Arch. Hydrobiol.	74	1	123—132	Stuttgart, August 1974

The Acute Toxicity of Three New Surfactant Mixtures to a Mayfly Larvae

J. MICHAEL DOLAN III, BENTLEY C. GREGG, JOHN CAIRNS, Jr, KENNETH L. DICKSON, and ALBERT C. HENDRICKS, Blacksburg, Virginia

With 5 figures and 5 tables in the text

Abstract

The individual toxicity of linear dodecyl benzene sulfonate (DBS), linear tridecyl benzene sulfonate (TBS), and a nonionic surfactant (a blend of two primary alcohol ethoxylates) to mayfly larvae (*Isonychia* sp.) were determined using static bioassays. Specimens were maintained for one week at 8—10° C with a photoperiod of 12L:12D and 40—70 ft. candles illumination in dechlorinated Blacksburg tap water (hardness = 50—65 mg/l). Tests were carried out in 3.8 liter glass jars containing a final test volume of 2.0 liters. Logarithmic — probability regression lines, EC_{50} and ET_{50} values were obtained using the BMD computer program and confidence limits, slopes and slope parallelism significance were determined using the LITCHFIELD-WILCOXON method. Results follow: for DBS 96 hr EC_{50} = 5.33 mg/l (LITCHFIELD-WILCOXON confidence limits 4.23—6.72); TBS 96 hr EC_{50} = 1.36 (1.06—1.76); nonionic blend 96 hr EC_{50} = 2.96 (2.44—3.58). At 18.0 mg/l the ET_{50} values were: DBS = 1230 min. (946—1598); TBS = 231 (202—264); nonionic blend = 685 (600—782). The main value of these preliminary tests was to determine the relative toxicity of each of the test materials under comparable conditions.

Introduction

MARCHETTI (1965) has attributed most of the toxicity of modern detergents to the surfactant portion of the detergent. Detergents consist primarily of a surface active agent (surfactant) and polyphosphate salts (builders). Many workers have studied the toxicity of surfactants to vertebrates but few have studied their effects on aquatic invertebrates. ARTHUR (1970) used continuous flow techniques to study toxicity to *Gammarus* of a detergent containing a "soft" (biodegradable) linear alkyl sulfonate (LAS) surfactant. He observed average 96 hr TL_m values of 7 mg/l LAS and in chronic studies of survival, growth, reproduction, feeding, and mobility the maximum acceptable concentration of LAS was 0.2—0.4 mg/l. DEGENS et al. (1950) and MANN (1955) surveyed the effects of surfactants and detergents on various other fish food organisms. DEGENS et al. (1950) observed that the anionic surfactants were toxic to *Daphnia* at 5 mg/l, but

that higher concentrations could be tolerated after acclimation to the surfactant. MANN (1955) described a series of experiments on *Tubifex, Chironomus, Carinogammus,* and *Asellus* with a toxicity range of 10—25 mg/l for the anionic and nonionic surfactants studied.

HEPWORTH (1961) and SURBER & THATCHER (1963) investigated toxicities of detergents containing "hard" (slightly biodegradable) alkyl benzene sulfonate (ABS) surfactant to some stream insects. HEPWORTH (1961) observed 96 hr LD_{50}'s of a nonionic detergent, Kyro-EO, on Ephemeroptera naiads, Plecoptera naiads, Odonata naiads, and Diptera larvae. These values are 5.2 ppm, 4.7 ppm, 4.9 ppm and $>$ 10 ppm, respectively. SURBER & THATCHER (1963) observed that the mayfly larvae *Stenonema ares* and *S. heterotarsale* and *Isonychia bicolor* were drastically reduced or eliminated at an ABS concentration of 8 ppm. The caddis fly larvae, *Cheumatopsyche*, had a 53 % survival at a concentration of 8 ppm and 29 % survival at 32 ppm.

The purpose of this study was to determine the relative acute toxicity of each of three new surfactant formulations to a mayfly larvae (*Isonychia* sp.) by using static bioassays.

Table 1. Formulation of Surfactants as Provided by the Soap and Detergent Association.

