionic surfactants made from plant oils. The physical/chemical properties of Agnique, a commercially produced MMF for mosquito control are listed in Table 1. Ethoxylated alcohols have been used in detergents and cosmetics for over 20 years. When applied to water, MMFs spread over the surface making an invisible layer that can be one molecule thick. MMFs unlike petroleum oils cannot be detected visually and thus an indicator oil must be used to determine the presence of the chemical on water surfaces. Petroleum oils work in much the same way as do MMFs. However, petroleum oils must be applied at higher rates (sometimes 10 times higher) than MMFs and are toxic to certain fish species and other nontarget organisms. Additionally, petroleum oils are much less expensive than MMFs on a weight basis, however this difference is often negated by the lower application rates required for MMFs.

TABLE 1: Physical properties of Agnique[1]

Appearance	Clear, viscous, light straw coloured liquid, mild odour
Chemical Name	Poly (oxy-1,2-ethanediyl), α-Isooctadecyl-ω-hydroxyl
CAS Number	52292-17-8
Water Solubility	Insoluble
Specific Gravity	0.906
Melting Point	-7 to –3°C
Vapour Pressure	Not Determined
Boiling Point	320°C

[1] Agnique Material Safety Data Sheet (Cognis Corporation 2005).

3.1 Chemistry and manufacturing of MMFs and Agnique

As previously mentioned, MMFs are alcohol ethoxylates (AE), a class of non-ionic surfactants that are developed by adding ethylene oxide to an organic alcohol, usually in the presence of a catalyst. AEs are the largest class of nonionic surfactants produced in the United States. In 1988, 632 million pounds of linear alcohol ethoxylates were produced in the United States. AEs are variously used in laundry detergent formulations, household and industrial cleaners, cosmetics, agricultural formulations, textiles and the paper and pulp industry. The environmental and human safety of AEs has been reviewed by Talmage (1993).

Agnique is developed by reacting isostearyl alcohol with ethylene oxide to yield isostearyl alcohol 2EO. Agnique is further treated by Cognis Corporation to remove polyethylene glycols that are a normal reaction product of this chemistry.

3.2 Toxicity

Poly (oxy-1,2-ethanediyl), α-Isooctadecyl-ω-hydroxyl, the active ingredient of Agnique and Arosurf is not a mutagen and is virtually non-toxic to mammals, birds, and fish (Table 2). The only effect in mammals is mild eye irritation, which is reversible.

Table 2. Toxicity of Arosurf/Agnique

Acute Toxicity	
Rat (oral LD50) [1,2]	> 20,000 mg/kg
Rabbit (dermal LD50) [2]	> 2,000 mg/kg
Rat (inhalation LC50) [1,2]	> 29 mg/l
Mallard duck (oral LD50) [2]	> 2,000 mg/kg
Bobwhite quail (dietary LC50) [2]	> 5,000 mg/kg
Bluegill sunfish (LC50) [2]	290 mg/l
Rainbow trout (LC50) [2]	98 mg/l
Daphnia (LC50) [1]	1.9 mg/l
Daphnia (EC50) [2]	51 mg/l
Rabbit (eye irritation) [1]	not an eye irritant
Rabbit (eye irritation) [2]	mild eye irritant
Human (human patch test) [1,2]	not a skin irritant
Chronic Toxicity	
Dog (2-year feeding study) [1]	NOEL = 1,250 mg/kg (mortality)
Monkey (10 month feeding study) [1]	NOEL = 1,000 mg/day (mortality)
Mutagenicity	
Salmonella microsomal	
Ames mutagencity assay [1]	negative

[1] data from Arosurf MSF Chemical fact sheet (Sherex Chemical Corporation 1984)
[2] data from Agnique Material Safety data sheet (Cognis Corporation 2005)

It is not clear from the data presented in Table 2 what the units presented in the dog and monkey study mean. Data generated from long-term feeding studies would usually be presented as mg active ingredient/body weight (kg)/day. These data were developed by Sherex Chemical Company and this company no longer exists.

The United States Environmental Protection Agency has approved the label which refers to the use of Agnique in potable (drinking) water. This indicates that Agnique is particularly safe for mammals.

3.3 Sublethal effects

No reports of sublethal effects of MMFs for any organism have been published. Because MMFs kill by physical means (suffocation) and are not chemical toxicants, sublethal effects are unlikely to be manifested in exposed organisms.

4. Monomolecular surface film products

Only two MMFs, Arosurf and Agnique have been commercialized for mosquito control.

4.1 Arosurf® MSF

Arosurf was the first commercial MMF product. Arosurf was originally produced by Sherex Chemical Company, Inc. Dublin, Ohio, USA, but this company no longer exists. Arosurf is now produced by Goldschmidt Chemical Corporation, Hopewell, Virginia, USA. The active ingredient in Arosurf is poly (oxy-1,2-ethanediyl), α-Isooctadecyl-ω-hydroxyl, an ethoxylated alcohol surfactant that is made from plant oils.

