THE BIOLOGICAL ACTIVITY OF AMINIMIDES ON MOSQUITO LARVAE AND PUPAE AND OTHER ARTHROPODS

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ABSTRACT

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By

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Aminimides or ammonio-amidates having the functional group, $R_1-\overset{O}{\overset{\|}{C}}-N^{-}-\overset{+}{N}-R_2$, have certain wetting and emulsifying properties which render them as good antibacterial agents. Because of their structural similarity to aliphatic fatty acids, amines, and amides which have antibacterial and insecticidal activity, their insecticidal activity has been investigated in this thesis.

Of all the aminimides tested, all were found to be ineffective against all terrestrial arthropods, and the pupae of Aedes aegypti when topically applied. Aminimides were most active against larvae and pupae of Culex pipiens and Aedes aegypti when tested in water, and activity was greatest when either acyl or alkyl substituent was C_{14} or C_{16}. Of the effective aminimides, the ranking of their biological activity was strongly correlated to the degree which they reduced the surface tension of water. Since the aminimides as a class of compounds have low mammalian toxicity and are effective

against mosquito larvae and pupae, it is considered that they are as worthy of attention as the amines which have been under investigation as a new group of mosquito larvicides.

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INTRODUCTION

Since the widespread use of second-generation insecticides, principally the organochlorines, organophosphates and carbamates, widespread resistance has been developed by a large number of insect pests (Brown, 1971). Many of these insect species have acquired resistance to the control regime in a matter of 3 years. This makes it likely that the usefulness of any new analogs of the already developed insecticides will be of limited value.

In order to maintain control of various insect pests, insecticides having new and different modes of toxic action and spectra of activity are urgently required. Aminimides or ammonio-amidates represent a relatively new functional group, $R_1 - \overset{O}{\overset{\|}{C}} - \overset{-}{N} - \overset{+}{N}(\,) - R_2$, of biologically active compounds. Because of their wetting properties and emulsifying characteristics this group has been investigated and found to have high antimicrobial activity by Kabara et al. (1975a). Similar compounds (fatty acids, amides and amines) have high activity as bactericides, and are promising mosquito ovicides, larvicides and pupacides. The potentialities of aminimides as mosquito larvicides and pupacides is reported in this paper.

For a number of years fatty acids have been known to have antimicrobial activity. Their structural relationship to antimicrobial activity has been investigated by Kabara et al. (1972a). On insects McFarlane (1968) found that they caused deformations and inhibited growth when applied to crickets in the nymphal stage. Curtis et al. (1970) assayed them against brine shrimp and found that their toxicity varied with chain length. Saxena and Thorsteinson (1971) tested fatty acids against larvae of the yellow fever mosquito and found them to be toxic and to affect moulting and metamorphosis.

Amides and amines were found by Kabara et al. (1972b) to have antimicrobial activity. Mulla (1967) discovered amides to be effective pupicides against several species of mosquitos. Alexander and Beroza (1963) tested aliphatic amides of cyclic amines and found several of them to be good mosquito repellents.

Amines were evaluated against bacteria by Fuller (1942), who found their activity varied with chain length and structural groups. Kindler (1934) reported the role of amines as chemotherapeutic agents.

Primary, secondary and tertiary amines were assessed against barnacle larvae by Christie and Crisp (1966) who found several of them to have good activity. Alkyl secondary amines having C_{12} or longer were toxic

to houseflies, and the activity could be correlated to chain length (Dahm and Kearns, 1941).

Many amines were evaluated against larvae of the malaria mosquito, Anopheles quadrimaculatus, (U.S. Department of Agriculture, 1947; King, 1954) and found to be active. Mulla (1967) and Mulla et al. (1970) tested various aliphatic primary amines, secondary amines, tertiary amines, beta amines, diamines, and beta diamines against larvae and pupae of several mosquito species; good activity was found and chain length could be correlated with activity. Cline et al. (1969) and Mulla and Chaudhury (1968) tested aliphatic amines against mosquito eggs and found them to be ovicidal. Mulla et al. (1971, 1975) considered that certain amines were so promising in mosquito control that field evaluations of them were made against immature mosquitos, and certain formulations and application techniques led to complete mosquito control.

Because of aminimides antimicrobial activity and structural similarities to various fatty acids, their potential as insecticides was therefore investigated and is described in this thesis.

LITERATURE REVIEW

Fatty acids are materials that are inexpensive to manufacture and have been found to have considerable biological activity. Their general formula is R-COOH. Maximum activity against gram-positive bacteria is reached with lauric acid (C_{12}) (Kabara et al., 1972a). The dienoic derivatives of octadecanoic acid (C 18:2) were more active than the monoenoic acid (C 18:1) which was more active than the saturated acid (C_{18}). In general esterification of the polar end group (carboxyl) led to a compound that was less active. Kabara et al. (1973) found that the unsaturated octadecanoates had maximum activity when the double bonds were at the *2 and *8 positions, while for lauric acid the greater effect was when the double bond was at the *10 position.

Saturated fatty acids reach their maximum effects against insects when their chain lengths are between C_{10} and C_{13}. Saxena and Thorsteinson (1971) tested synthetic "queen substance" and analogues on 4th-instar Aedes aeggpti larvae and found that the compound (9-oxo-2-decanoic acid) had four effects, namely (1) caused rapid mortality in the treated stage caused by "acute toxicity," (2) delayed

the moulting of larvae and (3) mechanically inhibited metamorphosis as a result of nondetachment of the exuvia during the larvae-pupal or pupal-adult molt and (4) biologically inhibited the imaginal differentiation and thus caused death. Increasing the chain length above C_{13} decreased the toxicity of saturated fatty acids, but increased the toxicity of unsaturated fatty acids, with linoleic and linolenic acids being the most toxic. The presence of an oxo-group in a saturated or unsaturated C_8 to C_{10} fatty acid remote from the carboxyl group reduced the acute toxicity and increased the inhibition of metamorphosis.

McFarlane and Henneberry (1965) showed that fatty acids when applied to the cuticle of the nymphal cricket Gryllodes sigillatus (Walk) inhibited growth and reduced wing development. Myristic and lauric acid methyl esters of fatty acids were tested, and the effective ones were methyl myristate and methyl stearate. When the fatty acids were applied by addition to the diet, only lauric acid inhibited growth and then when only applied at high doses. McFarlane (1968) further studied the effects of methyl palmitate and lauric acid when applied to nymphal Gryllodes at different time-intervals. He concluded that these compounds on insects probably affected either the neuroendocrine system itself or else one of its sites of

action. He found that the effects were greatest on the younger nymphs.

Levinson and Levinson (1973) tested the lipid depressant ethyl-p-chlorophenoxyisobutyrate (ECPIB) on Dermestes maculatus. When added to the diet ECPIB suppressed fecundity and retarded growth. This activity could eradicate the population of Dermestes maculatus so treated within 2 or 3 generations. The activity of ECPIB includes phagostimulation and increased fat metabolism which can be neutralized by ingestion of oleate. When oleic and linoleic acid were topically applied to the larvae and pupae of the western spruce budworm (Choristoneura occidentalis) these agents abolished the production of glucose-6-phosphatase or N-acetylglucosamine in pupae, but had only small effects on their production in larvae. This could have possibly been caused by increased lysosome activity (Andrews and Miskus, 1972).

When fatty acids were assayed by Curtis et al. (1970) against brine shrimp larvae, it was found that the most toxic compounds had a chain length of C_{10} to C_{13}. Unsaturated fatty acids were also tested and oleic, linoleic, and linolenic were found to be the most toxic.

Ikeshoji and Mulla (1974) found that several 2-alkyl fatty acids which occurred as a result of overcrowding mosquito larvae (Ikeshoji and Mulla, 1970, 1974a,b) had mosquito larvicidal properties. Of the

compounds tested, the most active fatty acids were 2-ethyl-octadecanoic acid, 3-methyloctadecanoic, and 2, 3-dimethyl-octadecanoic acid. These chemicals never killed the larvae in the same stadium as that when they were applied, but death superceded immediately after the next ecdyis. The 2nd instar larvae, after treatment, were unmelanized in their cuticles and head capsules. When treated before pupation, the larvae drowned while pupating and their cuticle was unmelanized. Final emergence of adults was inhibited when early instar larvae were treated. These compounds were also found to reduce larval heart-beat rates and were also bacteriostatic (Ikeshoji and Mulla, 1970). These substituted fatty acids possibly affect mosquito larvae by penetrating deep into the epidermal cells where the esterases for wax synthesis are located. Abrahamson et al. (1964) stated that the rates of esterification of alkyl branched fatty acids, and the rate of hydrolysis of their esters, are hindered when they are branched at the *2 and *3 positions. Because of the steric hindrance in enzymatic esterification, the mosquito larvae are endangered by not being able to synthesize a sufficient quantity of wax and triglycerides to meet the requirements in the formation of a new cuticle. The esterified lipids resulting from branched fatty acids do not provide a good barrier against water penetration (Ikeshoji and Mulla, 1974b).