	Linear Dodecyl Benzene Sulfonate (DBS)	Linear Tridecyl Benzene Sulfonate (TBS)	Nonionic
Solids	44.7 %	49.1 %	Blend of 2
Free Oil	0.8 %	0.4 %	primary alcohol
pH	8.8 %	11.8 %	ethoxylates
alcohol insolubles	2.2 %	3.6 %	Taken as
active ingredient	41.6 %	45.3 %	100 % active ingredient
Carbon Chain Length			
C_{10}	13.2	1.2	Chain lenghts
C_{11}	32.7	4.0	C_{12}—C_{15}
C_{12}	37.9	16.5	with 60 %
C_{13}	13.2	48.0	ethoxylation
C_{14}	3.0	30.2	
Phenyl Isomer distribution			
2 phenyl	19.6	15.6	
3 phenyl	19.2	17.0	
4 phenyl	18.5	16.5	
5 phenyl	25.9	19.8	
6+7 phenyl	16.8	31.0	

Methods and Materials

The three surfactants used in this study were linear dodecyl benzene sulfonate (DBS), linear tridecyl benzene sulfonate (TBS), and a nonionic surfactant (a blend of two primary alcohol ethoxylates) which were provided by the Soap and Detergent Association (SDA). Analyses of these surfactants were carried out by the SDA and are provided in Table 1.

Mayfly larvae (*Isonychia* sp.) were collected with a dip net from the New River near McCoy, Virginia and maintained for 1 week at a temperature of 8—10° C with a photoperiod of 12L:12D and 40—70 foot candles illumination. Bioassays with each surfactant were performed three times within a three week period. Before beginning a bioassay, mayflies were placed in 3.8 liter glass jars containing 1.5 l of dechlorinated Blacksburg tap water with a 4 cm square piece of plastic screen as a substrate. The tap water was dechlorinated by passage

Table 2. Means and standard deviations of chemical parameters in the highest concentration of surfactant and controls.

	Linear Dodecyl Benzene Sulfonate (DBS)		Linear Tridecyl Benzene Sulfonate (TBS)	
	32 mg l Before	32 mg/l After	18 mg/l Before	18 mg/l After
pH	7.8±0.2 (3)	7.5±0.2 (3)	7.7±0.3 (3)	7.4±0.3 (3)
Temperature (°C)	10.3±0.9 (3)	9.9±0.7 (3)	9.8±0.6 (3)	9.9±0.7 (3)
Dissolved Oxygen (mg/l)	9.1±0.9 (3)	8.9±1.0 (3)	9.0±1.1 (3)	8.7±1.1 (3)
Total Alkalinity as mg/l/$CaCO_3$	42.7±2.5 (3)	41.3±0.9 (3)	41.3±1.9 (3)	42.7±1.9 (3)
Total Hardness as mg/l $CaCO_3$	53.3±5.0 (3)	53.3±7.5 (3)	54.7±6.8 (3)	50.7±5.0 (3)

( ) = the numbers in parentheses represent the number of determinations.

Table 2. cont.

	Nonionic Blend		Controls	
	18 mg/l Before	18 mg/l After	Before	After
pH	7.8±0.1 (3)	7.2±0.3 (3)	7.5±0.2 (6)	7.4± 0.2 (6)
Temperature (°C)	10.5±0.5 (2)	10.0±0.8 (3)	9.5±0.4 (6)	9.8± 1.1 (6)
Dissolved Oxygen (mg/l)	9.4±1.4 (2)	5.3±1.7 (3)	9.1±1.3 (6)	8.8± 0.8 (6)
Total Alkalinity as mg/l/$CaCO_3$	40.7±0.9 (3)	44.7±3.4 (3)	38.7±3.8 (6)	34.7±10.3 (6)
Total Hardness as mg/l $CaCO_3$	60.0±8.6 (3)	84.0±42.5 (3)	54.7±6.0 (6)	63.3±23.7 (6)

( ) = the numbers in parentheses represent the number of determinations.

through an activated charcoal filter. The insects were acclimated to the jars for 24 hours before adding the surfactants, and the jars for both controls and tests were randomly placed in a water bath. At the beginning of each test, aliquots of the stock surfactant solutions were added to the jars to provide the desired concentrations, then additional dechlorinated tap water was added rapidly to mix the solution and to provide a final volume of 2 l. The jars were then gently swirled to insure complete mixing. A 100 ml sample of water for chemical analyses was removed from each test and control container at the beginning and end of each test. Dissolved oxygen and temperature were determined daily.