4.2 Aqnique

Agnique (EPA Reg. No. 53263-28, EPA Est. No. 53263-SC-01), is a MMF produced by Cognis Corporation, Cincinnati, Ohio, USA. The active ingredient in Agnique® MMF is the same as Arosurf, poly (oxy-1,2-ethanediyl), α-Isooctadecyl-ω-hydroxyl.

4.3 Application rates

MMFs are applied at rates 2-10 litres/hectare for mosquito control in fresh, brackish, and polluted waters (Cognis Corporation Agnique MMF Specimen Label 2005). Rates of 5-10 litres/hectare are recommended for control of midges in fresh, brackish and polluted waters.

4.4 Application methods

MMFs are generally applied by conventional methods such as backpack or vehicle-mounted sprayers or by aircraft. However, other methods of application and formulations have been evaluated such as injection systems, continuous drip methods and slow-release matrices. These methods and formulations are discussed in more detail in section 8.2.

5. Activity of monomolecular surface films

5.1 Mode of action and effect of Agnique applications

Agnique is an MMF that kills by a physical mode of action. Organisms that must make contact with the air-water interface to breath, such as mosquitoes, midges, and other aquatic insects may be susceptible. For example, larval and pupal mosquitoes use the surface tension of water to suspend for long periods of time when breathing and/or resting. MMFs kill by reducing the surface tension making it difficult for mosquito larvae and pupae to attach their siphon tubes to the water surface to breath. Wetting of trachael structures may also occur causing anoxia. The result is suffocation of the immature stages of mosquitoes. Additionally, adult mosquitoes that try to emerge from the pupal stage at the air-water interface become wetted with the MMF and drown. Adult mosquitoes that land on the surface of water to lay eggs may also drown. Unlike some of the more selective pesticides, such as S-methoprene and Bti, MMFs are not selective and kill all mosquito species and chironomid midge species that contact the air-water interface. Larvae of the following mosquitoes are not very susceptible to MMFs because they have little or no surface contact; *Coquillettidia*, *Culex pilosis*, *Culex erraticus* and *Manosonia*. Adults of these species may be susceptible when laying eggs or eclosing. *Coquillettidia* spp. can transmit Eastern Equine Encephalitis and West Nile Virus, while *Cx. erraticus* and *Mansonia tittilans* have been shown to transmit West Nile Virus.

Some species are more susceptible than others and certain instars are in general more susceptible than others. The fourth instar and the pupal stage appear to be the most susceptible stages to MMFs (Levy et al. 1986a, Das et al. 1986).

5.2 Developmental stages affected

Levy et al. (1980) have shown that all immature stages of mosquitoes are susceptible to MMFs at least to some degree. Several studies have indicated that fourth instars and pupae are more susceptible to MMFs than other immature stages of mosquitoes and that the pupal stage is the most susceptible (Mulla 1983, Das et al. 1986, Levy et al. 1986b, Perich et al. 1988). However, Levy et al. (1986a) found that second instars of *Culex quinquefasciatus* were more susceptible than fourth instars to Arosurf.

6.0 Studies with pest species

Mosquitoes and midges are the primary pest species controlled with MMFs because many species within these groups make contact with the air-water interface at some stage of their life cycle. Rates of 2-10 litres/hectare are often effective for control of many mosquito species and control usually lasts for at least 48 hours after application at low recommended dose rates.

6.1 Laboratory efficacy studies with mosquitoes

In one of the earliest research studies on the effects of MMFs on mosquitoes, White and Garrett (1977) evaluated the efficacy of MMFs as controls of *Anopheles* and *Aedes* species. Four MMFs, diethylene glycol monolaurate, sorbitan monooleate (75% + 25% 2-ethyl butane), sorbitan monooleate (37.5% + 50% lauryl ether and 12.5% ethyl butanol), and isosteryl alcohol containing 2 oxyethylene groups (ISA-20E) were evaluated in this study. Fourth instars of *Anopheles quadrimaculatus* and *Aedes taeniorhynchus* (now known as *Ochlerotatus taeniorhynchus*) were exposed to a concentration of 40 ul/m^2. The most effective product for control of *An. quadrimaculatus* was ISA-20E (100% control), followed by sorbitan monooleate (75% + 25% 2-ethyl butane) (approximately 82% control) 72 hours after treatment. None of these MMFs were effective controls for *Oc. taeniorhynchus* at the concentration tested. The highest level of control for this species was 36% with ISA-20E.

