JJH: JCL: TGG: RF

XF: N/A

Conoco Chemicals Company
A Division of Conoco Inc.
15990 North Barker's Landing Road
P.O. Box 19029
Houston, TX 77224

January 30, 1984

Mr. Bob Townsend
Kleen-Brite Labs
100 Fair Street
Brockport, NY 14420

Dear Mr. Townsend:

Per your request, enclosed is information on the aquatic toxicity of Linear Alkyl Benzene Sulfonates. No applicable information could be found on sulfonic acids. Conoco Chemicals has done no aquatic testing on these products. The enclosed articles are from open scientific literature.

Please contact us if you have questions on the enclosed.

Sincerely,

Thomas G. Grumbles, C.I.H.
Director, Industrial Hygiene

ajo

Enclosure

cc G. V. Curran

HUMAN SAFETY AND ENVIRONMENTAL ASPECTS OF MAJOR SURFACTANTS

A Report To The
SOAP AND DETERGENT ASSOCIATION

May 31, 1977

i.a

Arthur D Little Inc

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[redacted]ency of Gammarus was reduced at the lowest LAS concentration employed [redacted] mg/l). The possible toxicity of detergent components other than LAS [redacted] not considered.

The effect of 30 days' exposure of 4 species of fresh water fish to the [redacted] formulation described by Arthur (1970) above was examined by McKim et [redacted] (1975). Statistically significant reductions in the 30-day standing crop [redacted] noted (Table 1-I).

These few studies which only approach the problem of the possible chronic [redacted]icity of LAS to aquatic organisms indicate that long-term exposure may re[redacted]lt in toxic effects because of the increased sensitivity of early develop[redacted]tal stages; e.g., sac-fry and larvae, as compared to adult organisms. How[redacted]er, before any definitive evaluation of the chronic effects of LAS can be [redacted]empted, further work is necessary using test samples containing well char[redacted]terized LAS as the only surfactant and on typical environmental degradation [redacted]ples. Moreover, further chronic studies with fish and organisms of lower [redacted]onic levels would also be required.

3 Effects of Environmental Conditions on Toxicity

The toxicity of a chemical in an aquatic environment, natural or experi[redacted]al, is dependent on physical, chemical and biological conditions. Differ[redacted] species of fish exhibit various degrees of tolerance to toxic pollutants [redacted]nding on temperature, water hardness, dissolved oxygen and heavy metals.

2. Chronic Toxicity

One of the first reports of LAS chronic toxicity to fish was by Bardach et al. (1965) who found that LAS at concentrations of 0.5 mg/l for 24 days resulted in damage to the chemoreceptors of the taste buds of yellow bullheads (Ictalurus natalis). The study of Pickering and Thatcher (1970) on the effects of LAS (SDA Interim Reference Sample, LAS Lot No. 1-1, LAS-60.8%) to fish remains to the present the only effort to examine the chronic toxicity of LAS. They examined a number of responses with the fathead minnow (Pimephales promelas) in continuous flow systems including 5-week growth, egg production, hatchability and fry survival. Five-week growth, egg production and hatchability were not affected by mean LAS concentrations up to 2.7 mg/l. In agreement with other studies, the fry were more sensitive than other stages with deaths occurring at levels of LAS of 0.63 mg/l or above, and the greatest sensitivity at 7 to 14 days. The authors noted that even during the 96-hour TLm tests that 80 to 90% of the LAS as measured by MBAS was lost. For chronic studies, difficulty was encountered because of the increasing efficiency of biodegradation even though a dilution device was used to feed LAS. Standard deviations of MBAS values in 7-day composite samples amounted to 25% of the MBAS values.

Arthur (1970) studied the effects of a detergent containing LAS (14%), alcohol ethoxylate (2.3%), sodium soap (2.5%) and inorganic salts. The results were reported in terms of LAS concentrations alone for the invertebrate test organisms which were the amphipod (Gammarus pseudolimnaeus) and 2 species of snails (Campeloma decisum and Physa integra). The organisms were exposed acutely followed by a 6-week exposure of survivors. Survival of Physa was unaffected at LAS concentrations up to 4.4 mg/l, whereas Gammarus was affected at 0.4 mg/l and Campeloma at between 1.9 and 4.4 mg/l. Survival of F_1 and F_2

·:;4) found the 96-hour LC_{50} to larvae of the mayfly (Isonychia sp.) to be ·.33 mg/l (Litchfield-Wilcoxon confidence limits, 4.23-6.72) for a well defined ·:ple of LAS (C_{10}-13.2%, C_{11}-32.7%, C_{12}-37.9%, C_{13}-13.2%, C_{14}-3.0%).

Hendricks et al. (1974) found that a high molecular weight LAS (C_{13}, ·-362) was more toxic to the snail (Goniobasis sp.) than a low molecular ·eight LAS ($C_{11.6}$, MW-342). The respective 24 hr LC_{50} values were 19.4 ·:.6-38.9) and 92 mg/l.

Although algae are not aquatic fauna, their critical role as the lowest trophic level of the aquatic food chain makes their discussion appropriate ·t this point. Hall (1973) has examined the effects of surfactants on phytoplankton and finds that results from toxicity assays provide useful data for prediction of aquatic environmental safety. For the 3 species examined, Selenastrum capricornutum, Microcystis aeruginosa and Navicula seminulum, the 5-day minimum algistatic concentrations of LAS were 1000 mg/l, 50 mg/l and 50 mg/l, respectively.

Thus, for invertebrates, toxicities of LAS vary widely due in some measure to the protective morphological characteristics (exoskeletons, closure mechanisms) of many organisms. These traits allow organisms in the adult stage to resist exposure to toxicants from any source. On the other hand, early developmental forms of many bivalves, crustaceans and lower forms are susceptible to LAS at concentrations found toxic for sac-fry of fish. In some cases, toxic effects of LAS were noted on larval stages at concentrations as low as 0.05 mg/l.

At an LAS concentration of 5 mg/l for 6 hours, siphon retraction was completely abolished in the cockle, while the same exposure resulted in only a slight reduction in this response in the clam. Among the crustaceans, the swimming ability of larval stages of the spider crab and barnacle were reduced severely (100-fold) by LAS at a concentration of 10 mg/l.

In a subsequent examination of the mussel (Mytilus edulis) by Granmo (1972), fertilization and early developmental stages were inhibited at concentrations as low as 0.05 mg/l and larval growth was depressed at an LAS concentration of 0.1 mg/l.

As part of the chronic study on the effects of an LAS-containing detergent (LAS-14.0%, alcohol ethoxylate-2.3%, sodium soap-2.5%) on 3 invertebrate species, Arthur (1970) reports 96-hour TLm values for the amphipod Gammarus pseudolimnaeus and for the snails Physa integra and Campeloma decisum of 7, 9 and 27 mg/l, respectively, based on the LAS content of the detergent. The possible toxicity of other components of the detergent was not considered. Moffett and Grosch (1967) have reported that LAS induces "gross developmental abnormalities" in larvae of 5 genera of marine invertebrates at 1 to 3 mg/l LAS. The genera studied were Arbacia (sea urchin), Asterias (starfish), Spicul- (sponge), Chaetopteris (annelid) and Molgula (tunicate). The exact nature of the gross abnormalities was not described. In another report by these same authors (1968), brine shrimp (Artemia sp.) exhibited a 50% lethality at 22 hours following an 8-hour exposure to 5 mg/l LAS. Dolan et al.

significantly and the percentage survival and growth of larvae decreased significantly at 1.0 and 0.5 mg/l, respectively.

Swedmark et al. (1971), in a broad-ranging study of marine organisms, have studied the effects of LAS (uncharacterized) on a number of marine bivalves and crustaceans. The LC_{50} values at 6 to 8°C are shown in Table 1-H. Other than the cockle and scallop adults of the species examined were markedly more sensitive. These data parallel the findings in fish with respect to increased sensitivity of early developmental stages.