The mayflies were observed at the frequent intervals suggested by SPRAGUE (1972) and death was defined as the lack of response to gentle prodding with a small glass rod. Organisms were immediately removed when dead. Lengths, from the tip of the head to the base of the caudal filament, of 122 randomly selected mayflies were measured using a small plastic millimeter ruler. The mean length was 10.5 mm ± 1.8 S.E. Mean dry weight (90° C overnight) of the organisms was measured on a Mettler balance and was 2.6 mg ± 1.4 S.E.

Table 3. EC_{50}'s and 95 % confidence limits for 24 hr, 48 hr, and 96 hr for each surfactant.

	24 hr	48 hr	96 hr
Linear Dodecyl Benzene Sulfonate Mixture (DBS)	13.6 mg/l (11.0—16.9)	10.4 mg/l (8.8—12.4)	5.33 mg/l (4.23—6.72)
Linear Tridecyl Benzene Sulfonate Mixture (TBS)	4.19 mg/l (3.41—5.15)	2.47 mg/l (1.96—3.11)	1.36 mg/l (1.06—1.76)
Nonionic Blend	5.96 mg/l (4.73—7.52)	3.75 mg/l (2.95—4.76)	2.96 mg/l (2.44—3.58)

( ) = the values in parentheses represent 95 % confidence limits derived from LITCHFIELD & WILCOXON (1949).

Mortalities were analyzed to obtain the EC_{50} (Median Effective Concentration) and time-until-death ET_{50} (Median Effective Time) values. The data were evaluated by BMD03S Biological Assay, Probit Analysis — Revised computer analysis (DIXON, 1970). This program was intended for EC_{50} data, but will determine "best fit" log — probit lines for ET_{50}'s as well. Confidence limits of the computer obtained EC_{50} values and slopes were computed by the method described by LITCHFIELD & WILCOXON (1949). Confidence limits and slopes of the computer obtained ET_{50} values were determined by the method described by LITCHFIELD (1949).

Hardness, alkalinity, and pH were determined at the beginning and end of each bioassay. Dissolved oxygen concentration and temperature were measured daily using a YSI Model 54 D.O. meter. Hardness was measured by the titrimetric method described in Standard Methods 13th Edition (1971). Alkalinity was measured using the bromcresol green — methyl red titration also described in the 13th edition of Standard Methods. The pH was determined using a Fisher Accumet Model 210 pH meter (Table 2).

Table 4. Slopes and 95 % confidence limits for EC_{50} regression lines for each surfactant.

	24 hr	48 hr	96 hr
Linear Dodecyl Benzene Sulfonate Mixture (DBS)	2.04 (1.63—2.55)	1.94 (1.66—2.26)	2.32 (1.63—3.32)
Linear Tridecyl Benzene Sulfonate Mixture (TBS)	2.06 (1.72—2.46)	2.28 (1.73—3.01)	2.46 (1.80—3.35)
Nonionic Blend	2.31 (1.76—3.06)	1.84 (1.38—2.45)	1.88 (1.44—2.44)

( ) = 95 % confidence limits from LITCHFIELD & WILCOXON (1949).

