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# Structure–Activity Relationship of Alkanols as Mosquito Larvicides with Novel Findings Regarding their Mode of Action

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**Abstract**—Primary alcohols, from methanol to eicosanol, were applied to water for control of larval stage mosquitoes. By applying the alkanols as soluble solutions rather than as insoluble monolayers, and by trapping larvae under glass in assays that isolated them from the surface phenomena believed to be responsible for death by suffocation, we have shown that the action of alkanols against mosquito larvae is biochemical in nature, not just physical. Primary alcohols are known to act as general anesthetics, with increasing potency correlated to increasing chain length until a point of cutoff is reached, usually at dodecanol ($C_{12}$), after which activity disappears entirely. In mosquitoes, we found that activity levels off after undecanol ($C_{11}$) but does not disappear until after pentadecanol ($C_{15}$), that it is reversible, and that chain length plays a role not only in potency, but also in the time needed to manifest toxic effects. We used sonication, a surfactant, temperature, and the introduction of double bonds to manipulate activity around the cutoff, suggesting that it is at least partially a function of solubility. Mosquitoes appear to be the first animal for which cutoff has been demonstrated to occur at a chain length beyond $C_{12}$, offering new insights into the molecular basis of anesthetic cutoff and suggesting the possibility that alkanols might be used for selective pest control. Alkanols are stable, colorless, inexpensive, biodegradable and essentially non-toxic to humans, making them promising candidates for pest management programs.


## Introduction

Despite significant advances in the techniques used for its control during recent decades, the mosquito continues to pose serious public health problems as we approach the 21st Century. In addition to the persistant irritation they cause humans and animals simply by virtue of their blood-sucking behavior and the itching this causes, mosquitoes are also the principal vector of a variety of serious diseases, including malaria, yellow fever, dengue, and encephalitis. Worldwide, approximately 2.7 million human deaths occur each year solely as a result of malaria transmitted by mosquitoes.[1]

In this article we describe the use of primary alcohols (hereafter referred to as alkanols for simplicity) applied to water as soluble solutions for the control of mosquitoes in their larval stage. The species used for this study is *Culex tarsalis*, the principal vector of the Western equine and St. Louis encephalitis viruses common throughout the Western US, but preliminary assays against *Culiseta incidens* suggest that the alkanols tested show similar activity against members of other mosquito genera too. In an effort to understand the molecular basis of alkanol toxicity, a variety of commonly occurring unsaturated long-chain alcohols were also tested. Of these, farnesol has been reported previously as acting in some insects as a juvenile hormone mimic.[2]

Alkanols are important flavor and fragrance compounds that are found throughout the plant kingdom, occur naturally in everyday foods and beverages,[3] and are widely used as food additives.[4] Homologues containing 12 or more carbons are only sparingly soluble in water and their tendency to form monomolecular layers over the surface of a body of water was examined decades ago as a means to reduce evaporative losses.[5] Because monolayers lower surface tension, it was also suggested that they might be used in pest control to suffocate the aquatic stages of mosquitoes or to prevent freshly emerged adults from launching off the water's surface. Consequently, all earlier research employing alkanols against mosquitoes has attributed lethality entirely to reduced surface tension, wetting of their hydrofuge structures, or other surface-related phenomena that—over a period of many hours—eventually lead

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to drowning.[6] However, in this study we have shown that there is a more immediate and lethal effect of alkanols on mosquito larvae, that it is unrelated to surface tension, and is biochemical rather than physical.

A review of the literature indicates that this is the first comprehensive study of the structure–activity relationship for the full series of alkanols against mosquitoes. Primary alkanols are also known to act as general anaesthetics,[7] and increasing potency has been correlated to increasing chain length[8] until a point of cutoff is reached, usually at dodecanol ($C_{12}$), after which activity disappears entirely.[9] In mosquitoes, we found that activity levels off after undecanol ($C_{11}$) but does not disappear until after pentadecanol ($C_{15}$). Mosquitoes appear to be the first animal for which cutoff has been demonstrated to occur at a chain length beyond $C_{12}$, offering new insights into the molecular basis of anaesthetic cutoff and suggesting the possibility that alkanols might be used for selective pest control.