TABLE 1-H

ACUTE TOXICITY OF LAS TO MARINE BIVALVES AND CRUSTACEANS

Species	96-Hour LC_{50} (mg/l)
Mussel (*Mytilus edulis*)	>100
Clams (*Mya arenaria*)	70
Cockle (*Cardium edule*)	15
Scallop (*Pecten maximus*)	<5
Decapod (*Leander adspersus*)	50
Decapod (*Leander squilla*)	>100
Hermit crab (*Eupagurus bernhardus*)	>100
Spider crab (*Hyas areneus*), adult	>100
stage I zoea larvae	9
Shore crab (*Carcinus maenus*)	>100
Barnacle (*Balanus balanoides*), adult	50
stage II naupluis larvae	3

Swedmark et al. (1971)

Large amounts of MBAS alone do not appear to exert any adverse effects on various fresh water fish. Rainbow trout, golden orfe, goldfish, bream, tench, roach, perch, carp, raffe, pace, chib, pike, rudd, gudgeon, stoneloach, spined loach and bullhead fish have been reported to survive, grow and breed in two small, artificially created lakes (Colworth Lakes). The main source of water in the lakes is sewage effluent which, due to the nature of the site, contains high MBAS levels (3 mg/l), higher than normal BOD and a large amount of total dissolved solids (Unilever Ltd., unpublished data).

An additional study indicating that toxicity to aquatic organisms of sewage effluents cannot readily be attributed to LAS was carried out by Calabrese and David (1967) with oysters (Crassostrea virginica). Although not a fish study, the results are appropriately considered in this discussion. Effluents from treatment of sewage without and with biodegraded LAS (5 mg/l) had approximately the same toxicities to oysters with respect to survival of larvae, development of eggs and increase in length of larvae. Thus, biodegraded LAS did not contribute to the toxicity of the sewage effluents.

f. Acute Toxicity to Invertebrates

Daphnia, a commonly tested invertebrate, showed no effect from exposure to LAS (44.7% LAS) at concentrations less than 1 mg/l. The 24 hr LC_{50} for this species was 3.46 (2.31-5.22) mg/l (Shell Chemical Company, unpublished data).

The effects of LAS (supplied by SDA, 60.8% LAS) on the oyster (Crassostrea virginica) have been studied by Calabrese and Davis (1967). At concentrations of LAS greater than 0.025 mg/l, the development of fertile eggs was reduced

Esvelt et al. (1971), in a study of San Francisco Bay, determined toxicity to estuarine fish (golden shiner, Notemigonous chrysoleucas) related to sewage plant effluents and found significant reductions in MBAS levels after biological sewage treatment and concomitant reductions in toxicity. Toxicity attributable to MBAS in this study was difficult to separate from the overall toxicity of sewage effluents. There was a significant difference in concentrations of MBAS in effluents from primary and other more extensive treatment processes. Mean MBAS values in effluents from 4 primary treatment plants in the San Francisco Bay area were 10.9, 5.0, 7.2 and 7.3 mg/l. For activated sludge plants, the average was 1.1 mg/l, occasionally reaching levels of 6.7-7.3 mg/l. It was determined from a mathematical model that MBAS and ammonia nitrogen were significantly correlated with toxicity of primary effluents. However, the direct addition of LAS to primary effluents had little effect on its toxicity lending further support to the view that MBAS levels are not conclusive measurements related to acute toxicities of primary effluents.

In a study at the Elm Farm Sewage Treatment Plant, which employs an activated sludge treatment process, it was found that MBAS levels contributed by LAS were generally reduced by greater than 95%. Testing of this effluent for toxicity in fathead minnows (Pimephales promelas) resulted in complete survival (Renn, 1974). In an effluent solution of only 36% MBAS removal, all of the tested individuals died. In this instance, MBAS concentrations were 11.5 ppm (Colgate-Palmolive Company, unpublished data).

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- The most fish-toxic components of a LAS are also the ones most rapidly biodegradable.
- The biodegradation, even a partial one, reduces greatly the toxicity of the surface-active agent.
- Different LAS isomers with different initial fish toxicity tend to be reduced and to become equal upon biodegradation.
- The value of the LC_{50} of any LAS tends to increase considerably with the progress of biodegradation.

e. MBAS-Related Acute Toxicity to Fish in Sewage Effluents

The data summarized above clearly show that LAS is rapidly degraded in laboratory simulations of the activated sludge sewage treatment process. The biodegradation of LAS results in a 10- to 100-fold reduction in acute toxicity to fish. In the actual environment, the situation is considerably more complex because of wide variations in treatment of waste waters and in the extreme diversity of effluents reaching natural water bodies with respect to amount and characteristics of materials other than LAS. Thus, the toxicity to aquatic organisms of sewage effluents and waters containing these effluents cannot be readily attributed to LAS even though these waters contain MBAS. The problems surrourding the use of MBAS as an analytical tool for LAS in natural waterways, especially those which receive sewage effluents, have been considered above (Section II.B).

TABLE 1-G

ACUTE TOXICITY OF LAS AND PRESUMPTIVE BIODEGRADATION INTERMEDIATES

	48-Hour LC_{50} (mg/l)	
	Daphnia magna	Pimephales promelas
Intact LAS-C_{11}	5.7 ± 0.6	16.0
Sulfophenylundecanoic acid, disodium salt (mixed isomers, 6-through 10-phenyl)	208 ± 85	76.6 ± 12.4
3-(Sulfophenyl)butyric acid, disodium salt	~6,000	~10,000
4-(Sulfophenyl)valeric acid, disodium salt	~5,000	~10,000

Kimerle and Swisher, 1977

HLAS was 0.72 mg/l. After biodegradation to 25% of the initial surfactant concentration (MBAS), 96-hour LC_{50} for the bluegill was increased to 1.64 mg/l and to 2.3-7.2 mg/l with 92% degradation. Undiluted biodegraded HLAS resulted in LC_{50} values ranging from 4.6 to less than 4.6 mg/l for 24 and 48 hours. At 50% and 90% biodegradation levels, 24-hour LC_{50} values were 5.0 and greater than 5.0 mg/l. A test using intact LLAS gave a 3.89 mg/l LC_{50} in 96 hours for bluegills. This LAS product also showed decreased toxicities for biodegradation products, with LC_{50} of 10.3 mg/l MBAS.

Kimerle and Swisher (1977) obtained evidence that toxicity of a commercial LAS preparation (C_{12}-C_{14}) to Daphnia magna decreased from an LC_{50} of 3 mg/l for the parent product to a level of 6 mg/l for a partially (50%) degraded product. Further biodegradation resulting in 80 to 90% removal of the initial concentration of MBAS produced LC_{50} values of 20 to 35 mg/l. Moreover, they showed that certain presumptive LAS biodegradation intermediates have little or almost no toxicity to either Daphnia magna or fathead minnow (Pimephales promelas) (Table 1-G).

The preparation of a set of computations designed to predict the acute fish toxicity of complex mixtures of LAS from a knowledge of their molecular composition has led Divo (1974) to several conclusions with respect to the relationship of biodegradation of LAS to toxicity. These conclusions agree with the data from the few studies performed to date:

In the first systematic study of this problem, Swisher et al. (1964) examined the effect of biodegradation on toxicity to bluegill (Lepomis macrochirus) fingerlings. They found that addition to continuous flow activated sludge units of as much as 100 mg/l C_{12}- or C_{14}-LAS resulted in effluents that did not exhibit any lethal toxicity. MBAS concentrations in the test tanks ranged from 0.1 to 0.9 mg/l. Toxicity tests of effluents from acclimated, as well as unacclimated sludge yielded toxic levels of LAS at 1 to 2 mg/l. A minimally altered LAS, mixed isomers of sulfophenyl-undecanoic acid disodium salt, gave a 96-hour TLm of 75 mg/l indicating that even a single oxidative alteration of the alkyl chain of LAS is sufficient to markedly reduce toxicity.

Borstlap (1967) found that the acute toxicity (minimum lethal concentration) for guppies (Lebistes reticulatus) decreased markedly from 5 mg/l to >1000 mg/l with the biodegradation of the commercial LAS product DOBS-C-300 (sulfonate of Dobane C-300™). Similar sharp reductions in toxicity of other commercial LAS products following their biodegradation have been reported for guppies (Poecilia reticulatus) and harlequins (Rosboral spp.) (Shell Research Ltd., London, unpublished data) as well as for rainbow trout (Salmo gairdnerii) (Unilever Ltd., unpublished data).