Amines ($R-NH_2$) and amides ($R-CONH_2$) having chain lengths between C_9 and C_{14} are the most active antimicrobial agents (Kabara et al., 1972b; Fuller, 1942). Amides are active against only gram-positive bacteria while amines were active against gram-negative and gram-positive bacteria. It was also found by Kabara et al. (1972b) that mono-unsaturation failed to increase activity as it does in fatty acids. Alexander and Beroza (1963) reported that aliphatic amides of cyclic amines and tolyl maleimides worked as repellents against Aedes aegypti mosquitos.

Jensen and Liu (1963) tested primary, secondary, and tertiary amines on influenza virus in tissue culture, and found that they were inhibitory to the virus much the same way as ammonia was. Silver and Kralovic (1969) tested short chained triamines on bacteria, and found that they "paralyzed" the permeability of the cell wall. It was found that treated bacterial cells leaked potassium and thiomethyl galactoside through their cell membrane. Many years ago Dale (1920) pointed out that the effects of certain amines include certain physiological symptoms characteristic of nicotine poisoning. The polyamines tested by Tabor and Tabor (1964) neutralized, stabilized or labilized the lipids and the membrane structure of ganglionic cells, which is also a property of nicotine. Dahm and Kearns (1941) tested alkyl secondary amines against adult

houseflies (Musca domestica) and found that maximum activity was achieved at chain lengths between C_{11} to C_{17}.

Several amide derivatives of compounds related to farnesenic acid were tested by Cruikshank and Palmere (1971) and were found to have various degrees of juvenile hormone activity on Tenebrio molitor. Several amines structurally related to juvenile hormone mimics were tested by Robbins et al. (1975) on a number of insect species. These compounds exhibited juvenilizing affects on the tobacco hornworm Manduca sexta (L.), including formation of precocious "fourth-instar prepupae" and abnormal pupae which appeared to be prepupae-pupa intermediates. They also blocked development in the early larvae stages, and were lethal when applied at the time of molting. Similar juvenilizing and lethal effects were exhibited on larvae and pupae of Aedes aegypti and Tribolium confusum. These compounds were found to have reduced the cholesterol level of 80 - 85% to 5% of the total tissue sterols, and increased the demosterol content from 1.0 - 0.5% to 50% (representing total sterols of this insect). The toxicity and juvenile effects were stated as being caused by inhibiting metamorphosis and the *24-sterol reductase system in larvae which are involved in the conversion of plant sterols to cholesterol.

Christie and Crisp (1966) tested aliphatic amines on larvae of the barnacle Elminius modestus and found that

primary amines of C_8 to C_{14} chain length were the most active, while secondary and tertiary amines were active up to a chain length of C_{16}. They concluded that amines act upon metazoans in a different way or at a different site of action than the majority of organic substances. From their work on barnacles, they pointed out that the amines they tested exhibit four characteristics of compounds that are physically toxic, these being (1) reversible narcosis, (2) similar toxic effect with wide variations in time of exposure, (3) little dependence on temperature, and (4) similar toxicity measured on the thermodynamic scale.

Aliphatic amines were tested by Mulla (1967) against larvae and pupae of the southern house mosquito Culex pipiens quinquefasciatus (Say) and he found that lauryl, palmityl, t-stearyl-t-behenyl, oleyl, tallow, tall oil, and coco oil primary aliphatic amines were more toxic to larvae than pupae. Monoalkyl amines were more active than dialkyl amines and secondary amines were ineffective against larvae or pupae. The tertiary amines had little activity against larvae or pupae, while the alpha diamines showed the greatest activity of any group evaluated. Primary beta-amines were more effective against larvae than pupae, while the amides tested were more toxic to pupae than larvae. The tertiary beta-diamines were more active against larvae, and the beta-diamines were most active against larvae and pupae when their chain was greater

than C_{11}. When put in amine test solutions, the larvae immediately became hyperactive and began to curl up and touch their mouthparts to the anal gills and siphon. The larvae wriggle to the surface but soon after reaching the surface they repeated their past behavior. They also cannot rest at the surface as the untreated organisms can. Peak mortality was reached more rapidly than in the case of conventional insecticides. Pupae were also rapidly affected when exposed to lethal concentrations. The pupae would often snap their abdomens without darting from one place to another. Normal pupae dart after snapping their abdomens once, but those treated with amines had to snap several times before they moved. The treated pupae also became sluggish and did not respond to moving shadows, and shortly thereafter became paralyzed and remained on the air-water interface. Adults emerging from treated solutions had abnormal wings, lost their body- and wing-scales, and many were unable to take flight. The duration of the larvae and pupae stages was also lengthened by some of these aliphatic amines.

The biodegradable cationic surface-active agents produce symptoms on mosquito larvae and pupae unlike those observed in poisoning induced by organochlorine, organophosphorus, or carbamate insecticides. Their course of gross symptomatology is similar to that produced by treatment with petroleum oils, and when tested against

organophophate-resistant mosquito larvae (Mulla et al., 1970) there was no cross-resistance. Mulla (1967) stated that lowering of the surface tension of water did not seem to be a primary factor in their biocidal activity since some good surface-active agents tested did not kill larvae and pupae at much higher rates than those at which some of the aliphatic amines were effective.

Several theories as to the mode of action of amines on mosquito larvae and pupae were advanced by Mulla (1967), namely: (1) because of the substantive properties of aliphatic amines they probably change physical, electrical, and hydrophobic-hydrophilic characteristics of the cuticle; (2) the lipophilic portion of the aliphatic amines dissolves or disrupts the epidermal layer of larvae and pupae resulting in nutrient, chemical, and water imbalance accounting for the quick-acting power of the amines; (3) the aliphatic amines may interfere with or disrupt the membrane of the anal gills, thus resulting in salt and water imbalance, or they may disrupt or change the functional integrity of tracheae; (4) there may be interference in hormonal balance or amino-acid metabolism as evidenced by the abnormal eclosion of adults, appearance of abnormal structures, and shedding of scales in the emerging adult. Mulla also stated that from gross observations of symptoms, damage and death due to physical action seemed plausible; however, the type of physical action involved seemed to be complex

and may be followed by metabolic and chemical changes resulting in death, delayed development, or morphogenetic changes in the immature stages of the mosquitos.

Soaps and emulsifying agents can affect aquatic insects in several ways. The lipophilic side chain can attach itself to the waxy epicuticle which is responsible for prevention of water migration in and out of the insect (Wigglesworth, 1965) and can cause slight cuticular disruptions, while the hydrophilic portion can provide for migration of water into the insect (Wigglesworth, 1945; Beament, 1945). With the waxy layer thus affected, the insect is rendered more permeable to both water and insecticides.

Soaps and emulsifying agents are lethal to mosquito larvae and pupae by lowering the surface tension of the air-water interface. This causes the pupae or larvae to lose attachment of their respiratory trumpets to the surface film (Christophers, 1960). Early works by Russell and Rae (1941) give the range of 27 to 36 dynes/cm as being fatal to mosquito larvae and pupae, without the time-period it took to cause this mortality. Senior-White (1943) gave his test findings on mortality in relation to concentrations of various soaps versus time constants, but did not quantify the lowering of the surface tension in dynes/cm. Manzelli (1941) notes that some of the effects of reduced surface tension on mosquito life-stages are that emerging adults

and mosquito egg rafts sink. Whereas mosquito larvae are sclerotized only in the head capsule and anal siphon, the pupae have a cuticle that is entirely heavily sclerotized (Clements, 1963). Mosquito larvae, especially early instars resist submersion by being able to respire dissolved oxygen through their cuticle and anal papillae, but submerged pupae burn up the oxygen reserve in their tissue, become heavier than water and drown (Christophers, 1960). Because of the difference between larval and pupal cuticles, pupae are much more susceptible to lowering of the surface tension of the air-water interface.