## Results

### Toxicity of alkanols to mosquito larvae

The toxicity of the homologous series of primary alkanols against *C. tarsalis* larvae was recorded as mortality after 24 h and the results (Table 1) show that larvicidal activity peaks at dodecanol, with an $LD_{50}$ of 0.030 mM against 1st instar and 0.028 mM against mixed 3rd and 4th instar larvae. Methanol and ethanol were very weak agents and mortality did not reach 50% at the highest dose tested, 500 mM. Activity increases through the series as chain length is lengthened from propanol to dodecanol, tapers off slightly from dodecanol to pentadecanol, and then cuts off at hexadecanol. Alkanols with greater than 16 carbons were ineffective as mosquito larvicides because they did not display biochemical toxicity and did not consistently prevent larvae from surfacing; consequently, they did not produce 50% mortality up to the maximum practical dosage, even when assays were extended to 4 days.

The values cited in Table 1 for tridecanol through eicosanol exceed literature values for their maximum solubility in water[10] but experiments at high doses were included because these compounds are far more soluble in cell membranes—the putative active site—than in water. By creating a supersaturated suspension in the aqueous medium, even temporarily, we were able to deliver a higher dose to the mosquitoes themselves.

Addition of the surfactant Tween-80® to improve solubility increased the toxicity of tridecanol and tetradecanol

**Table 1.** $LD_{50}$ values of alkanols against *C. tarsalis* larvae and surface tension, $\gamma$, at $LD_{50}$ concentration. Up to decanol, the $LD_{50}$ concentration does not lower $\gamma$ sufficiently to prevent larvae from surfacing. Surface tension is given only up to undecanol because the longer species readily adsorb to the container walls and at high surface pressures a very small change in concentration can cause a large change in $\gamma$. Although values cited for tridecanol and longer alkanols exceed literature values for their maximum solubility in water,[10] this can be explained by (a) the fact that these compounds are far more soluble in lipid membranes than in water, and (b) the assay method, which was designed to allow delivery of a high dose by supersaturating the aqueous medium

| Alkan-1-ol | 1st instar $LD_{50}$, mM | 3rd and 4th instar $LD_{50}$, mM | $\gamma$[a] at $LD_{50}$ (dynes/cm) |
|---|---|---|---|
| Methanol | > 500 | | |
| Ethanol | > 500 | | |
| Propanol | 171 (±27.8) | | 58.8 |
| Butanol | 74.6 (±3.66) | | 58.8 |
| Pentanol | 20.0 (±4.10) | | 59.1 |
| Hexanol | 7.55 (±1.18) | | 54.7 |
| Heptanol | 2.64 (±0.322) | | 52.5 |
| Octanol | 1.12 (±0.081) | 1.02 (±0.139) | 47.1 |
| Nonanol | 0.260 (±0.061) | 0.278 (±0.026) | 50.0 |
| Decanol | 0.057 (±0.015) | 0.081 (±0.007) | 57.5 |
| Undecanol | 0.038 (±0.005) | 0.046 (±0.004) | 29.7 |
| Dodecanol | 0.030 (±0.004) | 0.028 (±0.002) | |
| Tridecanol | 0.034 (±0.003) | 0.042 (±0.006) | |
| Tetradecanol | 0.049 (±0.010) | 0.039 (±0.005) | |
| Pentadecanol | 0.302 (±0.058) | 0.088 (±0.028) | |
| Hexadecanol | inactive up to 0.6 | 0.222 (±0.047) | |
| Heptadecanol thru Eicosanol | inactive up to 0.6 | | |
| Tridecanol + Tween | 0.029 (±0.003) | | |
| Tetradecanol + Tween | 0.034 (±0.005) | | |
| Pentadecanol + Tween | 0.034 (±0.007) | | |
| Hexadecanol + Tween | inactive up to 0.6 | | |
| Heptadecanol thru Eicosanol + Tween | inactive up to 0.6 | | |
| Other Alcohols[b] | | | |
| *cis*-11-Hexadecen-1-ol | 0.032 (±0.002) | | |
| Farnesol | 0.036 (±0.004) | | |
| Linoleyl alcohol | 0.034 (±0.005) | | |
| Linolenyl alcohol | 0.031 (±0.002) | | |
| 1,10-Decanediol | inactive up to 4.6 | | |

[a]Surface tension at 20 °C.
[b]In combination with Tween-80 added at 100 μg/mL.

slightly, and of pentadecanol by nearly a full order of magnitude (Table 1). Activity of alkanols with less than 13 or more than 15 carbons was unaffected by the use of surfactant or sonication. Tween itself had no noticeable toxicity to larvae up to 400 μg/mL, four times the concentration used here.