Results and Discussion

EC_{50} values for each of the three surfactants are given in Table 3. Slopes of the curves are reported in Table 4. Statistically significant differences between any two parameters were determined by dividing the larger value by the smaller to obtain a potency ratio (PR) for the EC_{50}'s or a slope ratio (SR) for the slopes (LITCHFIELD & WILCOXON, 1949). The ratio was then compared with the appropriate f-factors using a nomograph in

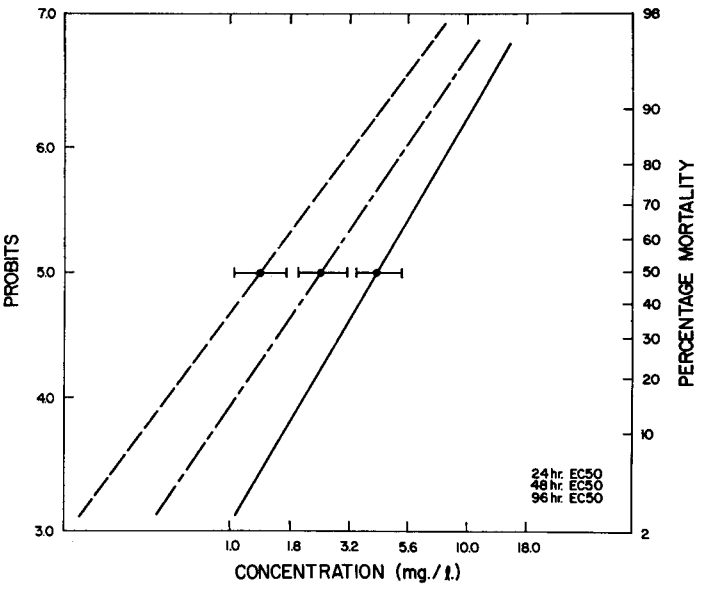

Fig. 1. Regression lines for determining EC_{50}'s of the linear tridecyl benzene sulfonate mixture (TBS) to *Isonychia* sp.

Litchfield & Wilcoxon (1949) constructed to determine the 95 % significance level.

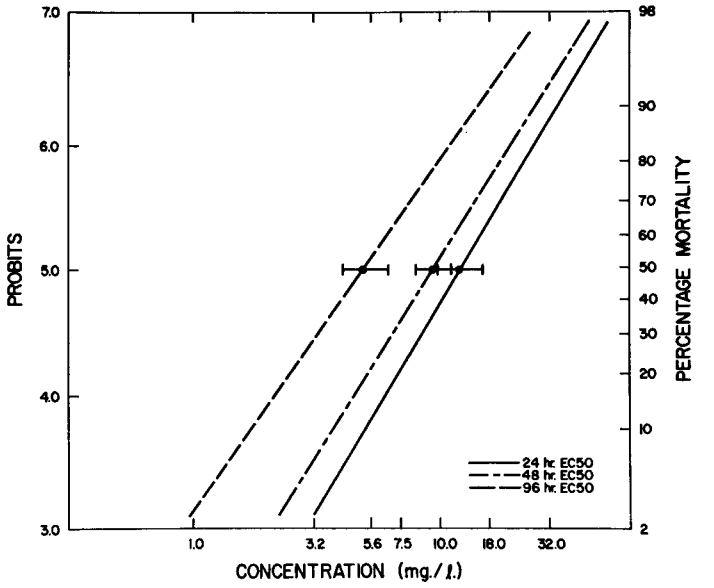

Fig. 2. Regression lines for determining EC_{50}'s of the linear dodecyl benzene sulfonate mixture (DBS) to *Isonychia* sp.

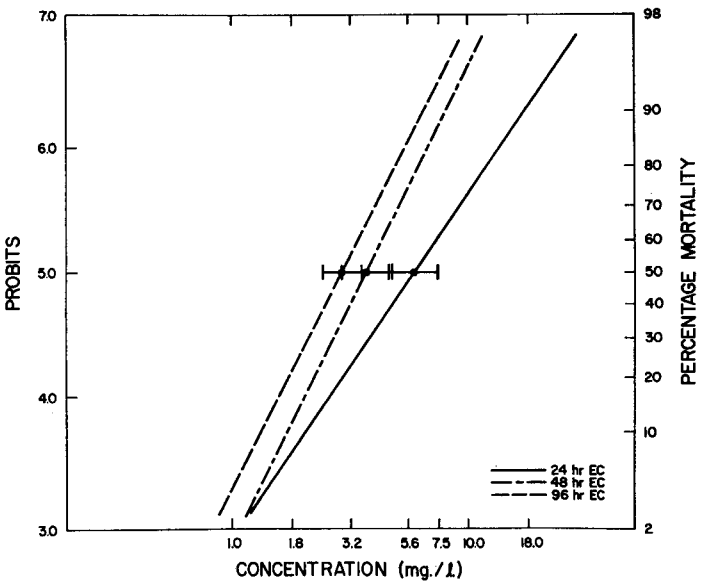

Fig. 3. Regression lines for determining EC_{50}'s of a nonionic surfactant to *Isonychia* sp.