Aminimides or ammonio-amidates are a relatively new group of fatty-acid derivatives. They are surface-active agents and exist as bipolar ions. Aminimides derived from C_{12} to C_{18} possess interesting wetting properties and emulsification characteristics (McKillip et al., 1973). These compounds were tested by Kabara et al. (1975a) and Kabara and Haitsma (1975b) and found to be very active against gram-positive bacteria and yeast organisms. Their activity against gram-negative bacteria is low or absent. The authors found that the aminimides, $R_1-\overset{O}{\overset{\|}{C}}-N^{-}-\overset{+}{N}-R_2$, were most active when the acyl (R_1) or alkyl (R_2) sides had a chain length of C_{14} or C_{16}. Among the aminimides they tested, the unsaturated compounds tended to be more active than the saturated ones.

METHODS AND MATERIALS

Test Organisms

The insects used in evaluating the biological activity of aminimides were: the large milkweed bug Oncopeltus fasciatus (Dallas), the confused flour beetle Tribolium confusum, the yellow fever mosquito Aedes aegypti, the common house or bird mosquito Culex pipiens pipiens, and the common orchard or two-spotted mite Tetranychus urticae.

The milkweed bug colony employed was a compound strain: individuals from Michigan, North Carolina, and California were interbred and selected for eating sunflower seeds. The strain was raised on sunflower seeds and distilled water, and kept in gallon jars at 30°C and 60-70% relative humidity. Only 5th-instar nymphs were used for susceptibility tests.

The confused flour beetle colony was a mixed strain obtained from Dr. Robert Mills at Kansas State University consisting of stock from Sacramento (B-25), San Bernadino (B-166) and Davis (B-28) California. They were raised on a diet of ball-milled yeast and whole wheat flour. Oviposition was obtained by adding adult beetles to whole wheat flour in 1 gallon jars, and after 1 week the adults

were sifted out. When the larvae were 2 weeks old they were used for testing the activity of the compounds. They were held at 30°C and 60-70% relative humidity while being tested.

The orchard mites were obtained from a greenhouse culture of mixed origin maintained by Dr. B. A. Croft at Michigan State University. Mites were raised on young pea plants at 30°C and 80% relative humidity. Only 1- or 2-day old females of uniform age were used in tests.

The yellow fever mosquito was the Rock strain obtained from the Department of Biology, University of Notre Dame. The larvae were reared in enamel-coated pans at 30°C. Adults were raised in collapsible aluminum cages held at 30°C and 70-80% relative humidity. Larvae were fed on guineapig barley pellets and adults were maintained on raisins and 10% sugar solution. Anesthetized guinea-pigs were the source of blood meals for the Aedes aegypti mosquitos, being exposed in dimmed light. For testing, only 4th-instar larvae and pupae less than one day old were used. The mosquito eggs used were stored in mason jars containing 20 ml of distilled water to maintain a high relative humidity and were used for tests after they had been stored for 3-18 days.

The Culex pipiens colony originated from sewage ponds at Belding, Michigan in 1973. They were raised in a fashion similar to that employed on Aedes aegypti larvae

and adults, the only differences being that the larvae were fed on dry barley pellets and ground Purina Cat Chow[R] and the adults were given blood meals from chickens. Only 4th-instar larvae and less than one day-old pupae were used for testing.

Experimental Chemicals

The aminimide compounds tested here were synthesized by The Ashland Chemical Company, Dublin, Ohio. The functional group is $R_1-\overset{O}{\overset{\|}{C}}-N^- - \overset{+}{N}-R_2$. Exemplifying one of the six active acyl aminimides, the structure of M-3 is, $CH_3(CH_2)_{14}\overset{O}{\overset{\|}{C}}-N^- - \overset{+CH_3}{\overset{|}{N}} - CH_2-\overset{OH}{\overset{|}{C}}H-CH_3$. M-57 is one of the three active long chain alkyl aminimides tested, and its structure is, $H_2C=\overset{CH_3}{\overset{|}{C}} - \overset{O}{\overset{\|}{C}}-N^- - \overset{CH_3}{\overset{|}{N}} - CH_2-\overset{OH}{\overset{|}{C}}H-(CH_2)_{13}-CH_3$. One of the seven ethoxylated acyl aminimides which had a fair degree of activity is M-116, its structural formula is, $CH_3-(CH_2)_{12}-\overset{O}{\overset{\|}{C}}-N^- - \overset{CH_3}{\overset{|}{N}} - CH_2-\overset{OH}{\overset{|}{C}}H-CH_3+EO$. Ethoxylated aminimides were exposed to ethylene or propylene oxide at the molar ratios given in Appendix A. M-6, $CH_3-\emptyset-SO_2N^- - \overset{CH_3}{\overset{|}{N}} - CH_3$ was the only sulfonated aminimide tested, while M-11, $CF_3-CF_2-\overset{O}{\overset{\|}{C}}- N^-N^+(CH_3)_3$, was the only fluorinated aminimide tested. Several quaternary aminimides were also tested, of the ones tested M-31 had a structural formula of, $\emptyset-CH_2-\underset{Cl^-}{\overset{CH_3}{\overset{|}{N}}}-CH_3 - CH_2-\overset{O}{\overset{\|}{C}}-N^- - \overset{CH_3}{\overset{|}{N}}^+ - CH_2-\overset{OH}{\overset{|}{C}}H-(CH_2)_{13}-CH_3$. These compounds exist as bipolar ions and their chemistry has been investigated by Timpe (1972) and McKillip et al.

(1973). They possess wetting properties and emulsifying characteristics, and behave like non-ionic surfactants with relation to cloud point (Kameyama et al., 1968, 1969). They exhibit a Kraft Point phenomenon similar to that exhibited by ionic surfactants (Corkill, 1970).

The aminimides used were recrystallized 5 or 6 times and were chromatographically pure. Several of their solubility constants are listed with their structural formulae in Appendix A. Kabara and Haitsma (1975b) found that on the laboratory CF-1 mice, aminimides were toxic at 200-400 mg/kg when interperitonially injected, and 2,000-4,000 mg/kg when given orally.

Test Techniques

Fifth instar nymphs of the large milkweed bug were anesthetized with CO_2 and the test compounds were topically applied to their abdomens as a 10% solution in acetone. The application of 1 microliter containing 100 micrograms of the compound was made with an ISCO applicator and a Hamilton 250-microliter syringe fitted with a 27-gauge needle. The solvent was allowed to evaporate and then the bugs were placed in pint sealers with water and sunflower seeds. Mortality counts were taken 24 hours later. Controls consisted of bugs treated with 1 microgram of acetone.

Two-week-old larvae of the confused flour beetle were used in the effectiveness tests for this species.

The candidate compounds were stirred into 20 grams of whole wheat flour and 40 ml of methylene chloride was added. This was stirred and mixed for 2 minutes in a round-bottom flask. The solvent was removed on a Rotavapor^{R} (Buchi) distillation apparatus. The treated flour was then placed on a sheet of aluminum foil and allowed to dry overnight. Controls consisted of methylene chloride alone and malathion. The flour was divided into two vials and inoculated with 25 two-week-old larvae (into each vial) and held at 30°C for 3 weeks when the percentage mortality was estimated from the number of adults that failed to emerge.

Tests on the two-spotted mite were performed according to the ESA slide-dip method for susceptibility levels (Anonymous, 1968). Two pieces of double-sided sticky tape were attached to a glass microscope slide. A piece of filament tape was placed, sticky side up, on top of the double-sided sticky tape. Twenty young female mites of uniform age were placed on their backs using a fine camel-hair brush. The slides were dipped and stirred in the acaricide solution for 5 seconds, taken out and allowed to drain for 15 minutes by placing them on their edge. Control tests were made with a known acaricide (Fundal) and also with the solvent (acetone) alone. The treated mites were stored for 24 hours at 30°C and 80-90% relative

humidity. Mites were judged dead if on being probed by a camel-hair brush they failed to move their legs.