Sublethal doses provoked symptoms of anaesthesia in mosquitoes (e.g. unresponsiveness to outside stimuli such as tapping on the container and cessation of characteristic diving and feeding behaviors).

Whereas there was no appreciable difference between the patterns of physiological toxicity of alkanols to larvae of different instars, the lethal effects of reduced surface tension were most evident against 3rd and 4th instar larvae, which were less able than 1st instars to tolerate prolonged periods without air—note the potency of pentadecanol and hexadecanol against late instars as compared to 1st instar. In short, the effectiveness of hexadecanol against late instars only is apparently the result of its physical properties for temporarily lowering surface tension, and not of inherent biochemical toxicity (see Results of suffocation experiments).

Statistical analysis of the data showed that the difference in potency of alkanols differing in length by one carbon was highly significant ($P < 0.01$) for $C_3$–$C_{10}$ and for $C_{14}$–$C_{16}$. The difference between pentadecanol alone and pentadecanol + Tween was also highly significant ($P < 0.01$). However, the differences among the most potent homologues ($C_{10}$–$C_{14}$) and the effects of combining each of these with Tween, were not significant for this dataset ($n = 5$).

### Unsaturated and branched alcohols

In order to better understand the significance of the cutoff in activity after pentadecanol, several other alcohols of relevant structure and chain length were tested as well, all in combination with Tween to ensure maximum solubility. It was interesting to note that whereas hexadecanol was not toxic to 1st instar mosquitoes at the highest concentrations tested, *cis*-11-hexadecenol was nearly as potent as the strongest of the saturated alkanols. Similarly, the introduction of two (in the case of linoleyl alcohol) or three (linolenyl alcohol) double bonds to octadecanol converted the compound from completely inactive to being among the most potent (Table 1).

The isoprenoid sesquiterpene farnesol, which has an overall length of 12 carbons, also showed toxicity comparable to that of dodecanol, leading us to infer that the toxicity depends on a particular balance between the hydrophobic and hydrophilic moieties. The head–tail type structure is apparently essential for larvicidal activity because whereas decanol was toxic to mosquitoes in the range of 0.06 mM, decane-1,10-diol was not toxic even at 4.6 mM.

Although the differences in relative toxicity were consistent among the four unsaturated alkenols tested, they were not statistically significant ($P < 0.01$ and $n = 5$).

### Surface tension experiments

The objective of the surface tension experiments was to test the hypothesis that the lethal concentration of a given alkanol can be positively correlated to the concentration required to lower surface tension enough to prevent larvae from surfacing for air. Dilute solutions of Ivory® soap produce low, yet stable surface tension values, and were used to confirm the results of earlier researchers that some mosquito larvae have difficulty surfacing for air when surface tension ($\gamma$)—normally about 72 dynes/cm—is decreased to 27–36 dynes/cm.[11] For *C. tarsalis* we found variability among individuals, but most first and second instar larvae were unable to attach to the surface when $\gamma$ was decreased to the range of 28 to 32 dynes/cm; fourth instar larvae were able to tolerate lower surface tensions, but none were able to surface when $\gamma$ fell below about 26 dynes/cm.

In contrast, the concentration of $C_3$–$C_{10}$ alkanol that caused 50% mortality in 1st instar larvae produced surface tensions of 62 to 47 dynes/cm (Table 1), which allowed even the smallest larvae to surface without difficulty. The concentration of each $C_3$–$C_{10}$ alkanol necessary to lower $\gamma$ to 30 dynes/cm was on average eight times higher than its respective $LD_{50}$ concentration (Fig. 1). Furthermore, for chain lengths up to 10 carbons, many of the dead larvae were found with their respiratory siphons still firmly suspended from the water's surface, unequivocally eliminating surface phenomena as the cause of death.