Cairns and Dickson (1973) have reported on a series of toxicity tests with intact and biodegraded LAS on bluegills and snails using high (HLAS) and low (LLAS) molecular weight products. The 96-hour LC_{50} value for intact

flow assays at 6-8°C, 96-hour LC_{50} values were 1.0, 1.5 and between 1.0 and 5.0 mg/l LAS, respectively. Tests conducted at 15-17°C gave 96-hour LC_{50} values of less than 1.0 mg/l for cod and plaice. As for fresh water fish, early developmental stages were more sensitive than adults. Concentrations of LAS of 0.1 and 0.3 mg/l significantly reduced survival time of cod and plaice, respectively, in the stages from hatching to yolk absorption. Sub-lethal responses such as impaired swimming activity and breathing rate as well as reduced opercular movement were observed in cod after exposure to 0.5 mg/l LAS for 24 hours. In contrast, flounder were more resistant, exhibiting normal swimming behavior after 21 days in 0.5 mg/l LAS.

Considering the available data on the acute toxicity of intact LAS to fish, the LC_{50} values for fingerlings and adults of a number of fresh water and marine species range from 1.0 to 10.0 mg/l. For those fresh water and marine species that have been examined, early developmental stages (e.g., sac-fry) are more sensitive to the acute toxic effects of LAS. Acute effects of LAS on sub-lethal manifestations of toxicity (swimming, breathing rate, opercular movement) occur at concentrations at or slightly below the LC_{50} values for the two species that have been examined for these responses.

d. Acute Toxicity to Fish-Biodegraded LAS

Although intact molecules of LAS are readily biodegraded in waste waters and waste water treatment plants as well as in natural waterways, there is a paucity of reliable information dealing with toxicity of degraded LAS to aquatic organisms.

$C_{10} - C_{14}$

TABLE 1-F

EFFECTS OF LAS ON BLUEGILL (LEPOMIS MACROCHIRUS)

Development Stage	TLm (mg/l)	Median Response (mg/l)
Fingerling	3.80 (24 hr)	
Sac-Fry		
5-day	3.4 (24 hr)	
1-day	2.3 (6 day)	
Newly hatched fry	>5.6 (24 hr)	
Fertilized eggs*		3.7-4.0 (hatching)
Fertilization		10
Sperm		5.4-5.7 (active swimming - gyration)

*Eggs burst at LAS concentrations >4.0 mg/l.

Hokanson and Smith, 1971

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C_{14} LAS necessary to reduce swimming activity to zero in 6 hours in the test system used by Marchetti were 4.7 and 3.2 mg/l, respectively. In this study, the lethal effects and subacute effects on locomotor activity appeared to be related.

Hokanson and Smith (1971) studied the toxicity of a well defined LAS sample (90% active; C_{10}-16.[illegible], C_{11}-[illegible], C_{12}-29.5%, C_{13}-18%, $\geq C_{14}$-2.5%) in Mississippi River water to various developmental stages of the bluegill (Lepomis macrochirus) ranging from sperm and unfertilized egg to fingerlings. They found that the feeding sac-fry were most sensitive to LAS: eggs and fingerlings exhibited intermediate sensitivity; egg fertilization was the least sensitive developmental step (Table 1-F).

Lubinski et al. (1974) also examined the toxicity of LAS to the bluegill (Lepomis macrochirus) in a continuous flow bioassay and found a 96-hour LC_{50} value of 6.5 mg/l. They also developed a concept of aquatic toxicity based on fractions of the 96-hour LC_{50} values of each of the identified toxicants in the Illinois River. They determined that the Illinois River water is not normally toxic to bluegills and that the major source of potential toxicity for fish would probably come from ammonia and cyanide, with LAS, copper, fluoride and zinc also contributing fractional toxicity.

In the only extensive study of marine fishes and their responses to LAS, Swedmark et al. (1971) investigated 3 species; i.e., cod (Gadus morrhua L.), flounder (Pleuronectes flesus L.) and plaice (P. platessa L.). In continuous

TABLE 1-E

ACUTE TOXICITY OF LAS TO FRESH WATER FISH

Species	Common Name	TLm (mg/l)	95% Confidence Limits (mg/l)
...opis atherinoides	Emerald shiner	3.0	2.96-3.56
...is macrochirus	Bluegill	4.0	3.70-4.30
...nales promelas	Fathead minnow	4.2	Not given
...opis cornutus	Common shiner	4.9	4.58-5.18
...alurus melas	Black bullhead	6.4	5.08-6.68

...ner and Santer, 1967.

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o several species of fresh water fish (Table 1-E). The results indicated hat "sufficient difference in sensitivity to LAS exists among species of ish to warrant attention to this factor when assessing the potential hazard f LAS to aquatic populations."

Pickering (1966) has investigated the effects of the same type of LAS reparation used by Thatcher and Santer (1967) on eggs of the fathead minnow Pimephales promelas) in a continuous-flow bioassay. The results expressed s 9-day TLm values for survival of hatched fry ranged from 2.3 to 2.6 mg/l n 4 replicate tests. The 1-day TLm value was 3.6 mg/l, with the threshold f mortality at 0.9 mg/l. The results with this species indicate that the egg and fry stages are more sensitive to LAS than are adults.

Dooley (1968) also examined the acute toxicity of this LAS sample obtained from the SDA on mosquito minnows (Gambusia affinis). At an LAS concentration of 0.1% (1000 mg/l), the survival time for males was 9 minutes and for females was 17 minutes. As the concentrations of LAS were reduced, survival times increased until the populations exhibited 72-hour survival at a level of 0.00078% (7.8 mg/l). The gills of fish killed by LAS were damaged showing a matted condition, occasional blood masses and loss of gill ucosa cells.

In addition to determining 6-hour LC_{50} values of 8.4 and 7 mg/l in goldfish (Carassius auratus L.) with C_{12}- and C_{14}-LAS products, respectively, archetti (1968) examined swimming activity. The concentrations of C_{12} and

en extensively studied for their aquatic toxicity. Divo (1974) found increased toxicity with an increase in chain lengths among a series of tetralins with chains from 10 to 13 carbon units. Kimerle and Swisher (1977) confirm this trend with 48-hour LC_{50} values in fathead minnow (Pimephales promelas) of 86.1, 21.5 and 5.3 mg/l for C_{10}, C_{12} and C_{14} dialkyltetralin-dane sulfonate mixtures, respectively. However, these side products are considerably less toxic than LAS isomers of comparable chain length.

c. Acute Toxicity to Fish - Intact LAS

In considering the reported data on the acute toxicity to fish of LAS and LAS-containing detergents, a number of factors should be weighed in the examination of experimental results, especially in relation to the use of these data to set aquatic safety standards. Abel (1974) has reviewed in detail the problems surrounding the assessment of toxicity of synthetic detergents to fish and aquatic invertebrates. In addition to wide variations in experimental protocols with respect to water temperature and chemistry and exposure patterns (static vs. continuous flow; water volume to organism mass ratio), the lack of adequate chemical characterization of the LAS samples tested and the wide range of susceptibilities among different aquatic species raise genuine difficulties for the comparative evaluation of acute toxicity studies.

In a series of continuous flow-through bioassays, Thatcher and Santer (1967) determined acute toxicities of an LAS preparation (SDA Interim Reference Sample: LAS Lot No. 1-1), 60.8% surfactant and 36.1% sodium sulfate,

TABLE 1-D

ACUTE TOXICITY OF INTACT LAS. EFFECT OF CHAIN LENGTH

LAS Homologues	48-Hour LC_{50} (mg/l)[1] Pimephales promelas	LC_{50} (mg/l)[2] Carassius auratus	LC_{50} (mg/l)[3] Lebistes reticulatus	LC_{50} (mg/l)[4] Idus melanotus	96-Hour LC_{50} (mg/l)[5]* Lepomis macrochirus
C_{10}	43.0	61.0	50	16.6	21.2-47.5
C_{11}	16.0	22.5	-	6.5	11.6
C_{12}	4.7	8.5	5	2.6	1.18-6.5
C_{13}	0.4	3.3	-	0.57	1.11
C_{14}	0.4	-	1	0.26	0.25-0.42
C_{16}			1	0.68	0.087
C_{18}			15		0.38

1. Kimerle and Swisher, 1977.
2. Gafa, 1974.
3. Borstlap, 1967.
4. Hirsch, 1963.
5. Procter and Gamble Company, unpublished data.

• LC_{50} values of individual LAS homologues dependent on phenyl group position. Lower LC_{50} values correspond to LAS with higher proportions of 2-phenyl isomers.