Effectiveness tests of the aminimides were made on Culex pipiens and Aedes aegypti mosquito larvae and pupae according to the WHO Standard Method for susceptibility testing (World Health Organization, 1970). Only early-4th-instar larvae and less than 1-day-old pupae were used in susceptibility testing. Lots of 25 larvae or pupae were placed in 50-ml beakers containing 25 ml of distilled water. Into 600-ml beakers were placed 225 ml of distilled water. At least 3 different concentrations each in duplicate were employed in each test. The solutions of the compounds were prepared to have a concentration of 100 ppm and were then serially diluted down. One ml of the compound in ethanol solution was pipetted into the 225-ml water in 600-ml beakers and stirred with a glass rod. The most dilute solutions were pipetted first and the most concentrated last. Controls were run with malathion (a known insecticide) and with the solvent (ethanol or acetone). After the compounds were pipetted they were allowed to equilibrate for 15 minutes. The mosquito larvae or pupae were added by tipping the contents of the small beakers into the large beakers. The temperature of the water during the tests was 21-23°C. After a 24-hour period, mortality counts were taken. Larvae and pupae that were scored as dead were those that failed to move if probed,

were discolored or were unable to rise to the surface or showed unnatural positions, tremors, uncoordination, rigor or failure to respond to shadows. The concentrations sought and chosen were those that gave partial mortality, and where possible three of them were used in multiples of two. If more than 10% of the larvae in the controls died or pupated, or more than 10% of the pupae tested subsequently emerged, the tests were rerun.

Pupae of Aedes aegypti were treated with aminimides by topical application, the method being similar to that used on the milkweed bug. The main difference was in the amount of solvent used. The aminimides were diluted in water instead of ethanol or acetone and were applied at a rate of 100 µg/pupae. Three replicates, each of 10 pupae were used, along with 2 control replicates. The pupae were placed on damp #2 Whatman filter paper and topically treated by means of an ISCO applicator; 15 minutes later they were placed in 100 ml of distilled water and held for 24 hours, when mortality counts were taken. All replicates were held until emergence to determine whether adult emergence was affected.

Three aminimide compounds (M-8, M-18, and M-20) were tested to determine their longer-term effects on Aedes aegypti larvae. Testing was similar to the WHO Standard Method, the difference being that the volume of water was increased to 500 ml. Four replicates using

4th-instar larvae were used on each solution. The test solutions were reinoculated every third day with fresh larvae, which were held for 24 hours before being recorded and removed. The concentration of 10 ppm was used, as being between the LC_{50} and LC_{90} of the candidate compounds.

To determine the possible after-effects on the adult mosquitos, after the treated larvae had pupated and emerged, three concentrations of M-3 were employed. These tests were similar to the WHO method but the Aedes aegypti larvae were held in the treatment solutions for up to five days. After 24 hours mortality counts were taken and survivors were held to determine the number of emergent adults.

Ovicidal tests were run according to the technique used by Cline et al. (1969) and Mulla and Chaudhury (1968). Aedes aegypti eggs were collected on #2 gauge roughened aluminum panels (9 x 2.4 cm.) taped into 400 or 600 ml beakers. Eggs used were between 3 and 18 days old. A 1% ethanolamine emulsifier was used with 0.4% of aminimide in distilled water. Controls were of 1% ethanolamine alone and a 0.4% solution of malathion. Exactly 2.5 mls. of solution were used per replicate and over 300 eggs per replicate were employed. Two replicates were used for each compound tested or control. A Potter Spray Tower was used to apply the compounds. The solutions were sprayed on the aluminum panels to the point of runoff (25 mls.) and then placed in beakers where they were stored for 24 hours at

29°C and at 60-70% relative humidity. A hatching medium of larval water was used to stimulate hatching and after 24 hours in this medium at 28°C hatch counts were recorded. The panels were removed from the hatching medium and allowed to dry before being counted. A WILD Heerburg stereoscope microscope (6-50X) was used to count the mosquito eggs. Intact eggs and partially emerged larvae were scored as dead eggs.

Aminimides were tested for their effect on adult Aedes aegypti by dissolving them in oil and impregnating them into filter paper. Solutions were made using the test chemicals and a 5:1 mixture of chloroform and olive oil, applied to Whatman #1 filter papers by means of a needle and syringe and allowed to dry overnight in a ventilated hood. The treated filter paper was fitted into a plexiglass cylinder which had a screen over one end and a removable slide at the other. The adult female Aedes to be used in the test were first aspirated into a holding cylinder where they were counted, and then lots of 25 were placed in each cylinder (2 replicates per solution per concentration) and held for 24 hours at 28°C and 80% relative humidity before mortality counts were taken. Malathion solution, and the olive oil-chloroform solvent alone, were used as controls.

Aminimides were tested as synergists for carbaryl against 4th-instar Culex pipiens larvae according to the

WHO method, and the solutions being made up in a ratio of 1:10 (carbaryl to aminimide). Controls consisted of ethanol, carbaryl, and carbaryl plus piperonyl butoxide.

In tests to see whether the aminimides were synergized by standard synergists (piperonyl butoxide, sesamex, and triphenylphosphate) against 4th-instar Culex pipiens larvae, a 1:1 ratio (aminimide to synergists) was employed for the mixture: the controls consisted of ethanol, of the M-8 aminimide alone, and each of the three synergists alone at their highest concentration (when used in the 1:1 mixture). The aminimide M-3 was also tested against Aedes aegypti pupae with the synergists triphenyl phosphate, TOCP, and piperonyl butoxide at a ratio of 1:1 (M-3 to synergist).

The aminimide M-3 was tested against Aedes aegypti larvae at 3 different pH's by means of phosphate buffers. In addition M-3 and M-8 were tested against Aedes aegypti larvae and pupae at an elevated temperature of 28°C.

To determine whether the reduction in surface tension caused by the addition of aminimides to water was a factor in their biological activity, tests were run at different concentrations of different aminimides (of a homologous series) to determine a possible correlation between reduced surface tension and larvae or pupae mortality. The hanging-ring method on a Model #20 Fisher Tensiometer was employed. The apparent surface tension

was read from the tensiometer and the true surface tension was then determined by using a correction factor chart. Distilled water was used as a test standard.

RESULTS

Aminimides had little or no toxic effect on Oncopeltus fasciatus (Table I), Tribolium confusum (Table II), or on Tetranychus urticae (Table III).

The aminimides that had LC_{50} values of less than 40 ppm on larvae or pupae of Aedes aegypti or Culex pipiens are given in Table IV. Aminimides failing to be active on mosquito immatures are listed in Table V along with the concentrations at which they were tested. Table VI reclassifies the aminimides according to acyl or alkyl families of different chain lengths and summarizes their activities accordingly. Three of the most active aminimides tested (M-3, M-8, and M-20) were sufficiently active to kill both larvae and pupae at concentrations between 1 and 10 ppm (Table IV). With the compounds M-3, M-4, M-18, M-20, M-57, M-71, M-108, M-116, M-122, and M-124 the activity was considerably greater against the pupae than against the larvae. For both mosquito strains, M-4 had very little effect on the larvae but was somewhat toxic to the pupae. M-2, M-3, M-8, M-20, M-57, M-108, M-116, M-122, and M-124 were more effective than malathion on the pupae of Culex pipiens and M-3, M-8, M-18, M-20, M-57, M-116, and M-122 were

TABLE I. Toxicity of topically applied aminimides to *Oncopeltus fasciatus* nymphs.

Compound	Dose (µg/nymph)	% Mortality
M-4	100	0
M-8	100	0
M-18	100	0
M-20	100	0
M-71	100	0
N,N dimethyl dodecylamide	100	45
Malathion	100	100
Control (Acetone)	---	0

TABLE II. Biological activity of aminimides against *Tribolium confusum* larvae in treated flour.

Compound (1% by Weight)	% Mortality
M-4	8
M-8	6
M-18	0
M-20	2
M-71	2
N,N dimethyl dodecylamide	28
Malathion	100
Control (Methylene chloride)	0

TABLE III. Biological activity of aminimides on Tetranychus urticae using the slide dip method.

Compounds	% Mortality at 1% Concentration*
M-4	56
M-8	44
M-18	62
M-20	16
Fundal+	100
Control (acetone, water 1:1)	0

*Held for 24 hours

+Held for 48 hours

TABLE IV. Concentration-mortality of biologically active aminimides tested on larvae and pupae of Culex pipiens and Aedes aegypti mosquitos.