Alkanols with 11–16 carbons, on the other hand, were found to be superior surface tension reducing agents and even at low doses, larvae attempting to surface for air simply bounce off the air–water interface and sink back downward. Nevertheless, the swiftness of death hinted that even these larvae were succumbing to a

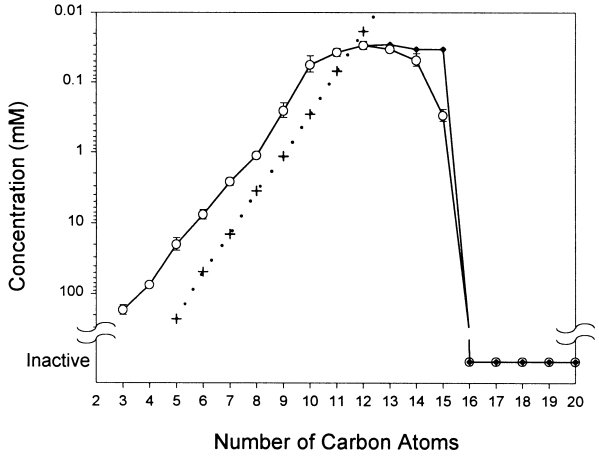


**Figure 1.** Twenty-four hour $LD_{50}$ concentration of alkanols against 1st instar *C. tarsalis* larvae. ○, Alkanols alone; ◆, Alkanols in combination with Tween-80 (100 μg/mL); +, Dose required to lower surface tension to 30 dynes/cm, illustrating that up to about decanol, the alkanol concentration needed to prevent larvae from surfacing is approximately eight times the $LD_{50}$ concentration. Surface tension data for $C_{10}$ through $C_{13}$ are estimates based on the pattern of experimental data for $C_3$ through $C_9$, whereby 30 dynes/cm was reached at approximately 85% of each alkanol's aqueous solubility.

biochemical phenomenon long before they could be expected to display the effects of suffocation. For these long-chain alkanols, the role of a biochemical mode of action was demonstrated by using a method that prevented contact with the surface for the course of the assay.

### Suffocation experiments

Mosquito larvae, especially early instars, can survive for extended periods without access to air, apparently by cutaneous respiration of dissolved oxygen in the water.[12] A glass ceiling such as a petri dish can be used to trap larvae without air. By creating a test system free of air and its associated interfaces, the container ceiling is effectively made identical to its walls and bottom and, most importantly, the larvae are specifically isolated from any surface-related phenomenon that might wet their siphon opening, reduce its hydrofuge properties, or allow leaks into their respiratory siphons during attempts to surface.

When we trapped twenty 1st instar *C. tarsalis* larvae under glass without air as a control, 100% were alive and active 16 h later. 4th instar populations survived only about 3–4 h, apparently due to their higher oxygen requirements and lower surface area-to-volume ratio. Survival time of 1st instar larvae under glass was approximately doubled to 32–48 h by lowering the temperature of the water to 8–10 °C.

In contrast, 1st instar larvae trapped without air in jars of water treated with decanol or undecanol were immobilized on the bottom and made no further efforts to surface after just 5 min; the same result was observed with dodecanol after about 12 min, with tridecanol after an hour, with tetradecanol after 3 h, and with pentadecanol after approximately 9 h. Extrapolation of this time dependency curve (Fig. 2) predicts activity for hexadecanol after about 18 h, but no mortality due to hexadecanol nor heptadecanol treatments occurred before control larvae themselves began to die of anoxia, after about 24–30 h. Nevertheless, we could not rule out the possibility that hexadecanol may act by a mechanism similar to—but weaker than—shorter chain homologues, for example as a partial anaesthetic.

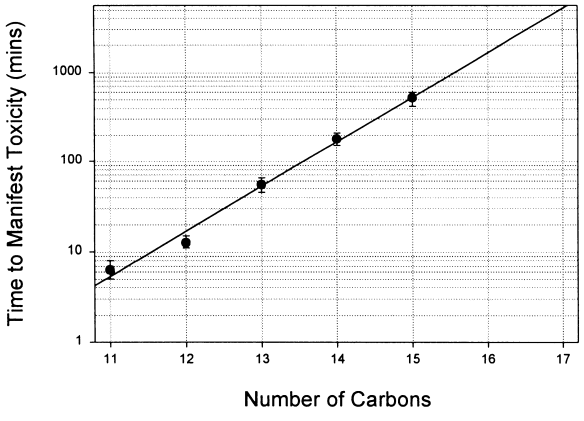


**Figure 2.** Time dependency of alkanol toxicity in mosquito larvae. Each point represents the mean time after treatment when the last bodily movement was observed from any of ten treated larvae when trapped without air under glass. Error bars show the range for three experiments. Alkanols of $C_{10}$ through $C_{14}$ were applied at 0.1 mM, $C_{15}$ through $C_{17}$ at 0.25 mM; there was no mortality for $C_{16}$ or $C_{17}$ treatments before controls themselves began to die, at 24–30 h. All solutions prepared with Tween (100 μg/mL) and sonication.