Levy et al. (1982a) conducted a series of laboratory and field studies to test the efficacy of ISA-20E against mosquitoes. The laboratory studies are discussed here and the field studies are discussed below in section 6.2. Three laboratory studies were conducted, the first to evaluate the efficacy of ISA-20E against larvae and pupae of *Culex quinquefasciatus*, the second to study the effects of treating egg rafts with ISA-20E on eclosion of *Cx. quinquefasciatus* larvae and the third to examine whether adult *Cx. quinquefasciatus* would drown when trying to oviposit on water treated with ISA-20E. In the first laboratory study, bioassays were conducted in 400 ml glass beakers. ISA-20E was applied at 0.25ml/m^2 and was not very effective in controlling *Cx. quinquefasciatus* larvae (instars 1-4). Mortality ranged from 13-80%, 96 hours after treatment. Pupae of *Cx. quinquefasciatus* were more susceptible, where 97-100% mortality was achieved 48 hours after treatment. The low efficacy observed in this study was attributable to the higher dissolved oxygen (3.8-4.8 ppm) in the water in the beakers relative to previous studies where dissolved oxygen ranged from 0.1-0.3 ppm (Levy et al. 1981). Highly oxygenated water makes MMFs less effective because larvae can absorb oxygen through the cuticle from the water and thus reduce contact with the surface of the water. In the second study, egg rafts were treated with 0.25 ml/m^2 ISA-20E and no effects on eclosion of *Cx. quinquefasciatus* were manifested. In the third study, caged adults were provided with untreated water and water treated with ISA-20E in oviposition dishes. Significantly greater numbers of adults drowned in the ISA-20E-treated dishes than in control dishes.

The toxicity and longevity of Arosurf against fourth instars and pupae of *Culex quinquefasciatus* was determined in the laboratory (Mulla et al. 1983). Field studies to evaluate the effects of Arosurf on *Cx. tarsalis* and *Cx. peus* in field ponds and dairy wash lagoons were also conducted. Nontarget effects in the field were also studied. Only the laboratory portion of this study is discussed in this section. Additionally, Mulla et al. (1983) explored the possibility of increasing efficacy and longevity of Arosurf by adding the film-forming substances, oleic (field study only) or nonanoic acid (lab and field study). Results of the laboratory study showed that fourth instars were susceptible to Arosurf, with an LC50 of 0.02-0.05 ul/cm^2 and an LC90 of 18.1-52.7 mg/l. Pupae were significantly more susceptible to Arosurf than fourth instars with LC50 and LC90 estimates of 0.0004-0.0008 ul/cm^2 and 0.20-0.40 mg/l, respectively. Nonanoic acid alone exhibited little or no toxicity to fourth instars or pupae and did not enhance toxicity of Arosurf under laboratory conditions.

Arosurf was evaluated as a control of *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti* in laboratory tests (Das et al. 1986). Arosurf produced high levels of mortality in all three species. Pupae were more susceptible than fourth instars. *An. stephensi* was more susceptible than the other two species. Furthermore, oviposition was disrupted when females attempted to oviposit in water treated with 0.56 ml/m^2. Females became trapped in the Arosurf film and drowned.

6.2 Field studies with MMFs for control of mosquitoes

White and Garrett (1977) evaluated the efficacy of four MMFs, diethylene glycol monolaurate, sorbitan monooleate (75% + 25% 2-ethyl butane), sorbitan monooleate (37.5% + 50% lauryl ether and 12.5% ethyl butanol), and isosteryl alcohol containing two oxyethylene groups (ISA-20E) as controls of *Anopheles quadrimaculatus* in artificial pools created in a woodland environment. ISA-20E and sorbitan monooleate (75% + 25% 2-ethyl butane) were further tested in large swimming pools. Applications of ISA-20E and sorbitan monooleate (75% + 25% 2-ethyl butane) resulted in 100% control of *An. quadrimaculatus* in the artificial pools and swimming pools. The other two products caused 71-87% mortality in the artificial pools.

Levy et al. (1980) evaluated three MMFs for control of *Culex* species in sewage treatment systems in southwestern Florida, USA. The first product tested was isosteryl alcohol containing two oxyethylene groups (ISA-20E). Two other products were also tested, sorbitan monooleate (75%) formulated with 2-ethyl butanol (25%) (SMO-75/2EB) and sorbitan monooleate formulated with 2-propanol (SMO-75/2P). Detection of the MMFs after application to water was made with an indicator oil made of a refined grade of oleyl alcohol (9-octadecen-1-o1, cis isomer). Several concentrations of these MMFs were applied to five settling, polishing and/or evaporation ponds at an industrial sewage treatment plant that had high populations of *Culex nigripalpus* and *Cx. quinquefasciatus*. Levels of control 24 hours after application varied from 0 to 96.9% and after 48 hours varied from 75.3 to 99.6%. Control was influenced by dosage, type of MMF, and sewage pond type (settling versus polishing versus evaporation ponds). However, most applications provided between 75 and 99% control 48 hours after treatment. For example, application rates of 0.55 and 0.71 ml SMO-75/2EB per m^2 resulted in 84.8 and 96.9% control, respectively of larvae and pupae of both mosquito species. Application of ISA-20E at 0.44ml/m^2 resulted in 90.1-94.4% control 24 hours after treatment. All larval instars (1-4) and the pupal stage were susceptible to the MMFs evaluated in this study. In an additional test with ISA-20E (0.56ml/m^2) in a sewage settling pond, control was lower than expected due to pocketing of larvae and pupae in dense mats of floating debris, scum and vegetation. All three MMFs used in this study could not be detected with the indicator oil 48 hours after application.