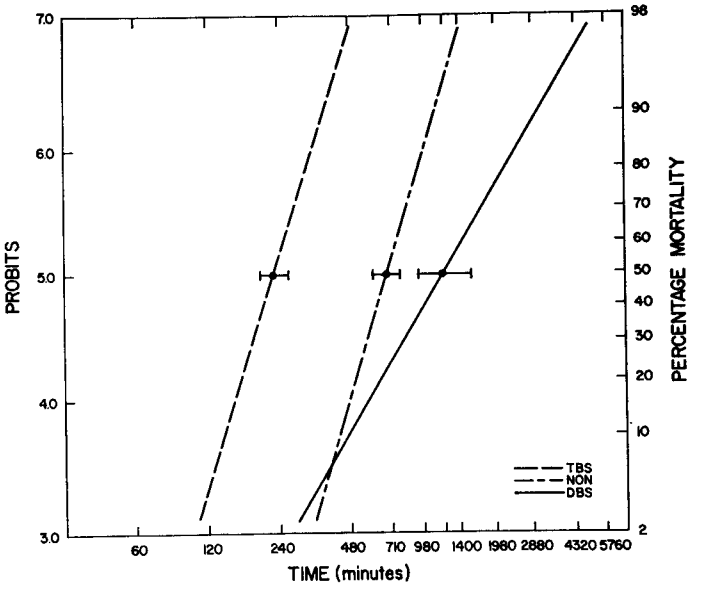

Fig. 4. Regression lines for determining ET_{50}'s for the three surfactants at a concentration of 18.0 mg/l to *Isonychia* sp.

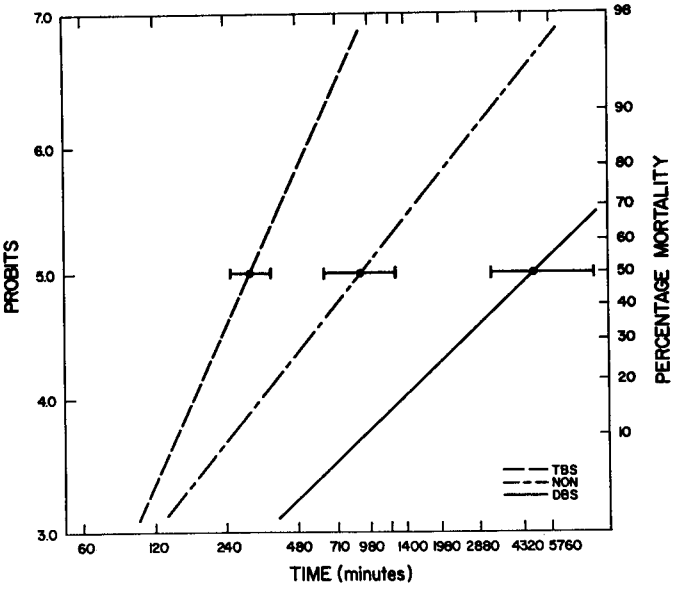

Fig. 5. Regression lines for determining ET_{50}'s for the three surfacants at a concentration of 10.0 mg/l to *Isonychia sp.*

The 24 hr., 48 hr., and 96 hr. EC_{50}'s for TBS are 4.19, 2.47 and 1.36 mg/l respectively (Fig. 1). There was a significant difference between all the EC_{50} values. However, the slopes for each of these time intervals were not significantly different from each other.

The EC_{50} curves for DBS are given in Fig. 2. Unlike the other surfactants, the 24 hr. and 48 hr. EC_{50} values were not different but the 48 hr. and 96 hr. EC_{50}'s were significantly different. Again the differences among the slopes were not significant.