In an effort to extend the survival of larvae trapped without air and thereby allow sufficient time for the possibility that hexadecanol might still show activity at longer exposures, the same air-free experiments were also carried out under refrigeration, at 6, 10, and 14 °C. Control larvae were thus able to survive up to 48 h, but we were surprised to find that 0.26 mM pentadecanol + Tween, which had caused 100% mortality at 20 °C, caused just 20% mortality at 14 °C, and toxicity disappeared entirely at 10 °C or less. Comparatively, the toxicity and time dependency of tetradecanol and the shorter chain alkanols were unaffected by these lower temperatures, suggesting that the loss of activity is at least partially a function of declining solubility.

### Reversibility

Subsequent experiments, in which larvae treated with lethal doses of $C_{10}$–$C_{15}$ alkanols were transferred to clean water well after the onset of anesthesia had become evident, confirmed that the effects were completely reversible. Within minutes or hours, again dependent on chain length, larvae returned to behavior indistinguishable from that of untreated larvae (data not shown).

## Discussion

The significance of our findings are twofold: First, they challenge the previously accepted belief that alkanols act as mosquitocides only via suffocation provoked by surface phenomena; second, for studies measuring biological activity of the alkanol series, this is the first documented instance of cutoff occurring in an animal at a chain length beyond $C_{12}$.

The speed with which alkanols $\leq C_{15}$ act as mosquito larvicides is in sharp contrast to the conclusions of previous researchers using insoluble monolayers of dodecanol,[6a,6d] hexadecanol,[6d] and lecithins,[6e] which were specifically described as producing larval death only after the dissolved oxygen content of the water was depleted, usually overnight. The increasingly time-dependent nature of toxicity as chain length is increased offers further evidence of the biochemical mode of action for alkanols, independent of any suffocation mechanism resulting from reduced surface tension at the air–water interface.

Action of farnesol was also rapid, so although its properties as a juvenile hormone mimic are known in other insects, hormonal disturbances take time, and its toxicity against mosquito larvae is apparently via the same mechanism as for unbranched, saturated alcohols.

When, for comparison purposes, we treated mosquitoes with Golden Bear Oil, a commercial product sold as a

surface-active mosquitocide, 4th instar larvae died after a period of several hours and 1st instar larvae were weakened, but still alive, 24 h later—both results consistent with an explanation of death via suffocation. Alkanols, in contrast, were generally observed to act more quickly on 1st instar than on 4th instar larvae. Also, whereas pupae have been shown previously to be more susceptible to monolayers than larvae,[6d,13] our preliminary findings indicate that solutions of long chain alkanols are more toxic to *Culex* larvae than to the pupae, possibly owing to the pupae's thicker cuticle and concomitant capacity to resist penetration by foreign substances.

Tests with animals in vivo are somewhat limited, but long chain alkanols are known to produce anesthesia in fathead minnows,[9b] tadpoles,[9a,14] and brine shrimp,[15] and to cause growth impairment in the ciliate protozoan *Tetrahymena pyriformis*.[16] In our own earlier studies, they showed activity against a variety of gram-positive bacteria and fungi, but not against gram-negative bacteria.[17]

A persistent quandary facing researchers has been the 'cutoff' phenomenon in the homologous series of alkanols, whereby potency increases with chain length until reaching a maximum, and the alkanol containing a single additional carbon shows no potency at all, even when duration of exposure lasts several days.[14b] Although attention has focused on instances where cutoff occurs immediately after dodecanol, the exact length of carbon chain where cutoff occurs clearly varies among genera (Fig. 3), and even among strains of the same species in the case of bacteria (data on *Streptococcus mutans*[17,18]).

