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Molluscicidal Properties and Selective Toxicity of Surface-Active Agents

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Of over 100 commercially produced surface-active agents tested against the bilharziasis vector snail Biomphalaria sudanica, *13 were found to possess considerable and highly selective molluscicidal properties at concentrations of less than 1 ppm for exposures of 48 hours. Against crustacea, fish, water plants, mosquito larvae, mice, and the eggs of* B. sudanica, *the toxicities of the 13 surfactants were slight. The chemicals did not appear to be absorbed by organic matter to any appreciable extent. It is thought that the toxicity to* B. sudanica *is of both a chemical and a physical nature.*

INTRODUCTION

Numerous publications have described the bactericidal properties of surface-active agents (surfactants), and these chemicals are known to have toxic effects on other forms of life also. For instance, the toxicity of synthetic surfactants to man has been reported by Gaulier & Fournier (1954) and Darby et al. (1956). These workers found that surface activity itself cannot be used as a measure of toxicity, which depends more on the chemical structure of the surfactant. Surfactants were found to produce both a rapid haemolysis of human erythrocytes owing to their action on free phospholipids in the cell wall and also a slow breakdown of a lipoprotein in the cell wall (Rideal & Taylor, 1957). Cutaneous toxicity was found to arise from the use (for example, in certain cosmetics) of surface-active agents in high concentrations. However, cutaneous toxicity of surfactants is on the whole negligible, both in man and in animals (Bidaux, 1954).

Various reports exist on the phytotoxicity of surfactants. Spurrier & Jakobs (1955) found that germination and plant growth were reduced by surfactant applications, and Mirimanoff & Bolle (1949) observed that certain surfactants were toxic to the cells of *Elodea canadensis*. The tested concentrations were all higher than 100 ppm. Furmidge (1959) found that, in any homologous series of surfactants, the phytotoxicity passed through a maximum as the molecular size increased. This maximum usually occurred at chain lengths of dodecyl or lower.

The molluscicidal activity of surfactants has been studied by several workers. Jaskowski (1954) conducted tests on freshly hatched *Stanicola reflexa*, specimens of which he placed in 0.5% solutions of ten different detergents. He found that in most of the detergents the snails died within half an hour of immersion. Lagrange, Scheecqmans & Sarkissian (1950) found that, of 185 chemical compounds investigated, two cationic types of detergent (Cetavlon and Zephyron) were lethal to snails at a concentration of 10 ppm. Vallejo-Freire et al. (1954) found that 18 commercial cationic types of detergent had pronounced molluscicidal properties, and Perlowagora-Szumlewicz & Almeida de Aguiar (1952) have reported the molluscicidal activity of rosin soap.

METHOD

The present study was undertaken as a sequel to previous work on a naturally occurring snail-growth inhibitor that affects *Biomphalaria sudanica* (Berrie & Visser, 1963). This work had indicated that the toxicity of the inhibitor was probably partly due to its surface activity. It was therefore decided to test the rate of survival of the same snail species in various commercially produced surfactants. As a result of a request to British manufacturers for samples of their surface-active products, over a hundred types were obtained.

Because little information was available on the composition of the surface-active products, especially with regard to possible additives, measure-

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ments were made of the pH value and the electrical conductivity at 24°C of 1% solutions in very pure distilled water. These properties appeared to be quite normal for most of the products and were therefore expected to have negligible effect on the comparative molluscicidal activities of the chemicals, especially at the lower concentrations. The manufacturers indicated that most of the surfactants were stable compounds, unaffected within fairly wide limits by acid or alkaline conditions.

The products were tested in the state in which they were received from the manufacturers, and no attempt was made to investigate whether the liquid products were solutions or pure chemicals.

Aqueous solutions of the surfactants were prepared in five different concentrations, ranging from 0.1 to 1000 ppm. The solvent used was artificial lake water of the same pH and electrical conductivity as the water in Lake Victoria (pH 7.0-7.2, conductivity 90×10^{-6} mho at 20°C) and containing a similar composition of dissolved ions: $NaHCO_3$ 47.9 ppm; KCl 4.7 ppm; $MgCl_2$ 16.4 ppm; and $CaCl_2$ 7.7 ppm. Artificial lake water was used because the natural water available contained appreciable amounts of surfactants. The artificial medium was also used for all control experiments. All the tested surfactants were highly soluble in water, with the exception of a few that did not dissolve entirely at the higher concentrations. In these cases suspensions were used.