The EC_{50} curves for the nonionic surfactant are provided in Figure 3. The EC_{50}'s for 24 hr. and 48 hr. were actually not significantly different, but the overlap of confidence limits was only 0.04 mg/l (Table 3), while the 48 hr. and 96 hr. EC_{50} values were definitely not significantly different, i.e. a large overlap. The slopes were not significantly different from each other.

The time-until-death analysis for the three surfactants at 18 mg/l and 10 mg/l are presented in Fig. 4 and Fig. 5, respectively. At both concentrations the relationship in these curves was similar. ET_{50}'s were all significantly different but the slope of the curves for TBS and the nonionic surfactant were significantly parallel at 18.0 mg/l, while the DBS slopes differed significantly from the other slopes (Table 5).

Table 5. ET_{50}'s and slopes and respective 95 % confidence intervals for time-until-death analyses with the three surfactants.

	Linear Dodecyl Benzene Sulfonate (DBS)	Linear Tridecyl Benzene Sulfonate (TBS)	Nonionic
18 mg/l ET_{50}	1230 min (946—1598)	231 min. (202—264)	685 (600—782)
18 mg/l Slopes	2.08 (1.71—2.56)	1.46 (1.33—1.64)	1.44 (1.32—1.58)
10 mg/l ET_{50}	5110 (3240—8080)	309 (255—374)	903 (635—1282)
10 mg/l Slopes	3.69 (2.52—5.40)	1.74 (1.51—2.00)	2.68 (2.10—3.44)

Based on the EC_{50}'s the order of toxicity follows: TBS (most toxic), Nonionic, and DBS (least toxic). The EC_{50}'s were all of the same order of magnitude (1.3—13.6 mg/l) but many significant differences existed between values. The time-until-death analyses confirm the same order of relative toxicity among the surfactants. The slopes of the time-until-death curves exhibited parallelism between the curves for TBS and the nonionic, but the curve for DBS was significantly different from the other two. This

similarity between the curves for TBS and the nonionic at 18.0 mg/l suggests a similar mode of action but additional study would be needed to determine the mode of action.

MARCHETTI (1965) in a review article reported that in both anionic and nonionic surfactants the toxicity to fish is generally related to molecular structure. Anionic surfactants are generally more toxic than nonionics, though anionics may exhibit considerable variability with the longer chain lengths being more toxic (MARCHETTI, 1965). The chain length relationship holds true for the two anionics in this study. The toxicity of the nonionic was intermediate to the anionics.

SURBER & THATCHER (1963) reported that mayfly nymphs (*Stenonema* and *Isonychia*) were killed by 10 days exposure to 16 ppm ABS and reduced in numbers by 10 days exposure to 8 ppm. HEPWORTH (1961) observed a 96 hr. LD_{50} of 5.2 ppm for mayfly nymphs using a nonionic, nonsulfonated detergent in a static test. Our results confirm those in both of these studies. SURBER & THATCHER (1963) used a "hard" anionic detergent which generally has a toxicity of about one-half, i.e. double the EC_{50}, of the straight chain anionics (MARCHETTI 1965). The 96 hr. ED_{50} of 5.96 ppm with the nonionic surfactant in these studies agreed very closely with the results of HEPWORTH (1961).

Acknowledgments

We gratefully acknowledge Mrs. HANNAH S. DOLAN for her assistance in performing the chemical analyses and the Soap and Detergent Association for their financial support.