Our review of the literature found no other animal (only microorganisms and a protozoan) for which cutoff has been reported at a chain length beyond dodecanol, which may be the result of differences in the membrane composition of test organisms. By comparison, tadpoles were fully anaesthetized by nonanol after 30 min, by decanol and undecanol after 60 min, by dodecanol after 120 min, but not by tridecanol, even when exposed for 96 h.[9a] Mosquitoes are also unusual in that the loss of activity is gradual, tapering off from the strongest compounds before disappearing, whereas for most organisms, maximum activity occurs at a given chain length of $n$, and absolutely no activity is present at the alkanol of chain length $n+1$. For example, the minimum bactericidal concentration against *Propionibacterium acnes* was 1.56 μg/mL for hexadecanol, but heptadecanol was completely inactive, even when tested at 800 μg/mL.[17]

The precise anaesthetic mechanism is still not well understood, and attempts to explain it have covered a wide array of biological functions, mostly related to structure and function of lipid membranes and/or proteins.[19] The

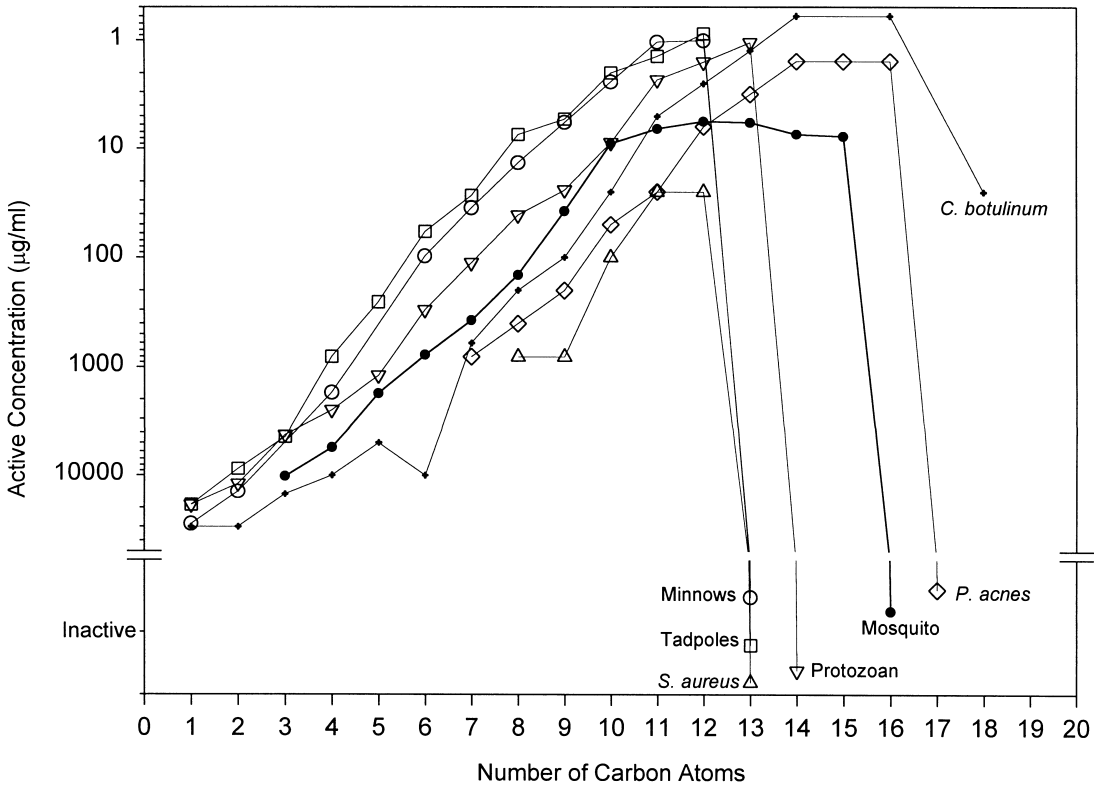


**Figure 3.** Comparison of biological activity of alkanols against various organisms. Cutoff in activity against fish and tadpoles occurs after $C_{12}$, but in mosquitoes not until after $C_{15}$. Although the endpoints measured are not identical in all test systems, sublethal doses provoked symptoms of anaesthesia in mosquitoes and growth inhibition in microorganisms. □, Tadpole[9a] (*Rana pipiens*), loss of righting reflex, $EC_{50}$; ○, Minnow[9b] (*Pimephales promelas*), 96 h $LC_{50}$; ▽, Protozoan[16] (*T. pyriformis*), inhibitory growth concentration, $EC_{50}$; △, *S. aureus*,[17] minimum bactericidal concentration, $EC_{50}$; ●, Mosquito (*C. tarsalis*), 24 h $LD_{50}$; Tween used to maximize solubility of alkanols $\geq C_{13}$; ◇, *P. acnes*,[17] minimum bactericidal concentration, $EC_{50}$; +, *Clostridium botulinum*,[27] minimum inhibitory concentration.