To provide a basis for comparison, solutions of Bayluscide (Gönnert & Schraufstätter, 1959), which is at present one of the most thoroughly investigated molluscicides, were tested in all experiments side by side with the surfactants.

Laboratory-bred *Biomphalaria sudanica* were used for the experiments. The snails were uniform in size, healthy, and selected at random. Five snails were tested for each dilution in 50-ml test tubes. The snails were not fed during their exposure to the surfactants. The solutions were kept at between 22°C and 25°C, and were not aerated. As it was noticed that the snails frequently tried to escape from the medium, the tops of the tubes were closed with one-holed rubber stoppers, and snails that had left the solutions were regularly returned to them by tapping the tubes against a hard surface. The snails were considered to be dead when they remained completely motionless even after repeated tapping, and had either completely retracted into their shells or had tried to leave them. In cases of doubt, the snails were transferred to clean lake water and observed for periods of up to 24 hours. Sometimes the phenomenon of haemolysis preceded the moment of death.

The solutions were considered to be toxic if 100% of the snails died and to be non-toxic if at least 80% survived. Gönnert (1961), in his experiments on Bayluscide, used 24-hour exposure times, but, since other authors used times of 5-6 hours, the present experiments were conducted with exposure times of 5 hours and 48 hours. The results of the 48-hour period were similar to those obtained with periods of 24-144 hours.

When the 48-hour toxic concentrations had been determined, solutions of the same concentration were made up with double-distilled water instead of artificial lake water and used for the measurement of surface tension. These measurements were carried out at 30°C ($\pm$0.001°C) by means of the drop-weight method of Harkins & Brown (1916, 1919), using apparatus practically identical to that described by Harkins (revised Alexander) (1959). If no information was available on the nature of the active component, the surface tension was not measured unless the toxic action seemed particularly interesting.

The ovicidal effect of the most effective surfactants was also tested, as was their toxicity to crustacea, fish, water plants, mosquito larvae and mice.

RESULTS

Molluscicidal effect

Of the 105 surfactants tested [1] at exposure times of 48 hours, five were non-toxic to *Biomphalaria sudanica*, even at concentrations of 1000 ppm, 21 were toxic at concentrations of 100-1000 ppm, 40 were toxic at 10-100 ppm, 26 at 1-10 ppm, 11 at 0.1-1 ppm, and two at concentrations lower than 0.1 ppm. These two last-mentioned surfactants were the tertiary amines Empigen AE3 and Empigen AN.

At 5-hour exposures, 21 of the surfactants were non-toxic at 1000 ppm, 50 were toxic at 100-1000 ppm, 31 at 10-100 ppm, and three at 1-10 ppm, the three last-mentioned being Ethylan CP, Ethylan OE and Ethylan TC.

The surfactants with the most effective molluscicidal properties are listed in Table 1.

[1] A table has been prepared showing in full detail the results obtained with the 105 surfactants. This has been deposited in the WHO Library, from which copies may be obtained on request.