The efficacy of ground- and aerially-applied MMFs for control of *Ochlerotatus taeniorhynchus* (formerly known as *Aedes taeniorhynchus*) in saltmarsh habitats in southwestern Florida, USA was evaluated by Levy et al. (1981). The MMF, ISA-20E described in the prior paragraph was also used in this study. Surface dosages of 0.30-0.45 ml/m^2 provided high levels of control under a wide range of field conditions. Reduction of mosquito larvae and pupae ranged from 49.3 to 100% depending upon the habitat treated and the time interval of evaluation, but control was usually above 90%.

Three field studies to test the efficacy of ISA-20E to various mosquito species in southern Florida, USA, were conducted (Levy et al. (1982a). The first study was conducted in a sewage treatment plant for control *of Culex* spp., the second in fresh/brackish water habitats and the third for control of *Psorophora* sp. in semi-permanent habitats (roadside ditches, drainage swales). In the first field study, four test sites in a sewage treatment plant were evaluated each receiving one of four concentrations of ISA-20E (0.25, 0.30, 0.33 and 0.45 ml/m^2). Control of mixed populations of *Cx. quinquefasciatus* and *Cx. nigripalpus* ranged from 2.6-98.4%. However, control was greater than 90% in three out of four tests. The low level of control in one of the tests was attributed to large areas of water covered by algae and high winds. This site was retreated when the algal population had declined and a high level of control resulted. In the second field trial, ISA-20E applications resulted in 68-100% control of *Cx. nigripalpus* in fresh and brackish water habitats. The final field trail showed that *Psorophora* sp. was quite susceptible to ISA-20E with 93-100% control occurring 72 hours after treatment.

The efficacy of Arosurf 66-E2 and the film-forming substances, oleic or nonanoic acid against *Cx. tarsalis* and *Cx. peus* in field ponds and dairy wash lagoons was evaluated by Mulla et al. (1983). In the field, Arosurf applied at 4.67-7.01 litres/hectare yielded 80-99% control of *Cx.*

tarsalis seven days after application. Oleic and nonanoic acid increased efficacy slightly and increased longevity of Arosurf in the field.

Mariappan et al. (1984) evaluated the efficacy of the MFF, Monox CI-FCM, for control of *Culex quinquefasciatus* in water tanks and pits in urban areas in India. Monox CI-FCM is described as a 15% w/v suspension of insoluble surfactant in water supplied by CI Insect Control Ltd., England. Applications of a 1% solution of this MMF resulting in a dose of 1.89 litres/m^2 produced high levels of inhibition of adult emergence. The effect was more prolonged in water tanks than in pits.

Small-scale field trials were conducted in Kenya to evaluate the effectiveness of Arosurf MSF as a control for *Anopheles arabiensis* in irrigated rice growing areas (Karanja et al. 1994). Arosurf was applied at a rate of 4 litres/hectare every 14 days for two months. Larvae of *An. arabiensis* were continuously present in the field during the study, but adult emergence was significantly reduced in Arosurf-treated plots compared to control plots due to high mortality of fourth instars.

Field tests of Arosurf for control of *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti* were conduced in India (Das et al. 1986). Arosurf applied at a rate of 11.2 litres/hectare provided effective control from 2-11 weeks after treatment in various breeding habitats.

Arosurf MSF was applied at 4.67 litres/hectare to a cattail marsh in Massachusetts, USA to evaluate control of *Coquillettidia perturbans* (Kenny and Ruber 1992). One helicopter application was made followed by three fixed wing applications. The helicopter application resulted in almost complete control for one week after treatment. However, high variation in data collected after the fixed wing applications resulted in no significant differences between treated and untreated sites.

6.3 Efficacy comparisons for mosquito control

Three laboratory studies were conducted to compare the efficacy of Arosurf MSF with diesel oil No. 2, diesel oil and isopropanol (1:1), and Abate (temephos) and/or combinations of these products for control of fourth instar *Culex quinquefasciatus* larvae (Levy et al. 1984a). In the first study, Arosurf was compared to diesel oil and the combination of both products. Only 0-10% mortality was achieved by diesel alone (9.63 litres/hectare). Arosurf MSF alone at a rate of 2.43 litres/hectare caused 36.7-73.3% mortality while the combination of both products (diesel, 9.63 litres/hectare and Arosurf, (2.43 litres/hectare) resulted in 96.7% mortality 72 hours after treatments were applied. In the second study, Arosurf was compared to diesel-isopropanol and the combination of both products. Exposure of *C. quinquefasciatus* to Arosurf (2.43 litres/hectare) alone resulted in only 23.3% mortality 48 hours after treatment. The diesel-isopropanol (9.63 litres/hectare) combination caused no mortality while the combination of Arosurf and diesel-isopropanol resulted in 100% mortality after 48 hours. In the third study, Arosurf was compared to Abate. Arosurf (2.43 litres/hectare) alone caused 83.3% mortality, Abate (0.07 litres/hectare) alone resulted in 53.3% mortality while the two products applied together caused 100% mortality after 48 hours.