increase in activity with chain lengthening has been widely correlated to each compound's octanol- or lipid-water partition coefficient, and thereby its relative tendency to accumulate in lipid regions of the cell membrane at concentrations sufficient to interfere with basic nutrient or ion transport processes.[20] Nevertheless, it has been difficult for lipid based theories to account for the cutoff effect and with this deficiency in mind, other researchers used alkanol inhibition of the lipid-free luciferase enzyme to build a case for anaesthetics acting by binding directly to a protein pocket of circumscribed dimension.[19,21] More recently, the effects of alkanols on ligand-gated ion channels in wild-type and mutated neurotransmitter receptors have also led some to conclude that alkanols act directly on membrane proteins, and do not depend on lipid-protein interactions.[22] Still, doubt remains as to whether these systems adequately model the site of general anaesthesia in animals,[9a,23] or for that matter whether anaesthetic activity of alkanols is limited to a single mechanism.

The fact that sonication, temperature and combination with a surfactant could be used to manipulate mosquitocidal activity nearly tenfold around the cutoff supports the notion that the solubility of alkanols in biological membranes and their capacity to perturb membrane lipids at the lipid–protein interface have some bearing on explanation of the cutoff phenomenon. Indeed, there are several recent studies to support this hypothesis. Anaesthetic chain lengths were shown to cause perturbation of membrane lipids whereas non-anaesthetic alkanols dissolved in membrane lipids without perturbing them.[14b] FTIR studies of lipid membrane vesicles in $D_2O$ showed that hydrogen bonding of the alkanol hydroxyl to the phosphate moiety in reversed micelles increases up to decanol, and then declines sharply at tetradecanol.[15] If alkanols bond to the phosphate moiety in biological membranes the way they do in membrane vesicles, then certainly they would affect the conformation and function of proteins normally held in place by hydrogen bonds at the lipid–protein interface.

Knowledge that introduction of a double bond shifts the cutoff to a longer chain length in tadpoles[14a]—and now in mosquitoes—is further evidence in support of explanations which consider the structural role of lipids rather than isolated action on a protein binding site, because if cutoff were due to a compound's hydrophobic moiety exceeding the limited size of a protein pocket, then since the addition of a *cis* double bond makes the compound even bulkier, it should theoretically provoke cutoff at shorter, rather than longer, alkyl chain lengths. We speculate that hydrogen bonding plays an important role in determining the molecular basis of alkanol activity in mosquitoes and other organisms.

## Conclusions

Alkanols are stable, colorless, inexpensive, biodegradable[24] and essentially nontoxic to humans.[25] The fact that they are already approved for use in food products at concentrations comparable to the doses used here[4] may facilitate their approval as insecticides. If further experience bears out the conclusions of earlier workers who reported a cutoff in activity after dodecanol for fish and amphibians, then the more narrow spectrum activity of tridecanol, tetradecanol, and pentadecanol against mosquitoes makes these promising candidates for environmentally sensitive pest management programs. The cutoff in anaesthetic potency occurs at different chain lengths in different organisms, lending new perspective to the molecular basis of anesthesia. We are currently studying the ability of alkanols to inhibit respiration at the cellular level.

## Experimental

### Mosquitoes

Eggs of *C. tarsalis* (Breckenridge strain) were generously supplied by Laura Kramer of UC Berkeley's School of Public Health and by Steve Schutz of the Contra Costa Mosquito and Vector Control District. Larvae were maintained at room temperature ($20 \pm 2\,^{\circ}C$) and fed on a slurry of TetraMin® flake food for tropical fish.