TABLE 1
MOLLUSCICIDAL PROPERTIES OF SURFACTANTS

Trade name	Active compound	Manufacturer	pH	Conductivity at 24°C ($\times 10^{-6}$ mho)	Surface tension of solution of 48-hour toxic concentration (dyn/cm)	Toxic concentration for 48-hour exposure (ppm)	Toxic concentration for 5-hour exposure (ppm)
Ace liquid detergent		Unilever Ltd	6.3	306	69.6-69.9	0.1-1	100-1000
Cibaphasol C		Ciba Clayton Ltd	9.2	436	68.7	0.1-1	10-100
Deciquam 222	didecyldimethyl ammonium bromide	British Hydrological Corporation	8.0	230	68.6-69.3	0.1-1	10-100
Empicol LXV	sodium lauryl sulfonate	Marchon Products Ltd	9.0	1 480	68.5	0.1-1	10-100
Empigen AE3	tert. lauryl ethoxydimethyl-amine	Marchon Products Ltd	9.9	32	< 70.8	< 0.1	10-100
Empigen AN	tert. lauryl/myristyl dimethyl-amine	Marchon Products Ltd	8.9	11	< 65.2	< 0.1	100-1000
Ethylan BCP	nonyl phenol/ethylene oxide condensate 1: 9	Lankro Chemicals Ltd	5.5	24	67.0-69.4	0.1-1	10-100
Ethylan CP	octyl phenol/ethylene oxide condensate 1: 9	Lankro Chemicals Ltd	6.2	22	46.6-63.9	1-10	1-10
Ethylan OE	cetyloleyl alcohol/ethylene oxide condensate 1: 14	Lankro Chemicals Ltd	6.4	20	67.6-70.7	0.1-1	1-10
Ethylan TC	lauryl amine/ethylene oxide condensate	Lankro Chemicals Ltd	9.3	37	64.4-67.5	1-10	1-10
Nonidet P40		Shell Chemical Co.	5.7	17	66.7-69.4	0.1-1	10-100
Perlankroll TL	triethanolamine lauryl sulfate	Lankro Chemicals Ltd	6.6	400	71.2-69.7	0.1-1	10-100
Sulphonated laurol liquid TA	lauryl alcohol triethanol-amine salt	Hickson and Welch Ltd	6.6	546	68.5-69.7	0.1-1	100-1000
Ultravon JU 400 %		Ciba Clayton Ltd	7.0	26	68.0-68.6	0.1-1	10-100
XL		Lancashire Tar Distillers Ltd	6.2	12	70.1-70.8	0.1-1	10-100

Death from haemolysis was sometimes, but not always, obvious, and the degree of haemolysis was certainly unrelated to the degree of toxicity.

Cationic detergents were, on the whole, more toxic than anionic or non-ionic detergents.

No direct correlation could be detected between the surface activity of the substances and their toxicity. Ethylan PC, for example, was not toxic, even though the solution had a surface tension as low as 36.9 dyn/cm—well below the average surface tension (60.8 dyn/cm) of the toxic solutions. It is likely, however, that the toxicity of surfactants is enhanced by their surface-active properties, which would, for instance, result in an increased absorption of the substances. When the surface activity of the surfactant solutions was reduced by the addition of Silicone MS Antifoam A, the toxicity was also reduced in most cases (Table 2). It is likely, therefore, that the toxic effect of most surfactants on *Biomphalaria sudanica* is of both a chemical and a physical nature.

Ovicidal effect

Investigations into the influence of surfactants on the eggs of *Biomphalaria sudanica* (Table 3) showed that the eggs were more resistant than were the snails themselves, while with Bayluscide the sensitivity of snail eggs had been found to be the same as, or slightly higher than, that of the adult snails (Gönnert, 1961). At concentrations of 10 ppm or less, none of the surfactants investigated had any noticeable effect on the snail embryos. However, at least one of the characteristics of surfactants is their effect on the permeability of membranes, and

TABLE 2
EFFECT ON TOXICITY OF DECREASE IN SURFACE ACTIVITY

Surfactant	48-hour toxic concentration (ppm)	
	Without antifoam	With antifoam
Ace liquid detergent	0-1.1	10-100
Cibaphasol C	0.1-1	10-100
Deciquam 222	0.1-1	0.1-1
Empicol LXV	0.1-1	10-100
Empigen AE3	< 0.1	0.1-1
Empigen AN	< 0.1	0.1-1
Ethylan BCP	0.1-1	0.1-1
Nonidet 40	0.1-1	1-10
Perlankroll TL	0.1-1	10-100
Sulphonated laurol liq. TA	0.1-1	10-100
Ultravon JU 400%	0.1-1	0.1-1
XL	0.1-1	1-10

it was observed that after immersion in the surfactants at higher concentrations swelling of the embryo took place, often followed by a bursting of its outer membrane.