Arch. Environm. Contam. Toxicol. 8. 599–612 (1979)

Archives of Environmental Contamination and Toxicology

Acute Toxicity Studies of Surfactants to *Daphnia magna* and *Daphnia pulex*

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Abstract. Several experiments were designed to examine the acute toxicity of surfactants to *Daphnia*. Specific tests were designed to develop comparisons between existing acute toxicity data for fish and similar data for *Daphnia*, and to provide data on the effects of various environmental factors on resultant toxicity of surfactants to *Daphnia*.

Acute toxicity data for a series of homologous linear alkyl benzene sulfonates (LAS) demonstrate increases of up to one order of magnitude in toxicity for each increase of two alkyl carbons. LC 50's obtained with *Daphnia magna* are similar to those obtained with bluegills, *L. Macrochirus*. Comparative tests with *D. magna* and *D. pulex* indicate no statistical differences in 48-hr LC 50 values for three anionic and two nonionic surfactants. A 50 mg/L concentration of suspended, naturally-occurring kaolin significantly reduced the toxicity of longer chain length LAS homologs and had no effect on nonionic surfactant toxicity.

In tests with variable hardness concentrations, the acute toxicity of LAS to *D. magna* is a combined function of both culture and test water hardness. The toxicity of a nonionic surfactant to *D. magna* was higher in soft water and was not affected by culture water hardness levels.

Unlike previously published data for fish, the results of acute toxicity tests with *D. magna* cultures previously exposed to 0.4 mg/L LAS for periods up to seven generations indicated no significant difference in LAS susceptibility compared to simultaneously tested unexposed controls.

The use of laboratory toxicity tests with aquatic species serves the important function of predicting the effects on aquatic life of chemical substances potentially released to surface water environments. As such, toxicity data for aquatic species is integral to the development of aquatic safety assessments for new or expanded-use chemicals and for the establishment of relevant water quality criteria. Traditionally, acute tests have been done with representative fish species since they are the most visible and economically significant component of most aquatic communities. Recently, as the utility of acute toxicity data for aquatic safety programs has been realized and the number of chemicals requir-

0090–4341/79/0008–0599 $02.80

ing the submission of acute data in support of use or registration has increased, the demand for such data has produced increased interest in the use of invertebrate species for the development of needed toxicity information. Representatives of the genus *Daphnia* have emerged as some of the more important test organisms and as such they typify the advantages of employing macroinvertebrates for development of toxicity data: ease of laboratory culture and maintenance in a strictly defined media, small culture space and water requirements when compared to fish, ease of handling and counting, small test volume requirements, significantly shorter life cycles, and greater disease resistance.

This program was designed to examine the application of *Daphnia* acute toxicity data to aquatic safety programs for the evaluation of effects of residual concentrations of surfactants potentially reaching surface water communities. Specific objectives were to:

1. Examine the effects of changes in surfactant chemical structure on the biological activity as measured by acute toxicity to *Daphnia*, and compare effect concentrations with those published for fish species.
2. Test the relative susceptibility of *D. magna* and *D. pulex* to surfactant toxicity.
3. Examine the effects of environmental variables such as presence of suspended solids, variable water hardness, and previous surfactant exposures or acclimation on the acute toxicity of several classes of surfactants.

Materials and Methods

Test Materials and Analyses

The test materials employed in these investigations included both anionic and nonionic surfactants. The identifications and chemical descriptions are listed in Table 1. Since the surfactants were of varying degrees of activity, a determination of percent active material was performed on each material immediately prior to toxicity test initiation. A standard curve for each anionic homolog was obtained utilizing the analysis for methylene blue active substances (Amer. Pub. Health Assoc. 1971).

Table 1. Chemical characterization of the test surfactants. Generic names are used throughout the text

Generic Name	Structure	Chemical Characterization
C_{10} LAS through C_{13} LAS	$CH_3 - (CH_2)_X - CH_3$ $\vdots$ SO_3Na^-	Anionics, alkyl chain length range: C_{10}–C_{13}, molecular weight range: 341–453
Linear alkyl ethoxylates $C_{14}AE_1$ through $C_{14}AE_9$	$CH_3 - (CH_2)_X - (C_2H_4O)_YH$	Nonionics X = 13 Y = 1 to 9

Table 2. Dilution water quality for all *Daphnia* toxicity tests with surfactants

Parameter	Concentration (mg/L)
Hardness	120 mg/L as $CaCO_3$
pH	7.4 ± 0.2
Dissolved oxygen	8.5 – 9.5
Nitrate	<0.05
Nitrite	<0.05
Copper	<0.001
Iron	<0.05
Lead	<0.01
Sodium	11.6
Zinc	<0.001

Water samples were taken from each test concentration at the termination of the 48-hr exposure period, preserved with 1% formaldehyde, analyzed by the MBAS procedure and compared with the standard curve for confirmation of expected nominal test concentrations. Results for the nonionic surfactants are based on nominal concentrations since accurate analytical methodologies for quantification of dilute aqueous concentrations of these materials are not available.

Test Procedure

The methods used for culture procedures and acute toxicity tests followed the guidelines established by the USEPA (EPA-660/3-75-009, 1975). The quality of the carbon-filtered well water used as dilution water for all tests is listed in Table 2. All tests were carried out at a constant temperature of 21 ± 1°C under a 16-hr illumination period.

Prior to a test, adult *Daphnia*, sorted by size, were isolated in separate aquaria and the young produced overnight were tested the following day. In this manner, known age individuals, 24-hr old or less, were used to initiate all tests. The test containers were 250 ml Pyrex beakers with a total solution volume of 200 ml. The beakers were cleaned and sterilized after each test. Prior to use, the beakers were rinsed in hot tap water, brushed in 95% reagent grade alcohol, and rinsed in deionized water and allowed to dry. All tests were of 48-hr duration and all concentrations were done in triplicate. Five *Daphnia* were added with a glass pipet to each beaker at the test initiation. Mortality was recorded after 24 and 48-hr intervals. The individuals were not fed during the test. Results were analyzed using a computerized probit analysis program providing for calculation of LC 50 values and associated 95% confidence intervals (Finney 1971).

Deviations from this standard method were employed for tests incorporating kaolin clay (Georgia origin, mean particle size 4μ). For these tests, 50 mg/L kaolin was added to a 4 L volume of dilution water and vigorously mixed. Five 1-L beakers were then filled with 600 ml of this suspension, placed on magnetic stirrers, and the required amounts of test surfactant added to achieve the desired test dilutions. The solutions were then vigorously mixed for 30 min and the three 200 ml replicates of each test concentration were decanted into the test containers.

For tests designed to determine the effects of prior exposure or acclimation to sublethal concentrations of the anionic surfactant $C_{11.8}$ LAS, *D. magna* was cultured in a 0.4 mg/L LAS concentration for periods of 24-hr (short-term exposures) up to seven continuous generations (long-term exposures) prior to comparative toxicity testing with unexposed individuals. During the long-term acclimation period, the 0.4 mg/L LAS concentrations in the culture chambers were replaced three times weekly to minimize degradation of the LAS. All tests were run simultaneously with unexposed individuals to examine the significance of the prior exposure on acute toxicity of LAS.