### Chemicals

All alcohols were purchased from Aldrich Chemical Co., with the exception of linoleyl alcohol (*cis*,*cis*-9,12,-octadecadien-1-ol) and linolenyl alcohol (9,12,15-octadecatrien-1-ol), which were both purchased from Sigma (St. Louis, MO), and octan-1-ol, which was a gift from Takasago, Inc. All chemicals were of at least 97–99% purity (with the exception of farnesol and *cis*-11-hexadecenol, which were at least 95% pure) and were used without further purification. Tween-80® was purchased from ICI (Wilmington, DE). Golden Bear Oil (GB-1111®), was obtained courtesy of the Alameda County Mosquito and Vector Control District, CA. Petroleum jelly (Vaseline®) was purchased at the local drugstore.

### Bioassays

Bioassays were conducted at room temperature ($20 \pm 2\,^{\circ}C$) using distilled water (20 mL) in 1 oz clear plastic containers made by Plastics Inc. (St. Paul, MN). Alcohols were applied by solubilizing them first in acetone, then diluting in water to the appropriate concentration and briefly shaking to ensure mixing. Ten larvae were then pipetted into each 20 mL volume and observed for a minimum of 24 h, when mortality was recorded. Controls were treated with the maximum amount of acetone applied in each alcohol assay, usually 0.1 mL. Acetone itself was found to have no effect on the larvae up to a concentration of 1%.

All compounds were assayed against 1st instar larvae and octan-1-ol through hexadecan-1-ol were also tested against mixed 3rd and 4th instar larvae. Assays were repeated at least five times for each compound.

Compounds were tested at a minimum of five concentrations following range finding tests.

Due to their excessive clumping and general insolubility in water, alkanols with 15 or more carbons were tested only up to 0.6 mM. Although this dose exceeds the measured solubility of long-chain alkanols in water,[10] the application of excess material was used as a way to supersaturate the water and thereby deliver a higher dose to the mosquito itself, in whose cell membranes these compounds are much more soluble. For alkanols greater than 10 carbons, bioassays were repeated with the addition of the commercial surfactant Tween-80 (100 μg/mL) to see if improved solubility might increase activity. For assays employing surfactant, shaking was impractical, so solutions were added while sonicating the test water, which helped to maximize solubility and mixing.

Larvae were considered dead or moribund if they stopped moving for a prolonged period, were unable to resurface after sinking to the bottom of the container, and were still unable to respond after gentle probing with a small spatula, as described in the World Health Organization's technical report series.[26] Mortality among controls was zero for more than 95% of the assays, and in no instance did it exceed 10%.

In assays where larvae were trapped without air and prevented from any contact with the surface, each test solution was prepared as described above, then used to fill a 250 mL glass jar above its brim so as to form a meniscus; a petri dish was then slid horizontally across the mouth of the jar so that no air bubbles remained. The edge was sealed with petroleum jelly to prevent drying and leakage. Time required to manifest toxicity is expressed as the time at which the last bodily movement could be detected from the last survivor among 10 larvae, and values cited are the mean of at least three experiments.

### Surface tension experiments

Surface tension measurements were taken at 20 °C with a CSC-DuNouy Precision Tensiometer (# 70535) from CSC Scientific, Inc. (Fairfax, Virginia). This instrument employs the upward pulling ring (6 cm iridium–platinum ring) method of measurement, is then corrected for gravity, and is accurate to about ±0.2 dynes/cm. Solutions were prepared by adding pure alkanols to 100 mL water, which was then shaken vigorously and allowed to rest until any bubbles present had subsided before measurements were taken. The ring was cleaned by flaming and the alkanol solution was mixed using a magnetic stirbar between each measurement. Values cited are the means of at least three consecutive measurements which were all within 0.5 dyne of one another.

### Data analysis

The $LD_{50}$ values reported are the means of an $LD_{50}$ calculated individually for each of at least five separate experiments, using nonlinear regression (SigmaPlot®), by fitting data to the 4 parameter sigmoidal logistic equation:

$$y = y^o + [a/(1 + (x/x^o)^b)], \quad (1)$$

where a = maximum mortality, x = concentration, $x^o$ = concentration at 50% amplitude, $y^o$ = minimum mortality, and b = the difference between the concentrations at 25% and 75% amplitude.

Statistical analysis was performed by calculating the difference between the standard errors of the compounds being compared, and correlating this value to a Normal Table for $P < 0.01$.

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