The efficacy of Arosurf and three commercial Bti products for control of larvae and pupae of *Culex quinquefasciatus* was investigated by Levy et al. (1984b). Results indicated that applications of Arosurf and Bti together provided better mosquito control than either product alone. Arosurf (2.43 litres/hectare) alone produced 67-83% mortality while Bti (1.17 litres/hectare) alone produced 37-50% mortality 48 hours after treatment. The application of Arosurf and Bti together killed 100% of the larvae and pupae within the same time frame.

Blends of Arosurf, diesel No. 2, and alcohol were tested as controls for *Culex quinquefasciatus* in laboratory tests (Levy et al. 1984d). Applications of diesel No. 2 with Arosurf resulted in higher levels of mortality than either product alone 24 hours after treatment. Alcohol-diesel blends with Arosurf also resulted in high levels of mortality of fourth instar *Cx. quinquefasciatus*.

Levy et al. (1986a) compared the toxicity of Arosurf MSF and *Bacillus sphaericus* to larvae and pupae of *Culex quinquefasciatus* in the laboratory. Forty-eight hours after treatment, Arosurf applied at a rate of 2.43 litres/hectare caused 83.3 and 73.3% mortality of second and fourth instars, respectively. Treatment with 2.43 litres/hectare of *B. sphaericus* resulted in 100 and 33.3% mortality of second and fourth instars, respectively. Application of Arosurf and *B. sphaericus* together at 2.43 litres/hectare resulted in 100% mortality of both second and fourth instars. There was no emergence of adults from surviving pupae over the course of this study.

The toxicity of Arosurf MSF and three formulations of Bti (Teknar, Bactimos and ABG-6193) alone or applied together as Arosurf + Teknar, Arosurf + Bactimos and Arosurf + ABG-6193 was determined for *Anopheles albimanus* in laboratory bioassays (Perich et al. 1987). Arosurf was the least effective product against first-third instar larvae but equally effective as the Bti formulations for controlling the pupal stage. Arosurf and the various Bti formulations applied together were very effective at controlling all stages of *An. albimanus* resulting in 100% mortality 72 hours after treatment. Arosurf was also effective at reducing egg eclosion. Eclosion was reduced 75% by Arosurf applied with the Bti formulations. However, Bti alone only reduced eclosion by 5-8%.

A laboratory evaluation to compare the efficacy of Duplex, a product containing both methoprene and Bti, Teknar (Bti) and Arosurf MSF combined with Teknar as controls for *Anopheles albimanus* and *An. stephensi* was conducted by Perich et al. (1988). High levels of control (usually 100%) with each product alone or Arosurf and Duplex applied together were obtained for all larval instars except late fourth instars which were not as susceptible (40-100% control). Pupae were most susceptible to the Arosurf-Teknar mixture. The shortcoming of this study however, was that Arosurf was not tested alone for comparison.

Arosurf MSF, Teknar (Bti), Arosurf and Teknar applied together, and SAN-809-I (a methoprene-Bti combination product) were evaluated in the field for control of *Anopheles albimanus* in Honduras (Perich et al 1990). All of the treatments reduced the mean number of larvae to zero within 48 hours after application. Control lasted for at least 10 days under field conditions.

6.4 MMFs for control of midges

Although midges are listed on the specimen label of Agnique MMF as a species that this product will control, only one study in the peer-review literature has been published on this subject.

Agnique was evaluated as a control for chironomid midges in man-made ponds in Florida (Ali 2000). Adult midge emergence was not significantly reduced by applications of 0.23 ml/m^2, but was significantly reduced (73-93%) for 1-2 weeks after treatment with 0.47 and 0.94 ml/m^2 Agnique.

7. Effects on nontarget organisms

The vast majority of studies indicate that MMFs have little effect on nontarget organisms. The only species that may be vulnerable are those that make contact with the air-water interface to breath or live on the water surface. However, even though a fairly large number of nontarget species have been evaluated, there is still a lack of information concerning the potential long-term impacts of repeated applications to wetlands. In particular, studies should be conducted that monitor arthropod biomass as well as bird and fish populations over several years. A number of bird species live in aquatic habitats in New Zealand including the rare species, the New Zealand dabchick and species unique to New Zealand such as the New Zealand scaup (Lindsey and Morris 2003). One could speculate that bird species that live in aquatic habitats may be susceptible to MMFs if the product contacts feathers. This could result in wetting of feathers leading to a reduction in the ability to float on water surfaces, to fly and negatively affecting thermoregulation.