Zusammenfassung

In Bioassay-Tests wurde die jeweilige Toxizität von geradkettigem Dodecylbenzolsulfonat (DBS), geradkettigem Tridecylbenzolsulfonat (TBS) und einer nichtionisierten, oberflächenaktiven Substanz (der Mischung aus zwei primären Alkohol-Äthoxylaten) für die Larven einer Eintagsfliege (Isonychia sp.) bestimmt. Die Versuchstiere wurden eine Woche lang bei 8—10° C und einer Photoperiode von 12 Stunden Licht zu 12 Stunden Dunkel bei 40—70 ft.cdls. Beleuchtungsstärke in entchlortem Blacksburger Leitungswasser (Härte 50—65 mg/l) gehalten. Die Tests wurden in Glasgefäßen mit 3,8 l Inhalt bei einem Endvolumen von 2,0 l durchgeführt. Logarithmische Regressionslinien, EC_{50}(Median effective concentration) und ET_{50}(Median effective time)-Werte wurden über das BMD-Computer-Programm errechnet und die Vertrauensgrenzen, Steigungen und Prüfungen der Steigungen auf Parallelität untereinander wurden mit der LICHTFIELD-WILCOXON-Methode ermittelt. Folgende Resultate wurden erhalten: für DBS 96 Std. EC_{50} = 5,33 mg/l (Vertrauensgrenzen nach LICHTFIELD-Wilcoxon 4,23 bis 6,72); TBS 96 Std. EC_{50} = 1,36 (1,06—1,76); nichtionisierte Mischung 96 Std. EC_{50} = 2,96 (2,44—3,58).

Bei 18,0 mg/l waren die ET_{50}-Werte: DBS = 1230 min (946—1598); TBS = 231 (202—264); nichtionisierte Mischung = 685 (600—782). Das Hauptziel dieser

vorläufigen Tests war die Bestimmung der relativen Toxizität der einzelnen Testsubstanzen unter vergleichbaren Bedingungen.

Literature Cited

American Public Health Association, American Water Works Association, Water Pollution Control Federation (1971): "Standard Methods for the Examination of Water and Wastewater", 13th ed. 874 pp.

ARTHUR, J. W. (1970): Chronic Effects of Linear Alkylate Sulfonate Detergent on *Gammarus pseudolimnaeus, Capeloma decisum* and *Physa integra.* — Water Res. **4:** 251—257.

DEGENS, P. N.; VANDERZEA, H.; KOMMER, J. D. & KAMPHIUS, A. H. (1950): Synthetic Detergents and Sewage Processing. V. The Effect of Synthetic Detergents on Certain Waterfauna. — J. Proc. Inst. Sewage Purif. **1:** 63—68.

DIXON, W. J. (1970): BMD Biomedical Computer Programs. — University of California Press, Berkeley.

HEPWORTH, W. G. (1961): Toxicity of the Non-Ionic Detergent Kyro-EO to Brook Trout, Rainbow Trout and Nymphal Mayflies, Stoneflies, Damselflies and Larval Midges. — Federal aid in Fish and Wildlife Restoration-Wyoming FW-3-R-8 Work Plan No. 9, Job No. 1F, Analysis No. **21:** 40—41.

LITCHFIELD, J. T., Jr. (1949): A Method for Rapid Graphic Solution of Time-Per Cent Effect Curves. — J. Pharmac. Experim. Therapeut. **97:** 399—408.

LITCHFIELD, J. T., Jr. & WILCOXON, H. (1949): A Simplified Method of Evaluating Dose-Effect Experiments. — J. Pharmac. Experim. Therapeut. **96:** 99—113.

MANN, H. (1955): The Action of Surface-Active Washing Agents on Fish and Animals Forming Fish Food. — Arch. Fisch Wiss. **6:** 131—137. (Water Pollution Abstracts **28:** 2569).

MARCHETTI, R. (1965): A Critical Review of the Effects of Synthetic Detergents on Aquatic Life. — Stud. Rev. Gen. Fish. Coun. Medit. **26.**

SPRAGUE, J. B. (1973): The ABC's of Pollutant Bioassay Using Fish. In: "Biological Methods for the Assessment of Water Quality", (J. CAIRNS, Jr. and K. L. DICKSON, eds.) — Amer. Soc. for Testing and Materials: 6—30.

SURBER, E. W. and THATCHER, T. O. (1963): Laboratory Studies of the Effects of Alkyl Benzene Sulfonate (ABS) on Aquatic Invertebrates. — Trans. Amer. Fish Soc. **92:** 152—160.

The address of the authors:

Biology Department and Center for Environmental Studies Virginia Polytechnic Institute and State University Blacksburg, Virginia, 24061, U.S.A.

Correspondence should be sent to BENTLEY C. GREGG.