Compound	Concentration (ppm)	% Mortality			
		A. aegypti		C. pipiens	
		Larvae	Pupae	Larvae	Pupae
M-2	25	94	83	98	92
M-2	12.5	42	38	52	44
M-2	6.25	0	2	6	6
M-3	25	88	100	61	100
M-3	12.5	52	97	32	100
M-3	6.25	48	93	30	97
M-3	3.0	17	75	20	60
M-3	1.5	6	55	0	7
M-3	0.75	0	25	0	0
M-4	20	0	75	0	100
M-4	10	0	50	0	100
M-4	5	0	16	0	100
M-4	1.0	0	2	0	7
M-9	25	78	100	63	85
M-9	12.5	0	12	15	0
M-9	6.25	0	0	0	0
M-8	20	84	100	80	100
M-8	10	51	100	35	100
M-8	5.0	30	90	0	100
M-8	1.0	2	8	0	5

TABLE IV. (Continued)

Compound	Concentration (ppm)	% Mortality			
		A. aegypti		*C. pipiens*	
		Larvae	Pupae	Larvae	Pupae
M-18	40	100	100	85	100
M-18	20	58	100	52	100
M-18	10	15	100	18	100
M-18	5.0	0	4	3	88
M-18	1.0	0	0	0	3
M-20	20	100	100	97	100
M-20	10	70	100	58	96
M-20	5.0	2	4	12	3
M-57	25	84	100	70	100
M-57	12.5	52	100	40	100
M-57	6.25	30	100	26	100
M-57	2.5	5	100	16	100
M-57	1.25	0	53	2	100
M-57	0.625	0	2	0	0
M-71	50	100	100	68	100
M-71	25	14	18	40	12
M-71	12.5	2	5	0	3
M-108	50	90	91	70	100
M-108	12.5	20	50	44	40
M-108	5.0	3	6	3	2
M-108	1.0	0	2	2	0
M-116	50	78	100	100	100
M-116	12.5	40	80	58	90
M-116	5.0	15	35	0	35
M-116	1.0	2	0	0	0

TABLE IV. (Continued)

Compound	Concentration (ppm)	% Mortality			
		A. aegypti		*C. pipiens*	
		Larvae	Pupae	Larvae	Pupae
M-122	50	84	100	78	100
M-122	12.5	4	100	4	100
M-122	5.0	0	62	0	65
M-122	1.0	0	0	0	0
M-124	50	84	100	40	100
M-124	12.5	4	90	40	94
M-124	5.0	0	33	10	48
M-124	1.0	0	5	0	0

TABLE V. Concentration-mortality test data of biologically inactive aminimides on larvae and pupae of Culex pipiens and Aedes aegypti mosquitos.

Compound	Concentration (ppm)	% Mortality			
		A. aegypti		C. pipiens	
		Larvae	Pupae	Larvae	Pupae
M-1	100	5			
M-1	50	0			
M-1	25	0			
M-6	50			0	
M-6	25			0	
M-6	12.5			0	
M-11	50			0	
M-11	25			0	
M-11	12.5			0	
M-16	100			0	
M-16	50			0	
M-16	25			0	
M-19	100	94			
M-19	50	10			
M-19	25	2			
M-25	50			0	
M-25	25			0	
M-25	12.5			0	

TABLE V. (Continued)

Compound	Concentration (ppm)	% Mortality			
		A. aegypti		C. pipiens	
		Larvae	Pupae	Larvae	Pupae
M-31	100			66	
M-31	50			42	
M-31	25			40	
M-31	12.5			32	
M-31	6.25			18	
M-34	100	16		10	
M-34	50	0		2	
M-34	25	0		0	
M-41	50			0	
M-41	25			0	
M-41	12.5			0	
M-43	50			0	
M-43	25			0	
M-43	12.5			0	
M-46	50			0	
M-46	25			0	
M-46	12.5			0	
M-48	50			0	
M-48	25			0	
M-48	12.5			0	

TABLE V. (Continued)

Compound	Concentration (ppm)	% Mortality			
		A. aegypti		C. pipiens	
		Larvae	Pupae	Larvae	Pupae
M-54	100		0	88	
M-54	50		0	34	
M-54	25		0	16	
M-62	100		2		
M-62	50		2		
M-62	25		0		
M-65	50			0	
M-65	25			0	
M-65	12.5			0	
M-121	100	16	23		
M-121	50	12	9		
M-121	25	5	9		
M-123	100	68	68		
M-123	50	29	43		
M-123	25	20	18		
M-125	100			90	
M-125	50			48	
M-125	25			34	
M-129	100	22			
M-129	50	20			
M-129	25	16			

TABLE V. (Continued)

Compound	Concentration (ppm)	% Mortality			
		A. aegypti		C. pipiens	
		Larvae	Pupae	Larvae	Pupae
M-132	50			0	
M-132	25			0	
M-132	12.5			0	
M-133	100	12		44	
M-133	50	10		36	
M-133	25	6		14	

TABLE VI. Summary of the biological activity of acyl, alkyl, and ethoxylated aminimides on larvae and pupae of Aedes aegypti and Culex pipiens (21°C).

Compound	Chemical Description	LC_{50} (ppm) Aedes aegypti		LC_{50} (ppm) Culex pipiens	
		Larvae	Pupae	Larvae	Pupae
A. Long chain acyl aminimides					
M-1	1, 1 dimethyl (2-hydroxypropyl) amine laurimide	NA*	NA	NA	NA
M-2	1, 1 dimethyl (2-hydroxypropyl) amine myristimide	13.5	15.5	12.5	13.5
M-3	1, 1 dimethyl (2-hydroxypropyl) amine palmitimide	8.0	1.65	12.0	2.73
M-4	1, 1 dimethyl (2-hydroxypropyl) amine stearimide	NA	1.0	NA	1.6
M-34	Trimethyl amine laurimide	NA	NA	NA	NA
M-9	Trimethyl amine myristimide	21.5	17.0	37.5	31.0
M-8	Trimethyl amine palmitimide	9.0	2.3	8.5	2.35
M-133	Trimethyl amine stearimide	NA	NA	NA	NA
M-16	Dimethyl-2-hydroxyethylamine laurimide	NA	NA	NA	NA

TABLE VI. (Continued)

Compound	Chemical Description	LC_{50} (ppm) Aedes aegypti Larvae	LC_{50} (ppm) Aedes aegypti Pupae	LC_{50} (ppm) Culex pipiens Larvae	LC_{50} (ppm) Culex pipiens Pupae
M-18	Dimethyl-2-hydroxyethylamine palmitimide	18	3.4	21	2.35
M-132	Dimethyl-2-hydroxyethylamine stearimide	NA	NA	NA	NA
B. Long chain alkyl aminimides					
M-19	Dimethyl-2-hydroxydodecamine methylacrylimide	NA	NA	NA	NA
M-20	Dimethyl-2-hydroxytetradecamine methylacrylimide	8.8	6.95	9.9	6.25
M-57	Dimethyl-2-hydroxyhexadecamine methylacrylimide	10.5	1.20	20	.79
M-129	Dimethyl-2-hydroxypalmityl methylacrylimide	NA	NA	NA	NA
M-71	Dimethyl-2-hydroxymyristylamine acetimide	32.0	31.0	35.5	30.0

TABLE VI. (Continued)

Compound	Chemical Description	LC_{50} (ppm) Aedes aegypti Larvae	LC_{50} (ppm) Aedes aegypti Pupae	LC_{50} (ppm) Culex pipiens Larvae	LC_{50} (ppm) Culex pipiens Pupae
C. Long chain ethoxylated acyl aminimides					
M-108	Dimethyl-2-hydroxypropyl laurimide +5EO	21.0	12.5	15.0	12.5
M-116	Dimethyl-2-hydroxypropyl myristimide +5EO	18.0	7.2	11.5	6.5
M-120	Dimethyl-2-hydroxypropyl palmitimide +5EO	NA	NA	NA	NA
M-121	Dimethyl-2-hydroxypropyl palmitimide +10EO	NA	NA	NA	NA
M-122	Dimethyl-2-hydroxypropyl palmitimide +10PO	37	4.8	36	4.2
M-123	Dimethyl-2-hydroxypropyl palmitimide +4EO	NA	NA	NA	NA
M-124	Dimethyl-2-hydroxypropyl palmitimide +4PO	35	9.4	NA	4.8

TABLE VI. (Continued)

Compound	Chemical Description	LC_{50} (ppm) Aedes aegypti Larvae	LC_{50} (ppm) Aedes aegypti Pupae	LC_{50} (ppm) Culex pipiens Larvae	LC_{50} (ppm) Culex pipiens Pupae
D. Control Substances					
N,N-dimethyl dodecylamide		6.0	17.5	6.8	16.8
Dodecylamine		NA	NA	NA	NA
Sodium oleate		NA	NA	NA	NA
Malathion		.125	2.6	.036	19
Carbaryl		.9	8.8	.71	1.65

*NA = No Activity

more effective than carbaryl was on the pupae of Aedes aegypti. The significance of these activities is that these compounds on the whole were more toxic to the pupal stage than to the larval stage.