Semi-field studies were conducted by White and Garrett (1977) to evaluate the efficacy of four MMFs, diethylene glycol monolaurate, sorbitan monooleate (75% + 25% 2-ethyl butane), sorbitan monooleate (37.5% + 50% lauryl ether and 12.5% ethyl butanol), and isosteryl alcohol containing two oxyethylene groups (ISA-2OE) as controls of *Anopheles quadrimaculatus* in artificial pools created in a woodland environment. Casual observations of the effects of these MMFs on water striders (Gerridae) and diving beetles (Dytiscidae) were also noted. No effects were observed in Dytiscidae, but Gerridae were seen to sink below the surface after contacting the MMFs.

As part of their study on the efficacy of MMFs for control of *Ochlerotatus taeniorhynchus* in saltmarshes in southwestern Florida, USA, Levy et al. (1981) made some observations on the effects of MMFs on several nontarget species. Dragonflies were observed ovipositing in water treated with ISA-20E with no apparent effect. Mosquito fish, *Gambusia* sp. were observed eating large lenses of ISA-20E with no apparent adverse effect. The planarian, *Dugesia dorotocephala* and a nematode that attacks mosquitoes, *Romanomermis culicivorax* were not adversely affected by ISA-20E applied at surface dosages of 0.4-0.5 ml/m^2.

In another study, Levy et al. (1982b) exposed the mosquito fish, *Gambusia affinis* and an aquatic snail, *Gyraulus* sp. to Arosurf 66-E2. Surface dosages of 0.5 and 1.0 ml/m^2 water surface were applied to outdoor concrete tanks. These dosages were higher than the recommended dosages for mosquito control in the State of Florida. Arosurf was reapplied to the tanks every 7-10 days. After 105 days, population numbers were recorded and no significant difference in the number of fish was detected. No differences in weight between treated and control fish was found. There were significantly higher numbers of snails in the Arosurf-treated tanks compared to the control tanks.

Mulla et al. (1983) studied the effects of Arosurf on several nontarget organisms. Mayflies, diving beetles and ostracods were not negatively affected after exposure to Arosurf applied at rates of 4.67-9.35 litres/hectare.

Unlike the majority of published studies that show no effects on nontarget species, Takahashi et al. (1984) conducted field studies with Arosurf at rates of 0.25, 0.5 and 1.0 ml/m^2 and found that this product killed hemipterans, Corixidae (*Corisella* spp.), Notonectidae (*Notonecta unifasciata*), clam shrimp (*Eulimnadia* sp.) and beetle adults, *Tropisternus lateralis*. All of these species with the exception of clam shrimp, have a ventral plastron for breathing or must make contact with the air-water interface to breath. The authors suggested that breathing may be disrupted in these species when they come in contact with MMFs. Takahashi et al. (1984) also found that mayfly naiads (*Callibaetis* spp.), chironomid larvae, and copepods were unaffected by Arosurf.

A study was conducted to determine the effects of Arosurf 66-E2 (ISA-20E) on several nontarget species (Webber and Cochran 1984). The effects of Arosurf on the freshwater green tree frog, *Hyla cinerea* (Hylidae), two fresh water fish species, *Hypostomus plecostomus* (Loricariidae) and *Gambusia affinis* (Poeciliidae) and five saline fish species, *Fundulus confluentus* (Cyprinodontidae), *Fundulus grandis* (Cyprinodontidae), *Cyprinodon variegatus* (Cyprinodontidae), *Poecilia latipinna* (Poeciliidae), and *Dormitator maculatus* (Eleotridae) were recorded. Each species was continuously exposed to Arosurf at a dosage of 0.68 ml/m^2 for six months. No detrimental effects were observed in any of these species. All species developed normally with no mortality. However, the flaw in this study was that only 3-5 individuals were evaluated for each fish species.

The effects of Arosurf on five nontarget aquatic plants were studied by Hester et al. (1989). Arosurf was applied once at a rate of 0.94 ml/m^2 to several fresh and saltwater habitats in Florida. Leaf length and new leaf addition was measured in black mangrove (*Avicenna germinan*), saltwort (*Batis martima*), cordgrass (*Spartina alterniflora*), arrowhead (*Sagittaria* sp.) and commercially grown rice (*Oryza sativa*) after exposure. No significant effects on plant growth or leaf addition were found in this study.