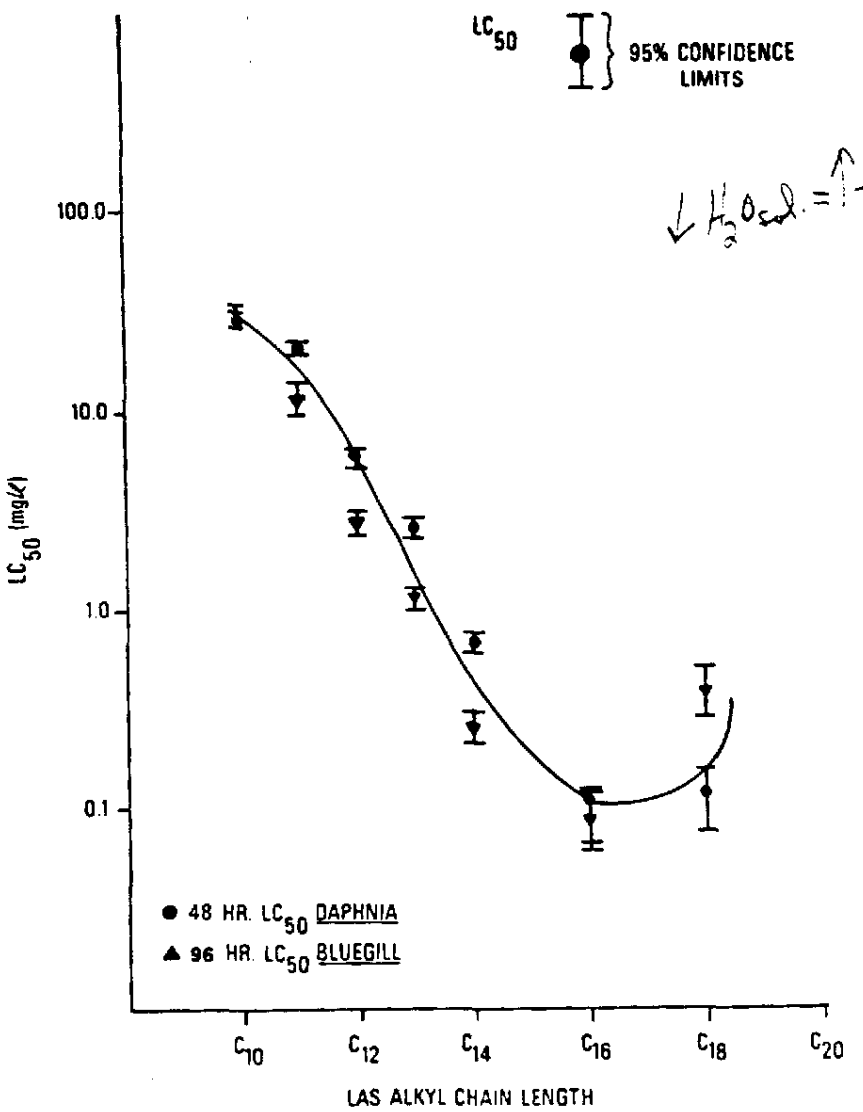

Fig. 1. The effect of variations in the alkyl carbon chain length of linear alkyl benzene sulfonate on resultant acute toxicity to *Daphnia magna*.

Results and Discussion

Structure: Activity Correlations

The effect of an increase in the alkyl carbon chain length of the anionic surfactant, LAS, on resulting acute toxicity is summarized in Figure 1. An increase in

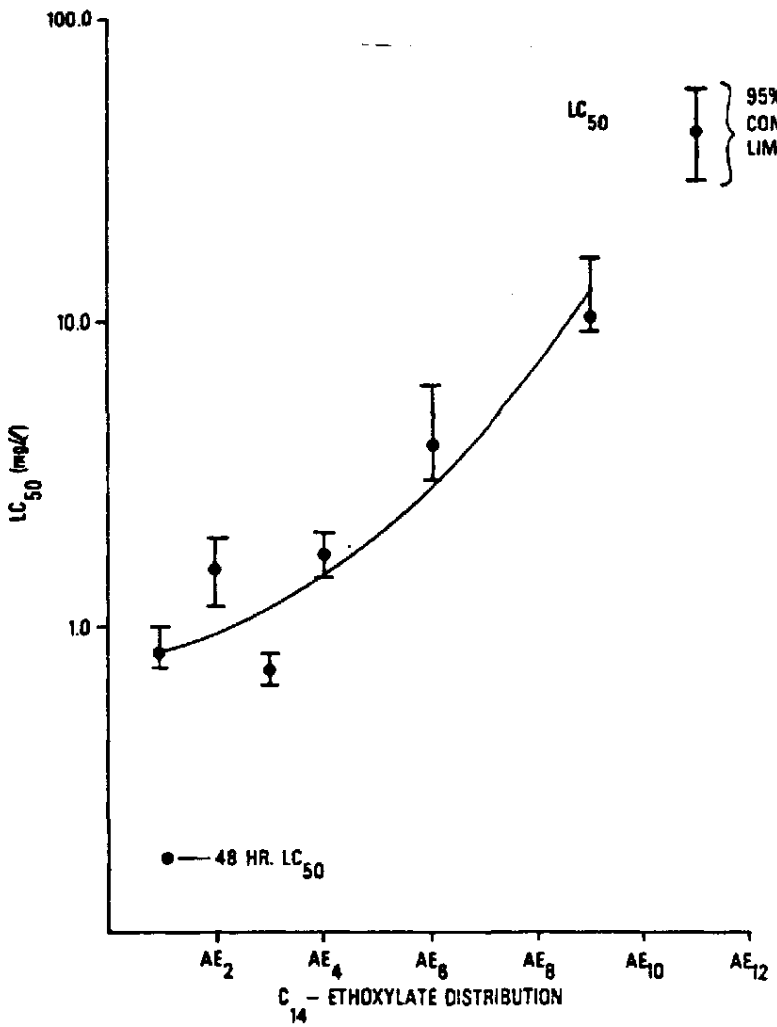

Fig. 2. The effect of variations in the ethoxylate distribution of nonionic surfactants on the resultant acute toxicity to *Daphnia magna*.

toxicity of approximately one order of magnitude is demonstrated for each additional two carbons between C_{10} and C_{16}, with a subsequent slight decrease at C_{18}. The acute toxicity measured as 48-hr LC 50 values drops consistently from 29.5 mg/L at C_{10} to 0.11 mg/L at C_{16}, demonstrating a slight but statistically insignificant increase to 0.12 mg/L at C_{18}.

Also plotted in Figure 1 are the bluegill 96-hr LC 50 values for the same homologous series of anionic surfactants (A.D. Little 1977). These data demonstrate a similar relationship between increased toxicity and increasing alkyl

carbon chain length. Acute toxicity values for bluegill and *Daphnia* indicate that anionic surfactant toxicity data obtained for representatives of one trophic level may be extended to species of different trophic status within aquatic communities.

Similar *Daphnia* toxicity tests completed with nonionic, linear alcohol ethoxylate surfactants with a constant alkyl carbon chain length of C_{14} and an ethoxylate distribution ranging from AE_1 to AE_9 indicate that increasing ethoxylate distribution causes a decrease in acute toxicity to *Daphnia* (Figure 2). The 48-hr LC 50 value for $C_{14}AE_1$ is 0.83 mg/L, while the corresponding value for $C_{14}AE_9$ is 10.1 mg/L, demonstrating greater than one order of magnitude decrease in toxicity over this increased ethoxylate composition (Table 3).

Consistent changes in biological activity, measured as acute toxicity to *Daphnia*, resulted from variation in chemical composition within these homologous series of anionic and nonionic surfactants. These relationships demonstrate that it may be possible to predict subsequent toxicity of formulation changes for new materials by knowing precise chemical structure and basic toxicity data for representatives of the homologous series. Thus, individuals attempting to design safety assessment programs for new chemicals or individuals attempting to establish water quality criteria have much to gain from an examination of data for similarly structured chemical substances.

Species Comparison Testing

Five surfactants representing three anionics and two nonionics were utilized during comparative acute toxicity tests with *D. magna* and *D. pulex*. All tests with a particular material were run simultaneously under similar conditions with young daphnids 24-hr old in an attempt to control any extraneous variables

Table 3. Acute toxicity of homologous series of surfactants to *Daphnia magna*

Anionic Surfactants	48 hr LC50 (ppm)	95% Confidence limits (ppm)
C_{10} LAS	29.55	27.94 – 31.05
C_{11} LAS	21.15	18.49 – 22.25
C_{12} LAS	5.88	5.24 – 6.49
C_{13} LAS	2.63	2.37 – 2.85
C_{14} LAS	0.68	0.58 – 0.77
C_{16} LAS	0.11	0.068 – 0.126
C_{18} LAS	0.12	0.074 – 0.154
Nonionic Surfactants	**48 hr LC50 (ppm)**	**95% Confidence Limits (ppm)**
$C_{14}AE_1$	0.83	0.73 – 0.91
$C_{14}AE_2$	1.53	1.18 – 1.97
$C_{14}AE_3$	0.73	0.64 – 0.81
$C_{14}AE_4$	1.76	1.43 – 2.03
$C_{14}AE_6$	4.17	3.05 – 6.02
$C_{14}AE_9$	10.07	9.46 – 10.66

Table 4. Results of 48 hr acute toxicity tests with surfactants and two species of *Daphnia*

Test Material	48 hr LC50 (mg/L)	
	Daphnia magna	Daphnia pulex
C_{12} LAS	6.84 (5.29 – 8.46)	8.62 (7.28 – 9.91)
C_{14} LAS	0.80 (0.69 – 0.91)	0.59 (0.51 – 0.69)
C_{16} LAS	0.20 (0.17 – 0.22)	0.15 (0.13 – 0.17)
$C_{14}AE_1$	0.14 (0.11 – 0.16)	0.10 (0.07 – 0.12)
$C_{14}AE_4$	0.24 (0.20 – 0.27)	0.21 (0.18 – 0.23)

otherwise influencing directional susceptibility of the two test species. Results of these comparative tests with anionics ranging from C_{12} to C_{16} LAS, and the nonionics $C_{14}EA_1$ and $C_{14}AE_4$, indicate that no statistically significant difference can be shown between the 48-hr LC 50 values for the two species (Table 4). Slight differences existing between the two species are most likely due to the sample size—15 individuals from three replicates of each test concentration. These differences would most likely be normalized with a higher number of replicates.