Effect on crustacea

No toxic effect of the surfactants on the crustacea *Daphnia* and *Cyclops* was observed at concentrations of up to 10 ppm and for periods of contact of up to 24 hours.

Toxicity to fish

Table 4 shows the times of survival of various types of fish in different solutions of the 13 surfactants that were toxic to *Biomphalaria sudanica* at concentrations below 1 ppm. The surfactants were only very rarely toxic to the fish at concentrations below 10 ppm, which is in most cases at least ten times higher than the concentration at which snails died.

Tests were also conducted on *Gambusia affinis*, *Haplochromis versicolor*, and *Aphelochilichthys pumilic*. These species of fish are all known to eat mosquito larvae. Insufficient numbers were available for the results of exposure to surfactants to be fully reliable, but in each case the toxic concentration was considerably higher than that necessary to kill snails. Bayluscide killed the different types of fish within one hour at concentrations as low as 1 ppm.

Solutions of Empigen AE3 and Empigen AN retained their effectiveness for periods of up to three weeks in aquaria with much organic debris on the bottom, and it therefore appears that these surfactants are not absorbed by decomposing organic matter to any appreciable extent.

Phytotoxicity

The 13 surfactants were tested against two types of water plant—*Azolla* sp. and *Ceratophyllum demersum*. The first plant is known to be especially sensitive to adverse conditions (Denny—personal communication). For both plants it is easy to fix a criterion of death. The experiments were conducted over a period of two weeks, and, as Table 5 shows, the toxic concentration of most of the surfactants proved to be 10-100 ppm. Bayluscide killed both types of water plant at a concentration of slightly over 1 ppm.

Toxicity to mosquito larvae

MacGregor (1924) showed that soap introduced into streams from washing activities, even when extremely diluted, inhibits the breeding of mosquitos. Hopkins (1952) confirmed the observation in Uganda.

TABLE 3
TIMES OF SURVIVAL OF *BIOMPHALARIA* EGGS IN SOLUTIONS OF SURFACTANTS

Surfactant	Time of survival of *Biomphalaria* eggs (hours)		
	1 ppm	100 ppm	10 000 ppm
Ace liquid detergent	—	—	< 5
Cibaphasol C	—	24	< 5
Deciquam 222	—	< 5	< 5
Empicol LXV	—	—	< 5
Empigen AE3	—	< 5	< 5
Empigen AN	—	—	< 5
Ethylan BCP	—	24	< 5
Nonidet P 40	—	< 5	< 5
Perlankroll TL	—	< 5	< 5
Sulphonated laurol liq. TA	—	—	< 5
Ultravon JU 400 %	—	—	24
XL	—	< 5	< 5

TABLE 4
TIMES OF SURVIVAL OF FISH IN SOLUTIONS OF SURFACTANTS

Surfactant	Time of survival (hours)								
	Astatoreochromis alluaudi			Tilapia nilotica			Tilapia zillii		
	1 ppm	10 ppm	100 ppm	1 ppm	10 ppm	100 ppm	1 ppm	10 ppm	100 ppm
Ace liquid detergent	—	—	0.5	—	—	1	—	9	1
Cibaphasol C	—	17	1	—	—	0.5	—	—	1
Deciquam 222	—	1.5	1	—	7.5	0.5	20	1.5	1
Empicol LXV	—	—	0.5	—	—	1	—	18	0.5
Empigen AE3	—	1.5	0.5	2.5	0.5	0.5	—	1	0.5
Empigen AN	—	—	2.5	—	—	—	—	—	2.5
Ethylan BCP	—	2.5	0.5	—	—	0.5	—	—	0.5
Ethylan OE	—	18	1.5	—	9	1.5	—	2.5	1.5
Nonidet P 40	—	—	0.5	—	5	0.5	—	2.5	0.5
Perlankroll TL	—	—	5	—	—	2.5	—	—	2.5
Sulphonated laurol liq. TA	—	—	9	—	—	9	—	—	9
Ultravon JU 400 %	—	—	0.5	—	—	0.5	—	9	0.5
XL	—	—	1.5	—	—	0.5	—	9	1.5
Bayer 73	1	0.5	0.5	1	0.5	0.5	1	0.5	0.5