The effect of Arosurf on several nontarget organisms was examined in laboratory studies (Hester et al. 1991). Ninety-six hour static toxicity testes were conducted with longnose killifish (*Fundulus similus*), grass shrimp (*Palaemonetes pugio*), freshwater shrimp (*Palaemonetes paludosus*), fiddler crab *Uca* sp.), crayfish (*Procambarus* sp.), freshwater amphipod (*Gammarus* sp.), freshwater isopod (*Asellus* sp.), fairy shrimp (*Streptocephalus seali*), snail (*Physa* sp.), polychaete (*Laeonereis culveri*) and an unidentified amphipod. These species were exposed to Arosurf at a rate of 47 ml/m^2 and no acute toxicity was observed in any of the species after 96 hours.

Zooplankton was censused after application of Arosurf to a marsh in Massachusetts, USA to control *Coquillettidia perturbans* (Kenny and Ruber (1992). No population changes were detected between treated and untreated areas.

In another study by Kenny and Ruber (1993), the effects of Arosurf on microcrustacea associated with the cattail mosquito, *Coquillettidia perturbans* were assessed. Arosurf was applied by aircraft to a cattail marsh habitat at a rate of 4.67 litres/hectare. Populations of copepods and cladocerans (*Daphnia*) were monitored on four dates each during 1989 and 1990. No significant reduction in numbers of either group of crustaceans was detected.

The effects of Arosurf on 15 species of aquatic invertebrates were monitored during a field trial in Kenya to evaluate the effectiveness of Arosurf MSF as a control for *Anopheles arabiensis* in irrigated rice growing areas (Karanja et al. 1994). Arosurf was applied at a rate of 4 litres/hectare every 14 days for two months. None of the nontarget species was significantly reduced in Arosurf-treated plots compared to controls.

8. Persistence and activity in the environment

The persistence of MMFs can vary in the field and these products are often undetectable 48 hours after application. Cognis Corportion lists the persistence of Agnique at 5-22 days on their Specimen Label and 5-14 days on their Material Safety Data Sheet. The different persistence ranges are due to different application rates and temperatures that Cognis Corporation used to develop the persistence data. Control of mosquitoes can last as long as 10 days under certain environmental conditions. The persistence of MMFs varies depending upon application rate, microbial concentration, wind speed and direction, water temperature, water level, tidal fluctuations, and the amount of debris and vegetation floating on water surfaces.

8.1 Persistence

Levy et al (1980) monitored residues of an MMF with an indicator oil, oleyl alcohol (9-octadececen-1-ol,cis isomer, and could not detect the MMF 48 hours after application to sewage settling ponds for control of *Culex* species in southwestern Florida, USA. The inability to detect the MMF after 48 hours was due to wind blowing the product to one corner of a treatment pond. Wind velocity as low as 3.2 kilometres per hour pushed the film downwind. However, frequent changes in wind direction continually redistributed the film to other areas of the treatment ponds.

The persistence of the MMF, ISA-20E was evaluated after ground and aerial application to control *Ochlerotatus taeniorhynchus* in saltmarsh habitats in southwestern Florida, USA (Levy et al. 1981). Persistence was monitored with an indicator oil. When applied at a dosage of 0.30 ml/m^2, ISA-20E was fairly stable providing control for up to six days. However, wind gusts and rain greatly reduced persistence. Lower temperatures were found to increase persistence.

Arosurf has been reported to persist from 2-10 days (Levy et al. 1985). One way to increase the persistence of MMFs is with the use of a drip-dispensing device or commercial oiler. These devices are calibrated to continuously drip-dispense a small quantity of MMF per day. Levy et al. (1985) utilized a drip-dispenser to apply Arosurf at a rate of 0.19-0.76 litres per sewage pond per day. This continuous drip-dispenser controlled 30-100% of the first-fourth instars of *Culex* species. The level of control was dependent upon the amount of duckweed, cattails and other emergent and floating vegetation present. Additionally, wind reduced effectiveness.

While conducting field trials with Arosurf for control of several mosquito species in India, Das et al. (1986) observed that this product performed well in stagnant water, but that efficacy was greatly reduced in systems with flowing water.

Agnique applied to man-made ponds in Florida could not be detected with an indicator oil seven days after treatment (Ali 2000). Addition of Agnique to these ponds did not significantly change water pH, dissolved oxygen, conductance, or water temperature compared to untreated ponds.

8.2 Effect of formulation and application method on persistence and efficacy

In most of the field trials mentioned above, MMFs were applied by conventional methods such as backpack or vehicle-mounted sprayers or by aircraft. However, other methods of application and formulations have been evaluated such as injection systems, continuous drip methods and slow-release matrices.

Hertlein et al. (1985) and Burgess et al. (1985) evaluated a Dema injection system as a means to improve application of proper dose rates for control of mosquitoes. The system was able to meter a precise quantity of MMF at recommended rates at high pressures and volumes without the need for tank agitation.

Slow-release matrices consisting of floating, multiporous biodegradable pellets have been shown to increase the persistence of MMFs (Levy et al. 1985). However, no commercial product formulated as a slow-release matrix is being marketed today.