When treated at acute toxic levels (not less than the LC_{50}), the larvae died much more rapidly with the aminimides than with the malathion or carbaryl controls. They showed adverse symptoms within the first half hour, and after three hours 50% were dead. Their diving reactions to light were much impaired and they had a difficult time maintaining their contact with the air-water interface. White fluffy material, probably the peritrophic membrane (Abedi and Brown, 1961) was excreted through the anus when the treatment concentration was at 10 ppm or greater. The larvae would often curl over as if trying to bite off this material. One to three hours after death the larvae were slightly swollen and in a markedly extended and relaxed state, with some discolorations on the dorsal surface. Of the aminimide compounds, the pupicidal activity was greatest if the chain length was C_{16} on either the amine (alkyl) or amide (acyl) side. The C_{14} aminimides displayed some activity but usually only at greater concentrations. Of the alkyl aminimides tested M-20 (C_{14}) had LC_{50} values for larvae and pupae between 1 and 10 ppm.

Exposed pupae were affected even more rapidly than the larvae. Instead of spending most of the time at the

water surface, they would lie on the bottom of the beaker. After several hours of exposure they could rise to the surface only with great difficulty. The pupae in most cases could not maintain themselves at the air-water interface, and had to snap many times in order to regain the surface. Many abnormalities were noted in the treated pupae. When treated only in the pupal stage the pupal wing-pads, mouthparts, and thoracic air case was disrupted. If treatment was begun at the late larvae to pupal stage, the pupae were formed with mouthparts and wing-pads sticking straight out instead of being glued to the thorax, and they were untanned. The treated pupae were examined under a light microscope to determine if any cuticular entrance could be discerned, but the pupal cuticle, although abnormal in appearance had no disruptions or other detectable points of entry. As with the larvae, the aminimides having the chain length of C_{16} were the most active on the pupae, followed by those having a C_{14} chain length.

The ethoxylated aminimides evaluated for their activity against Culex pipiens and Aedes aegypti larvae and pupae were found to be less effective than their parent compounds (Table VI).

Raising the temperature from 21°C to 28°C only had a minimal effect on the aminimides' toxicity to Aedes

aegypti larvae and pupae (Table VII). The aminimides were more toxic when tested at higher pH than in acidic solutions.

When aminimides were tested as synergists for carbaryl (Table VIII) they failed to show the synergizing characteristics of piperonyl butoxide, but showed an independent joint action. This combination follows the equation $P_c = P_a + P_b - P_aP_b$ where:

P_c = proportion of animals killed by the combination of poison a and b,

P_a = proportion killed by poison a,

P_b = proportion killed by poison b,

P_aP_b = proportion killed by both a and b (from Bliss, 1939).

When the standard synergists were added to the aminimides (Table IX) there was no true synergism. The aminimides' toxicity was apparently enhanced but not significantly.

When tested by standard methods the aminimides failed to have any ovicidal effects on Aedes aegypti eggs (Table X).

Aminimides dissolved in oil impregnated into filter paper (Table XI) did not kill adult female Aedes aegypti; thus they showed no contact toxicity.

TABLE VII. Effects of temperature and pH on the activities of aminimides against larvae or pupae of Aedes aegypti.

	LC_{50} (ppm)	
	Aedes aegypti	
	Larvae	Pupae
M-3 (21°C)		1.65
M-3 (28°C)		1.60
M-8 (21°C)	9.0	
M-8 (28°C)	8.0	
M-3 (pH 6.5)		1.8
M-3 (pH 7.0)		1.65
M-3 (pH 7.5)		0.625

TABLE VIII. Effects of aminimides used with carbaryl on 4th instar Culex pipiens larvae.

Compounds	LC_{50} (ppm PB)	LC_{50} (ppm carbaryl)
Carbaryl		.71
Piperonyl Butoxide (PB)	20	
Compounds in mix (10:1 carbaryl)		
PB		.14
M-4		.44
M-8		.28
M-18		.34
M-20		.41
M-31		.45
M-54		.54
M-62		.43
M-71		.50
M-125		.54

TABLE IX. Effects of standard synergists used with aminimides on 4th instar larvae of Culex pipiens and pupae of Aedes aegypti.

	LC_{50} (ppm aminimide)
A. M-8 used on larvae of Culex pipiens	
M-8	13.5
M-8 + Piperonyl Butoxide (PB)	8.6
M-8 + Sesamex (1:1)	9.2
M-8 + Tri-phenyl phosphate (TPP)	6.8
B. M-3 used on Aedes aegypti pupae	
M-3	2.2
M-3 + Tri-O-Tolyl Phosphate (1:1)	1.25
M-3 + TPP (1:1)	2.6
M-3 + PB (1:1)	1.45
Control	% Mortality at 20 ppm
TOCP	0
PB	2
Sesamex	0
TPP	0

TABLE X. Percent hatch of Aedes aegypti eggs laid on aluminum panels after spray applications of aminimides in 1% ethanolamine in distilled water.

Compounds	% Hatch	No. Eggs Tested
M-4	95.2	878
M-8	86.1	748
M-18	93	785
M-20	95.2	415
M-71	94.5	835
M-116	58.3	1,105
M-120	81.9	856
Malathion	94.6	687
Control (1% ethanol amine in distilled water)	94.2	639

TABLE XI. Biological activity of aminimides against adult *Aedes aegypti* using impregnated filter-paper technique.

Test Compounds	Mortality (5% Test Compound)
M-1	1/25, 2/25
M-2	6/25, 3/25
M-3	0/25, 1/25
M-132	0/25, 0/25
Malathion	25/25, 26/26
Olive Oil	0/25, 2/25

When M-3 was topically applied to the pupae, the aminimide had no effect, in contrast to malathion which caused 100% kill at the same concentration (Table XII).

In the adult after-effect tests (Table XIII) using M-3 at 10 ppm, 55% of the larvae were killed and adults failed to emerge. At 5 ppm the larval mortality was greater than 50%, but at 1 ppm was nearly all the adults emergenced.

Water containing M-18 and M-20 at 10 ppm remained toxic to Aedes aegypti larvae for 12 days, whereas M-8 (10 ppm) was only effective for 5 days (Table XIV).

Measurements of the surface tension (dynes/cm) for comparison with the biological activity obtained showed that aminimides toxicity to Aedes aegypti pupae was in direct proportion to which the surface tension of the water had been lowered (Table XV). When the surface tension values (X-axis) were plotted against the percent mortalities (Y-axis) a definite negative correlation was made evident, and the correlation coefficient obtained by calculation ($r=-.78$) was of a high value. The most effective compound M-3 (chain length C_{16}) lowered the surface tension the most, while M-2 (chain length C_{14}) which was nearly as effective, reduced the surface tension nearly as much.

TABLE XII. Results of topically applied aminimides to Aedes aegypti pupae at a rate of 100 µg/pupae at 90% relative humidity.

Compound	% Mortality	No. Emerged
M-3	0/10, 0/10, 0/10	10/10, 10/10, 10/10
Malathion	0/10, 10/10	0/10, 0/10
Control (Distilled water)	0/10	10/10

TABLE XIII. Adult after effect tests of M-3 on late 4th instar larvae of Aedes aegypti.

M-3	10 ppm	5 ppm	1 ppm	Control
24-Hour Mortality of Larvae	20/35, 20/35, 18/35	15/35, 13/35, 12/35	2/35, 11/35, 1/35	1/35, 0/35, 0/35
(% Mortality)	55%	38%	4%	1%
Number of Emergent Adults after 4 days in Solution	0/35, 0/35, 0/35	1/35, 5/35, 2/35	20/35, 20/35, 20/35	29/35, 23/35, 25/35

TABLE XIV. Biological activity of aminimides tested every third day against 4th instar Aedes aegypti larvae.

Compound (10 ppm)	Effective Time Span
M-8	5 days
M-18	12 days
M-20	12 days

TABLE XV. Effects of aminimides on the surface tension and their relation to larval and pupal mortality in Aedes aegypti.