Representatives of the genus *Daphnia* have become some of the more important test organisms for aquatic safety evaluation programs, due to the relative ease of laboratory culture and short generation times of approximately eight days. Traditionally, the most popular *Daphnia* test species has been *D. magna;* however, recently, questions have arisen over the most appropriate *Daphnia* species for testing. *Daphnia magna* has a somewhat limited geographical distribution, being typically found in small, hard water lakes (Brooks 1957). It has been argued that the more cosmopolitan species, *D. pulex*, is a more valid test organism for water quality demonstrations (Buikema et al. 1976). Such a conclusion, however, is not warranted by the available data. Using LC 50 values as a measure of acute toxicity, *D. magna* and *D. pulex* have been reported to exhibit virtually identical sensitivities to a wide variety of organic and inorganic compounds (Canton and Adema 1978; Winner and Farrell 1976; Buikema et al. 1976). The similarity of response for *D. magna* and *D. pulex* demonstrated in this investigation indicates that both species are equally sensitive to anionic and nonionic surfactants.

Effects of Suspended Solids on Surfactant Toxicity

Numerous investigators have examined the effects of various physical and chemical environmental factors on the toxicity of surfactants to aquatic life. These studies have demonstrated that acute toxicity thresholds are generally

observed at lower concentrations of nonionic surfactants (Swedmark et al. 1971) and anionic surfactants (Hokanson and Smith 1971) when test temperature is increased. Similar studies examining the effects of variable concentrations of dissolved oxygen (Herbert et al. 1957; Hokanson and Smith 1971) and salinity concentrations (Eisler 1965) on the acute toxicity of surfactants have shown effect levels to be significantly lower at reduced oxygen concentrations and when tested at high and low salinity extremes. Little information, however, concerning the interactions of suspended solids with surfactant toxicity is currently available. Since natural surface waters typically contain measurable concentrations of suspended inorganic sediments and intact surfactants are known to interact with these solids, it is reasonable to assume that toxicity tests completed with the presence of suspended sediments would closely model the chemical/physical complex likely to exist in surface waters to which undegraded surfactants may have been discharged.

The results of the present tests with *Daphnia magna* demonstrate variable toxicity, with interactive effects between the 50 mg/L suspension of kaolin clay, the chemical class of surfactant tested, and individual alkyl chain lengths (Table 5). For the anionic surfactants tested, the 48-hr LC 50 values were observed at significantly higher concentrations of both the $C_{14}$and C_{18} homologs, but no significant difference was observed with C_{11} LAS (Figure 3). The acute toxicity of the nonionic surfactants tested varied as a function of alkyl carbon chain length; however, there was no effect on the 48-hr LC 50 values when kaolin clay was present (Table 5).

The results of these tests with surfactants demonstrate that the presence of suspensions of purified, naturally occurring kaolin clay, significantly alters the

Table 5. Effects of a 50 mg/L suspension of kaolin clay on the acute toxicity of a homologous series of anionic surfactants to *Daphnia magna*

		48 hr LC50 and Associated 95% Confidence Limits (ppm)	
Anionic Surfactants		Without Kaolin	With Kaolin
C_{11} LAS		19.3 (14.3 – 23.5)	13.0 (5.1 – 17.4)
C_{14} LAS		1.0 (0.94 – 1.1)	1.4 (1.2 – 1.5)
C_{18} LAS		0.09 (0.07 – 0.11)	0.18 (0.12 – 0.24)
Nonionic Surfactants			
$C_{10}AE_3$		1.9 (1.0 – 2.6)	1.7 (1.1 – 2.3)
$C_{14}AE_3$		0.12 (0.08 – 0.16)	0.12 (0.05 – 0.18)
$C_{18}AE_3$	young	5.0 – 20.0	<5.0
	adults	>80.0	>80.0

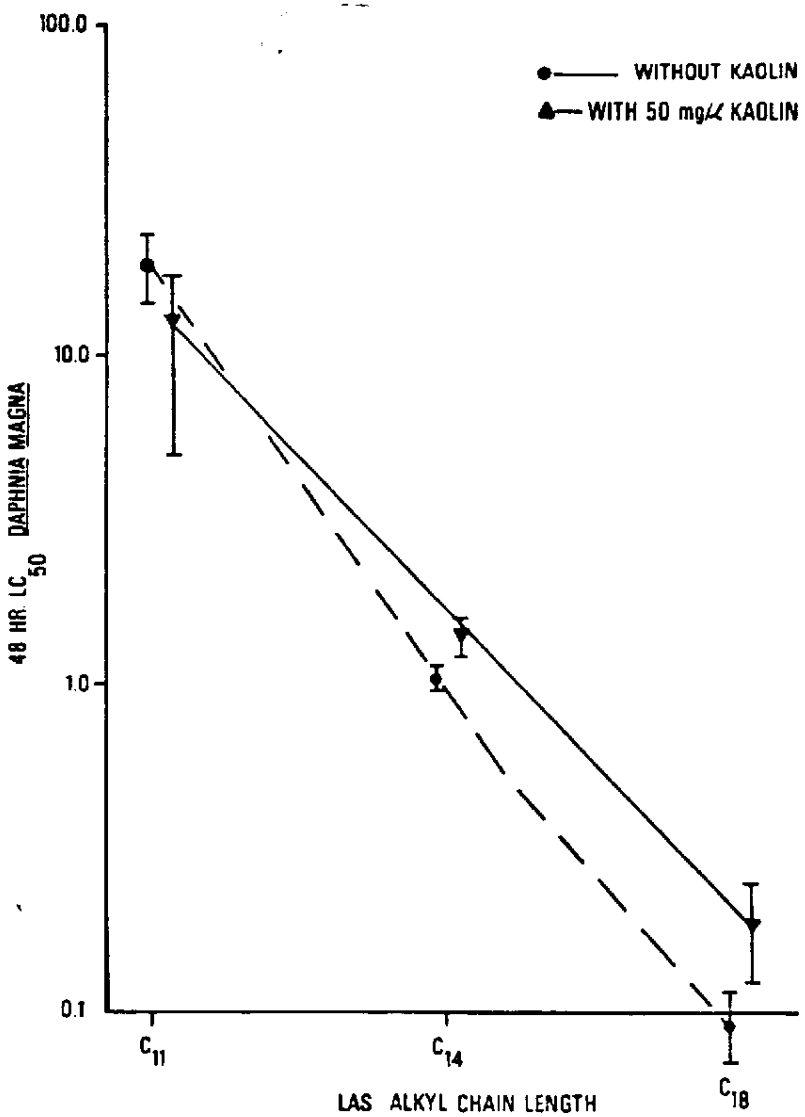

Fig. 3. The influence of suspensions of naturally-occurring Kaolin clay on the acute toxicity of linear alkyl benzene sulfonate to *Daphnia magna*.

observed acute toxicity threshold and LC 50 concentration. The proposed mechanism by which toxicity is reduced is believed to result from absorption of the surfactant on the clay and subsequent settling of this suspension in the test container, with resultant loss of active surfactant from solution. The degree of adsorption and subsequent observed loss of toxicity is dependent on the chemical class and specific composition of the surfactant. In general, the longer alkyl

chain length homologs of anionic surfactants demonstrate significant reductions in toxicity in the presence of kaolin, while nonionic surfactants representing a wide range of alkyl chain lengths appear unaffected by the suspended solids additions. These results lead to the conclusion that the addition of suspended solids to a toxicity test chamber can be important to testing designed to determine acceptable or nonhazardous concentrations of surfactants to aquatic life, but that the decision to employ solids during the testing procedure should be predicated on the demonstrated ability of the material in question to strongly adsorb to natural solids. In these instances, the addition of known solids is advisable to more closely simulate the physical/chemical form of test material existing in the environment.