The efficacy of Arosurf (100% technical) was compared with Arosurf that had been mixed with water to make a final concentration of Arosurf in water of 5.2%. These two formulations were evaluated in the laboratory against first and fourth instar *Ochlerotatus taeniorhynchus* (Levy et al. (1986b). Each formulation was applied at a rate of 2.43 litres/hectare. Results of this study showed that the water-based formulation was more effective than the technical product producing 47-97% mortality in fourth instars compared to 0-63% mortality by the technical product 24 hours after treatment.

Arosurf-water mixtures have been shown to be more effective at controlling mosquitoes than Arosurf technical (Levy et al. 1986b). The effect of the quality of water used for making Arosurf-water mixtures on the efficacy of these mixtures was determined by Levy and Miller (1987). Four types of water, well, tap, distilled and water subjected to reverse osmosis were evaluated. Comparative bioassays with 2.43 litres/hectare against fourth instar *Ochlerotatus taeniorhynchus* indicated that the type of water used to make the Arosurf mixture had no effect on efficacy.

9. Resistance

Because MMFs kill by physical means, it is generally agreed that resistance cannot be developed to these products. There are no published reports documenting the development of resistance to MMFs. However, resistance can take many generations to develop and

these products have not been widely used to date. Development of behavioural resistance (avoidance) to MMFs may be possible.

10. Environmental factors affecting efficacy

Temperature has been shown to affect the efficacy and spread of MMFs over the surface of the water. MMFs work in cold temperatures, but effects are delayed when compared to applications at higher temperatures. Vegetative cover such as emergent and floating aquatic plants, algal mats and debris have been shown to greatly reduce the efficacy of MMFs. When the water surface is covered, immature mosquitoes have refuges where they do not come into contact with the MMF.

10.1 Temperature

MMFs have been shown to be more effective at warmer temperatures (Levy et al. 1981). However, MMFs do work at colder temperatures but it takes a longer time for them to work. Levy et al. (1981) found that the persistence of ISA-20E increased as temperature declined to 14°C. Unfortunately, Levy et al. (1981) never mention the initial temperature in their study. The effect of temperature on the rate of spread of Arosurf over the surface of water was determined by Levy et al. (1984c). The rate of spreading of Arosurf was evaluated at 2, 4, 5, 10 and 32°C. As temperature increased, the time to spread a set distance decreased.

10.2 Vegetative cover and debris

As mentioned in sections 6.2 and 8.1, vegetative cover and surface debris can greatly reduce the efficacy of MMFs (Levy et al. 1980, Levy et al. 1982a, Levy et al. 1985). Treatment of water bodies with surface and emergent vegetation, algal mats and/or debris results in areas that are essentially untreated and serve as protected sanctuaries for mosquitoes.

11. Discussion and conclusions

Agnique is a reasonably effective mosquito control agent, but there are limitations to its use. High dissolved oxygen levels can reduce the efficacy of MMFs. MMFs are not very effective in windy conditions or where emergent and floating vegetation, algae and debris are present. Sustained winds >16 km/hour can blow these products into one corner of a body of water while debris and vegetation can act as untreated shelters where mosquitoes are not exposed to the MMF. Additionally, mosquitoes that are present under vegetation and debris can wait-out the residue of an MMF because these products breakdown quickly. MMFs are most effective when applied to standing water in containers or small, stagnant, sheltered bodies of water such as ditches, puddles, and temporary pools. MMFs perform better when a large proportion of the immature mosquito population has reached the fourth instar and/or pupal stages. MMFs appear to be relatively safe for mammals, fish, birds, amphibians, many arthropod groups and plants. However, in many of the laboratory studies dealing with nontarget effects, low numbers of organisms were

evaluated over very short periods of time. No long-term environmental studies have been conducted where MMFs have been repeatedly applied to wetlands and nontarget organisms monitored. Therefore, the long-term impact on ecosystems after repeated application of MMFs is unknown. Much of the data on MMFs is very old and there are obvious data gaps. For example, the units presented in the toxicology data developed for dogs and monkeys is confusing and needs to be clarified. Because S-methoprene and Bti have proven to be such effective mosquito control agents that are relatively safe to the environment compared to other insecticides, they should be considered first for mosquito control and/or eradication. However, Agnique, if used under appropriate environmental conditions can be a valuable addition to the other control agents available for mosquito control. Agnique may be very useful for localised control of recently arrived or established mosquito species in the immediate vicinity of ports in areas where water may collect. The use of Agnique in combination with Bti and/or S-methoprene may also be a useful approach to mosquito control in certain circumstances.

12. Acknowledgements

I appreciate the help of E.R. Frampton, Critique Limited, Christchurch, New Zealand, with this project. I thank Dean Oester, Cognis Corporation, Cincinnati, Ohio, USA for providing information on Agnique and reviewing this document.

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