Compound	Concentration (ppm)	Surface Tension (dynes/cm)	% Mortality on Aedes Aegypti at Given Concentration (24 hr LD_{50} Test)	
			Larvae	Pupae
M-1	10 ppm	68.1	0%	0%
M-2	10 ppm	47.4	20%	18%
M-2	1 ppm	60.1	0%	0%
M-2	0.1 ppm	73.0	0%	0%
M-3	10 ppm	39.7	58%	95%
M-3	1 ppm	49.7	0%	38%
M-3	0.1 ppm	73.0	0%	0%
M-4	10 ppm	59.6	0%	50%
Distilled water		73.0	0%	0%

x axis surface tension (dynes/cm)
y axis pupal mortality
correlation coefficient - 0.78
slope - 2.178
y intercept 153.29

DISCUSSION

The common feature among the test species to which the aminimides were non-toxic, i.e., Oncopeltus fasciatus nymphs, Aedes aegypti adults, Tribolium confusum larvae and pupae, and young Tetranychus urticae, is that they are all terrestrial organisms. In their type and spectrum of activity the aminimides have the greatest similarity to the various amines and amides tested by Mulla (1967) which are also organo-nitrogen compounds whose maximum biological activity is reached against aquatic organisms and life-stages.

Had the biological action of aminimides been exerted on some target enzyme, then it is likely that their activity would have been synergized by one or more of the synergists tested (Table IX). However, since none of these enzyme synergists were effective when used with the aminimides, it is likely that these compounds have a non-enzymatic mode of action.

Of the 4 criteria advanced by Christie and Crisp (1966) for compounds that exert their toxic effects by physical action (reversible narcosis, little dependence on temperature, toxicity independent of time of exposure and similar toxicity on the thermodynamic scale), the

aminimides demonstrate the first three. When removed from the test solutions, the affected larvae soon recovered. Increasing the temperature of the test solutions from 21°C to 28°C had only minimal effects on aminimides' toxicity to mosquito larvae or pupae.

Mulla (1967), however, concluded that amines have a more complex mode of action on mosquito larvae and pupae, including a change in the properties of the cuticle and possible interference with hormonal or amino-acid metabolism. He ruled out the possibility of the toxicity of amines being simply due to lowering of surface tension at the air-water interface, but did not take any surface tension readings of any of the amine test solutions. Kabara and Haitsma (1975b) noted that all surface-active agents, which are efficient bactericides, have been found to possess a marked ability to reduce surface tension although the converse is not necessarily true. Furthermore an increase in the chain length of the carbon chain from C_6 to C_{16} progressively increases the surface activity of the compound with maximum activity being reached at C_{14} or C_{16}. Table XVI (from Kabara et al., 1975a) shows the antimicrobial activity of several series of homologous acyl aminimides.

The measurements made on the reduction in surface tension of a homologous series of aminimides (M-1, M-2, M-3 and M-4) verifies the statements of Kabara and Haitsma

TABLE XVI. The minimal inhibitory concentration (μg/ml) for long chain acyl aminimides tested on microorganisms.

Aminimide[a]	Organism[b]								
	Gram (-)				Gram (+)			Yeast	
Acyl Derivatives	1	2	3	4	5	6	7	8	9
1, 1, 1 Trimethyl									
C_{12}	NI[c]	NI	1000	1000	1000	1000	1000	1000	1000
C_{14}	NI	NI	100	10	10	10	10	100	10
C_{16}	NI	NI	10	10	10	10	10	10	10
C_{18}	NI	NI	100	10	100	10	100	NI	100
1, 1-Dimethyl-1-(1-hydroxyethyl)-									
C_{12}	NI	NI	100	100	100	100	100	100	100
C_{14}	NI	NI	10	10	10	10	10	10	10
C_{16}	NI	NI	100	10	10	10	10	10	10
C_{18}	NI	NI	1000	10	100	100	10	NI	1000
1, 1-Dimethyl-1-(2-hydroxypropyl)-									
C_{12}	NI	NI	100	10	100	10	100	100	100
C_{14}	NI	NI	100	10	10	10	10	10	10
C_{16}	NI	NI	100	10	10	10	10	10	10
C_{18}	NI	NI	100	10	100	10	100	1000	100
$C_{18:1}$	NI	1000	1000	1	10	10	100	1000	10
Hexachlorophene	NI	1000	10	1	1	1	10	10	100

[a] C_n = Carbon number of acyl chain.

[b] 1) Escherichia coli, 2) Pseudomonas aeruginosa, 3) Streptococcus faecalis (Group D), 4) Streptococcus pyrogenes, 5) Staphylococcus aureus 6) Corynebacterium sp., 7) Nocardia asteroides, 8) Candida albicans, and 9) Saccharomyces cerevisiae.

[c] NI = Non-inhibitory.

SOURCE: Kabara et al., 1975a

(1975b) on surface tension. When tested at 10 ppm the acyl derivatives lowered the surface tension in proportion to their chain length (Table XV). At C_{12} (M-1) the surface tension was lowered from 73.0 dynes/cm (distilled water) to 68.1 dynes/cm, C_{14} (M-2) lowered the surface tension to 47.4 dynes/cm, C_{16} (M-3) lowered the surface tension to 39.7 dynes/cm, while C_{18} (M-4) only lowered the surface tension to 59.6 dynes/cm. Christophers (1960) has stated that compounds which reduce the surface tension make good pupicides by causing the pupae to lose attachment to the air-water interface and drowning. The high value of the correlation coefficient ($r = -.78$) for the aminimides verifies the relation between 24-hour mortality of mosquito pupae and reduced surface tension. Furthermore these aminimides had no lethal effect when topically applied to pupae of Aedes aegypti, nor did they cause any abnormal adult emergence. For substances which reduce the surface tension of the air-water interface, LC_{50} values can be stated either by their concentration (ppm) or by their surface tension in dynes/cm.

A succession of cuticular abnormalities was observed when treatment was maintained through the larvae and pupal stages by aminimides. The cuticular abnormalities were most frequent with acyl or alkyl aminimides having chain lengths of C_{14} and C_{16}. The air bubble responsible for the hydrostatic balance of mosquito pupae

in water (Christophers, 1960) is formed by the cementing together of the wing-pads, mouthparts, and thorax enclosing a small air pocket. Since it is at C_{14} and C_{16} that the aminimides have their peak emulsifying properties, it is therefore theoretically possible that aminimides emulsify off the cement layer, disrupting the air bubble and causing the pupae to lose their hydrostatic balance. The cuticular abnormalities evident in the larval-pupal stage may derive from the fact that it is just before or just after molting that the cement is poured over the wax surface (Wigglesworth, 1965). This would give the aminimides a "window" or time period where they could compete with the insect for the cement. At high concentrations aminimides could conceivably prevent enough cement from reaching the cuticle to allow the formation of a properly formed pupal integument. The abnormalities that occur in the pupal-adult stage at lethal concentrations may have a similar explanation. The emerging adult cannot maintain itself on the air-water interface because of the reduced surface tension (Manzeli, 1943). The resulting "wetting" of the untanned and unhardened new adult cuticle would result in the loss of its cement to the aminimide solution, preventing the development of a normal integument and also drowning the adult mosquito.

Aminimides possess potential as good mosquito larvicides and pupicides. Being more effective pupicides than carbaryl or malathion and having lethal effects on

mosquito larvae, and in the transition between larvae and pupae, and between pupae and adults, their effectiveness and specificity renders them potential mosquito control agents. Since mosquito larvae feed on bacteria (Christophers, 1960), the bactericidal qualities of aminimides would have a further negative-growth effect on mosquito larvae. The unhinging effect of the spiracular apparatus at the air-water interface caused by reducing the surface tension is a very specific type of activity which would be useful in combatting mosquitos of medical importance that are resistant to standard insecticides. The low toxicities of these compounds (200-400 mg/kg) allow therefore to be easily handled. Aliphatic amines, which have toxicities and exert symptoms similar to those observed with aminimides, have been tested in California (Mulla et al., 1970; Mulla and Darwazeh, 1971, 1975) at rates between 0.25 and 1.0 lbs./acre, and gave good control of mosquito larvae.

Because these compounds are most active in water, their effects on other types of aquatic metazoans should be tested. Their effects on such pests as nematodes or protozoa, and their effectiveness when field-tested against mosquito immatures should be investigated. The possible use as wetting agents for standard insecticides is another way these compounds could be used.

SUMMARY AND CONCLUSIONS

1. Of 35 aminimides tested in water against larvae and pupae of the mosquitos Aedes aegypti and Culex pipiens, 3 of them killed both larvae and pupae of either species at concentrations between 1 and 10 ppm. In 2 of these the acyl substituent was of chain length C_{16}, and in the third the alkyl substituent was of chain length C_{14}. Ten of them killed pupae at doses lower than the larvicides now in use.