Effects of Hardness Ions

Water hardness affects the toxicity of many chemicals in solution and the toxicity of surfactants has been variously reported to increase, decrease, or be unaffected by water hardness. (Abel 1974). Henderson et al. (1959) found nonionic toxicity to be unaffected by water hardness, but reported the anionic sodium alkyl sulphate to be more toxic in soft water. Hokanson and Smith (1971) reported the toxicity of LAS to be significantly greater in waters of 290 mg/L $CaCO_3$ than in waters of 15 mg/L $CaCO_3$.

The toxicity and bioconcentration of the anionic surfactant, sodium lauryl sulphate, were directly proportional to the hardness of the test solution and to the hardness of the water in which the fish were acclimated. (Tovell et al. 1974). Toxicity and bioconcentration were related to the concentration of divalent cations (Ca^{+2} of Mg^{+2}) in the test solution. The toxicity and bioconcentration of a nonionic surfactant, C_{12} alkyl ethoxylate, were inversely proportional to the test water hardness, and not related to the cation composition of the test solution or to the hardness of the water in which the fish were acclimated (Tovell et al. 1975).

The acute toxicity of LAS to *D. magna* is a function of both culture and

Table 6. *Effects of culture and test water hardness on the acute toxicity of an anionic and nonionic surfactant on Daphnia magna*

Culture Water		Test Water		48 hr LC50 (95% Confidence Interval)[b]	
Type	Hardness[a]	Type	Hardness	$C_{11.8}$ LAS	Neodol 45–7
Soft	50	Soft	25	7.1 (5.4–16.9)[b]	.36 (.28–.45)
Soft	50	Very hard	350	3.5 (2.9–4.1)	.65 (.52–.79)
Moderately hard	125	Soft	25	4.2 (3.2–6.0)	.56 (.52–.74)
Moderately hard	125	Very hard	350	4.0 (3.4–4.8)	.62 (.48–.77)
Hard	225	Soft	25	2.0 (.5–2.9)	.36 (.28–.45)
Hard	225	Very hard	350	3.2 (2.6–3.8)	.88 (.64–1.90)
Very hard	350	Soft	25	1.8 (.6–2.4)	.36 (.28–.45)
Very hard	350	Very hard	350	4.0 (3.1–5.0)	.90 (.72–1.30)

[a] mg/L as $CaCO_3$
[b] mg/L, based on nominal concentrations

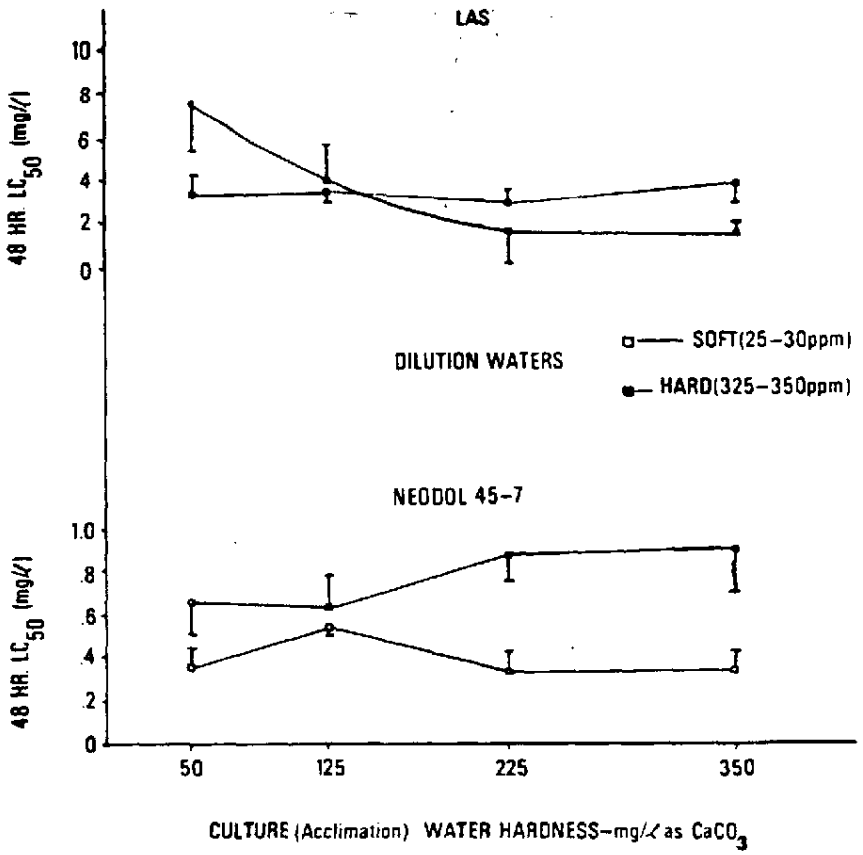

Fig. 4. The influence of variations in culture and dilution water hardness on the acute toxicity of an anionic and nonionic surfactant to *Daphnia magna*.

test water hardness (Table 6). In hard water the toxicity in LAS is independent of culture conditions. In soft water, however, the toxicity of LAS is directly related to culture water hardness levels. That is, in soft water LAS is significantly more toxic to *Daphnia* cultured at high hardness levels (figure 4). This apparent discrepancy may indicate a significant additional physiological stress induced by testing high hardness acclimated *Daphnia* in low hardness test solutions. By allowing a sufficient time for acclimation to low hardness, a significant reduction of LAS toxicity in soft water may be affected (Table 6).

The nonionic surfactant, Neodol[1] 45-7, was more toxic to *Daphnia* in soft water (Table 6). The compound has a mixed alkyl chain length of linear C_{14} and C_{15} and an average of seven moles of ethylene oxide per mole of alcohol. Furthermore, the sensitivity of *Daphnia* to Neodol was not conditioned by the

[1] Neodol is a registered trademark of the Shell Chemical Company

Table 7. Acute toxicity of $C_{11.8}$ LAS to acclimated and unacclimated *D. magna*

	Acclimated[a]	Unacclimated
Acclimation Period	48 hr LC50[b] (95% Confidence Interval)	48 hr LC50[b] (95% Confidence Interval)
24 hr	2.4 (2.1 – 2.7)	2.9 (2.5 – 3.2)
24 hr	2.8 (2.1 – 3.3)	2.5 (1.7 – 3.1)
5 generations	3.2 (2.0 – 4.0)	4.3 (3.7 – 4.9)
7 generations	2.6 (1.8 – 3.3)	3.0 (2.0 – 3.8)

[a] Nominal LAS concentration = .4 mg/L
[b] mg/L

hardness of the water in which the organisms were cultured (Figure 4). The results of these investigations underscore the importance of documenting the quality of test dilution waters and the culture conditions of aquatic species used in toxicity evaluation programs.

Effects of Prior Surfactant Exposures

Existing data indicate that fish are able to acclimate to surfactant concentrations. A 30-day exposure of bluegill, *L. macrochirus*, to 3 mg/L ABS (alkyl benzene sulphonate) produced a 48-hr LC 50 63% higher than that of unacclimated control fish tested simultaneously (Lemke and Mount 1965). Hokanson and Smith (1971) also found that acclimation of bluegill to 0.5 mg/L LAS increased lethal threshold concentrations by 50 to 400% that of unexposed controls at several levels of oxygen saturation. If aquatic life is able to acclimate to previous surfactant exposure with no effects on survival or reproductive parameters, this capability could have significance to natural surface water communities existing in receiving waters continuously exposed to low background concentrations of the surfactant, indicating that results of laboratory toxicity tests may yield unnecessarily restrictive values when projected to these real world populations.