TABLE 5
TOXICITY OF SURFACTANTS TO TWO TYPES OF WATER PLANT

Surfactant	48-hour toxic concentration (ppm)	
	Azolla sp.	*Ceratophyllum demersum*
Ace liquid detergent	⩾ 100	⩾ 100
Cibaphasol C	> 10	> 10
Deciquam 222	> 1	> 10
Empicol LXV	⩾ 100	> 10
Empigen AE3	> 10	> 10
Empigen AN	> 10	> 10
Ethylan BCP	> 10	> 10
Ethylan OE	⩾ 100	⩾ 100
Nonidet P 40	> 10	> 10
Perlankroll TL	⩾ 100	⩾ 100
Sulphonated laurol liq. TA	⩾ 100	⩾ 100
Ultravon JU 400 %	> 10	> 10
XL	⩾ 100	> 10
Bayer 73	> 1	> 1

The toxicity of the 13 surfactants was tested on larvae of *Aedes (Stegomyia) aegypti* and *Anopheles gambiae* during times of contact of up to 24 hours. Some difference might have been expected in the toxic effects on these two species since they have different floating and respiratory mechanisms.[1] In fact, however, the two species exhibited similar susceptibility to the surfactants, both surviving concentrations of up to 10 ppm. At a concentration of 100 ppm, Empigen AE3 proved to be the most toxic agent, killing both kinds of larvae within one hour, while Deciquam 222 caused the death of the larvae within 18 hours. Bayluscide at 100 ppm killed *Aedes aegypti* after 3½ hours of contact, while at the same concentration it seemed to be harmless to *Anopheles gambiae*.

Toxicity to warm-blooded animals

Mice were fed for seven days on food containing 1-100 ppm of the 13 surfactants. During this period no harmful effects were observed. Although the period of contact was admittedly too short to draw

[1] Maple (1945) reported that DDT appeared to kill *Anopheles quadrimaculatus* larvae not by outright toxicity but by producing symptoms that caused death by drowning.

any definite conclusions on the effect of surfactants on mice, it is unlikely that the time of direct contact with surfactants in practice would ever be as long as that of the test period.

DISCUSSION

The two most interesting surface-active agents from the point of view of their molluscicidal action were found to be the tertiary amines Empigen AE3 and Empigen AN, which both kill snails after two days of contact at a concentration of 0.1 ppm. One of the advantages of these two substances over other molluscicides would seem to be their specific toxicity to snails. Although these agents will not kill snails' eggs at the exposures and concentrations investigated, neither are they likely to kill fish, crustacea or water plants. Also, they do not appear to be absorbed by organic matter to any appreciable extent.

The reason for the toxicity of surfactants is not clear, but it is unlikely to be related solely to the surface activity, because the surface tension of 0.1 ppm solutions was lower by only 7 dyn/cm than that of pure water. Experiments with mixtures of these surfactants with other surface-active compounds or with an antifoaming agent also suggest toxicity effects other than those caused by surface-active properties.

The molluscicidal value of surfactants may usefully be borne in mind when selecting a surface-active agent for the dispersion of other less soluble molluscicides.

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RÉSUMÉ

Sur plus de 100 produits tensio-actifs du commerce dont les auteurs ont étudié, en laboratoire, les propriétés molluscicides chez *Biomphalaria sudanica*, 13 se sont montrés efficaces à des concentrations inférieures à 1 ppm, pour une durée d'application de 48 heures. L'Empigen AE 3 et l'Empigen AN étaient actifs à la concentration de 0,1 ppm.

L'action toxique de ces 13 préparations a d'autre part été remarquablement spécifique: elles n'ont affecté que médiocrement les crustacés (*Daphnia* et *Cyclops*), les plantes aquatiques (*Azolla* sp. et *Ceratophyllum demersum*), les larves de moustiques, les souris, les poissons et les œufs de *B. sudanica*.

Il semble que ces produits tensio-actifs ne soient pas absorbés par les matières organiques dans une mesure appréciable, et que l'action toxique sur *B. sudanica* soit de nature à la fois chimique et physique.

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