2. The aminimides were found to be ineffective against terrestrial arthropods such as Tetranychus urticae, Oncopeltus fasciatus, Aedes aegypti adults, and Tribolium confusum. They were also inactive against pupae of Aedes aegypti when topically applied in a test chamber.

3. Of the effective aminimides, the ranking of their biological activity was strongly correlated to the degree to which they reduced the surface tension of water. The symptoms shown by the mosquito larvae and pupae in water treated with aminimides strongly suggests that these compounds acted as physical poisons preventing the insects from maintaining contact with the air-water interface.

4. Since the aminimides as a class of compounds are of low toxicity to higher animals, and since certain of them are active against pupae as well as larvae, it is considered that they are as worthy of attention as the amines which have recently been under investigation as a new group of mosquito larvicides.

APPENDICES

APPENDIX A

EXPERIMENTAL CHEMICALS AND THEIR PHYSICAL PROPERTIES

APPENDIX A

EXPERIMENTAL CHEMICALS AND THEIR PHYSICAL PROPERTIES

Compounds	M.P. °C	Solubilities* 95% EtOH	Heptane	H_2O
M-1, $CH_3(CH_2)_{10}CON_2(CH_3)_2CH_2(CH)OHCH_3$, 1, 1 dimethyl (2-hydroxypropyl) amine laurimide,				
M-2, $CH_3(CH_2)_{12}CON_2(CH_3)_2CH_2(CH)OHCH_3$, 1, 1 dimethyl (2-hydroxypropyl) amine myristimide,				
M-3, $CH_3(CH_2)_{14}CON_2(CH_3)_2CH_2(CH)OHCH_3$, 1, 1 dimethyl (2-hydroxypropyl) amine palmitimide,				
M-4, $CH_3(CH_2)_{16}CON_2(CH_3)_2CH_2(CH)OHCH_3$, 1, 1 dimethyl (2-hydroxypropyl) amine stearimide,	70-72	70	insol.	insol.
M-6, $CH_3(C_6H_4)S(O_2)N_2(CH_3)_3$, Trimethylamine p-Toluyl sulfonimide,				
M-8, $CH_3(CH_2)_{14}CON_2(CH_3)_3$, Trimethylamine palmitimide,	71-72	S	insol.	S
M-9, $CH_3(CH_2)_{12}CON_2(CH_3)_3$, Trimethylamine myristimide,	62-64	S	insol.	S

APPENDIX A (Continued)

Compounds	M.P. °C	Solubilities* 95% EtOH	Heptane	H_2O
M-11, $CF_3(CF_2)CON_2(CH_3)_3$, Trimethylamine perfluor propionimide,				
M-16, $CH_3(CH_2)_{10}CON_2(CH_3)_2CH_2CH_2OH$, Dimethyl-2-hydroxyethylamine laurimide,				
M-18, $CH_3(CH_2)_{14}CON_2(CH_3)_2CH_2CH_2OH$, Dimethyl-2-hydroxyethylamine palmitimide	72-74	S	insol.	insol.
M-19, $(CH_2)CH_3(C)CON_2(CH_3)_2CH_2CH(OH)(CH_2)_9CH_3$, Dimethyl-2-hydroxydodecamine methylacrylamide,	64-66	S	insol.	very slightly soluble
M-20, $(CH_2)CH_3(C)CON_2(CH_3)_2CH_2CH(OH)(CH_2)_{11}CH_3$, Dimethyl-2-hydroxymyristylamine methylacrylamide,	67-68	S	insol.	insol.
M-25, $(NH_2)(C_6H_4CON_2(CH_3)_3$, Trimethyamine anthranilimide,				
M-31, $(C_6H_5)CH_2N^+(Cl)-(CH_3)_2CON_2(CH_3)_2$, $CH_2CH(OH)(CH_2)_{12}CH_3$, Monobenzyl quaternary acetimide,				

APPENDIX A (Continued)

Compounds	M.P. °C	Solubilities* 95% EtOH	Heptane	H_2O
M-34, $CH_3(CH_2)_{10}CON_2(CH_3)_3$,	53-54	S	3	S
M-43, $(C_6H_5)NH(CH_2)CH_2CON_2(CH_3)_2CH_2CH(OH)CH_3$, β-anilino dimethyl-2-hydroxy propyl propionimide,				
M-46, $(C_3N_2H_3)CH_2CH_2CON_2(CH_3)_2CH_2CH(OH)CH_3$, Imidazolyl aminimide,				
M-48, $(C_5NH_{10})CH_2CH_2CON_2(CH_3)_2CH_2CH(OH)CH_{3-2}$, Bis, (n-piperidine) dimethyl-2-hydroxy propylamine propionimide,				
M-54, $OHCH_2CH_2N^+Cl^-(CH_3)_2CH_2CON_2(CH_3)_2CH_2CH(OH)(CH_2)_{13}CH_3$, P-nonyl phenoxy DMHP acetimide,				
M-57, $(CH_2)CH_3(C)CON_2(CH_3)_2CH_2CH(OH)(CH_2)_{13}CH_3$, Dimethyl-2-hydroxypalmitylamine methylacrylimide,	72-73.5	S	insol.	insol.
M-62, $(C_5NH_{10})CH_3CH_2CON_2(CH_3)_2CH_2CH(OH)(CH_2)_{13}CH_3$, Dimethyl-2-hydroxypalmitylamine pyridine N-methyl acetimide,				

APPENDIX A (Continued)

Compounds	M.P.°C	Solubilities* 95% EtOH	Heptane	H_2O
M-65, $(C_6H_5)CH_2Cl^-(C_5N^+H_4)CON_2(CH_3)_2CH_2CH(OH)(CH_2)_{13}CH_3$, Dimethyl-2-hydroxypalmitylamine N-benzyl isonicotinimide,				
M-71, $CH_3CON_2(CH_3)_2CH_2CH(OH)(CH_2)_{11}CH_3$, Dimethyl-2-hydroxymyristylamine acetimide,	63-65	S	insol.	insol.
M-125, $(C_5N^+H_5)Cl^-CH_2CON_2(CH_3)_2CH_2CH(OH)(CH_2)_{13}CH_3$, Dimethyl-2-hydroxypalmitylamine n-benzyl pyridineamide,				
M-129, $(CH_2)CH_3(C)CON_2(CH_3)_2CH_2CH(OH)(CH_2)_{15}CH_3$, Dimethyl-2-hydroxy stearyl methylacrylamide,	77-78	S	insol.	insol.
M-132, $CH_3(CH_2)_{16}CON_2(CH_3)_2CH_2OH$, Dimethyl-2-hydroxyethylamine stearimide,				
M-133, $CH_3(CH_2)_{16}CON_2(CH_3)_3$, Trimethylamine stearimide,				
Ethoxy Compounds				
M-108, $CH_3(CH_2)_{10}CON_2(CH_3)_2CH_2CH(OH)CH_3$+5EO, Dimethyl-2-hydroxypropyl laurimide,	77-78	S	insol.	insol.

APPENDIX A (Continued)

Ethoxy Compounds	M.P.°C	Solubilities* 95% EtOH	Heptane	H_2O
M-116, $CH_3(CH_2)_{12}CON_2(CH_3)_2CH_2CH(OH)CH_3$+5EO, Dimethyl-2-hydroxy propyl myristimide,				
M-120, $CH_3(CH_2)_{14}CON_2(CH_3)_2CH_2CH(OH)CH_3$+5EO, Dimethyl-2-hydroxy propyl palmitimide,				
M-121, $CH_3(CH_2)_{14}CON_2(CH_3)_2CH_2CH(OH)CH_3$+10EO, Dimethyl-2-hydroxy propyl palmitimide,				
M-122, $CH_3(CH_2)_{14}CON_2(CH_3)_2CH_2CH(OH)CH_3$+10PO, Dimethyl-2-hydroxy propyl palmitimide,				
M-123, $CH_3(CH_2)_{14}CON_2(CH_3)_2CH_2CH(OH)CH_3$+4EO, Dimethyl-2-hydroxy propyl palmitimide,				
M-124, $CH_3(CH_2)_{14}CON_2(CH_3)_2CH(OH)CH_3$+4PO, Dimethyl-2-hydroxy propyl palmitimide,				

*gms/100g solvent where available, S = Soluble, insol. = insoluble

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