To determine if this acclimation effect also occurs in *D. magna*, cultures were exposed to $C_{11.8}$ LAS at a residual concentration of 0.4 mg/L, selected as approximately 1/10 the 48-hr LC 50 concentration, for up to seven generations (approximatley 14 weeks). Subsequent tests with unexposed controls indicate no significant difference in the acute toxicity of $C_{11.8}$ LAS to acclimated or unacclimated cultures (Table 7), leading to the conclusion that previous exposure of *D. magna* to concentrations of LAS as high as 1/10 the 48-hr LC 50 does not alter the sensitivity of the organisms to acute effects. Caution should be used, therefore, in extrapolating results of acclimation reported for fish, to aquatic life in general.

Conclusions

1. Data for a series of linear alkyl benzene sulfonates and a series of linear alkyl ethoxylates show regular and predictable effects of variation in chemical structure on subsequent acute toxicity to *D. magna*.
2. Comparative toxicity testing with *D. magna* and *D. pulex* and several anionic and nonionic surfactants indicated that both species have statistically similar 48-hr LC 50 values and that both species are equally valid test organisms for investigators attempting to develop water quality information.
3. Acute toxicity tests with *D. magna* and two surfactant types carried out in solutions with and without suspended kaolin clay, indicate that observed loss of toxicity is dependent on the chemical class and specific composition of the surfactant. While nonionics were generally unaffected, the C_{14} and C_{18} alkyl chain lengths of LAS have reduced toxicity in the presence of kaolin.
4. In hard water, the acute toxicity of LAS to *D. magna* is independent of culture conditions, while in soft water toxicity is directly related to culture water hardness. The acute toxicity of a nonionic surfactant was greater in soft water and was not affected by culture conditions.
5. Acute toxicity tests with *D. magna* cultures previously exposed to 0.4 mg/L LAS for periods of up to seven generations indicated no significant difference in LAS susceptibility compared to simultaneously tested unexposed controls.

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Prioritization of Chemicals According to the Degree of Hazard in the Aquatic Environment

by Dean R. Branson*

Chemicals designated as "priority pollutants" or "toxics" have received special attention recently because the discharge of these compounds into public water is to be restricted to the maximum possible with little regard to water quality or economics. The selection of many of the 129 priority cemicals was not based on an objective scientific assessment of the exposure and effect data. In fact, for some compounds, including acenaphthene and 4-chlorophenyl-phenyl ether, the necessary data for listing were non-existent.

As an alternative to arbitrarily listing or delisting chemicals for the purpose of prioity control, this paper suggests a promising scientific approach to selecting priority chemicals based on the principles of hazard assessment for chemicals in the aquatic environment. According o the hypothesis, the highest priority ch-nicals are those with the least margin of safety, defined as the gap between the no-observable-effect cuncentrations and the ambient exposure concentrations.

The no-observable-effect concenrations are based on the results of chronic or sensitive life stage tests with aquatic organisms and the acceptable daily intake rate for fish eates. The ambient exposure concentrations are levels either measured in fish and water, or roughly estimated from a simple nomogram that requires only two of the following three factors: environmental release rate, ratio of dissipation to biococentration potential, or ambient residues in fish.

The chemicals studied to illustrate this approach to prioritizing chemicals based on hazard assessment are: polychlorinated biphenyls, di-2-ethylhexyl phthalate, linear alkylbenzene sulfonate, and pentachlorophenol.

Introduction

The principles of hazard assessment used in this paper are those developed at a recent workshop in Pellston, Michigan (*1*). At this workshop, representatives of government, industries, and universities reached a consensus that hazard in water could be assessed only after both effect and exposure of the chemical substance had been taken into account. Figure 1 illustrates the relationship. In general, the margin between the no-effect concentration and the expected exposure concentration in water is the statement of hazard or margin of safety. Estimates of these two concentrations are made in a sequential fashion, and the error limits on these two estimates narrow as the amount of information about biological effects and exposures increases. It is important to recognize that the essential environmental information can be estimated even from preliminary screening studies but the error limits on these estimates are usually quite large. To illustrate the use of hazard assessment techniques in prescreening priority chemicals, no emphasis on error limits was made.

This concept of hazard assessment is fairly new in the field of aquatic toxicology. Another new concept in our field is regulation of those specific chemicals which represent the greatest cause for concern for pollution in the aquatic environment. The 1977 amendments to the Clean Water Act contained a list of 65 categories of substances, which was later defined in terms of 129 specific priority chemicals (*2*). These chemicals are to receive the maximum possible discharge control in effluents. This paper suggests combining these two concepts into an objective selection criterion for prescreening priority pollutants. Such selection criteria are needed, since Congress provided a process for adding and sub-

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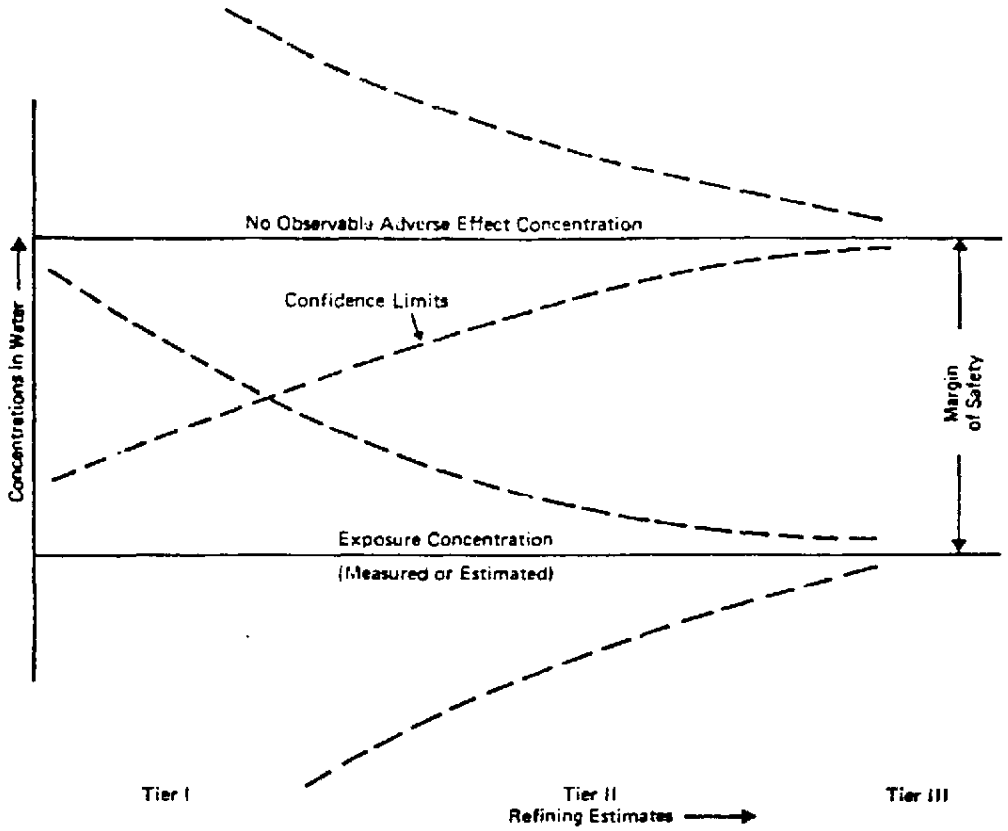

FIGURE 1. Principle of sequential hazard assessment of chemicals in the aquatic environment. Large gaps between the no observable adverse effect concentration and the exposure concentration indicate a low degree of potential hazard and vice versa.

tracting chemicals from the list of priority chemicals. If the hypothesis is correct that principles of hazard assessment can be employed as a prescreen for selecting priority chemicals, then it may be possible to distinguish between priority chemicals and those chemicals whiih more appropriately should be classified as chemicals with less potential to cause harm to man or the environment. The discharge of chemicals that might be removed from the list will also be regulated but to the extent that water quality is protected rather than to the maximum possible extent with little regard to economics.

Case Study Chemicals

Four chemicals were selected to illustrate the principles of hazard assessment—two with fairly high environmental release rates, di-2-ethylhexyl phthalate (DEHP) and linear alkylbenzene sulfonate (LAS), and two other chemicals with moderately high environmental release rates, polychlorinated biphenyls (PCB), and pentachlorophenol (PCP).

The environmental release rate, according to a National Science Foundation (NSF) workshop (*3*), is the maximum amount of material which may enter public receiving water and is calculated from the amount produced minus the amount consumed, destroyed, or contained. The estimates of environmental release rates in the NSF workshop were based on 1972 production records. For DEHP and LAS, the maximum environmental release rate in 1972 was 100 million to 500 million lb/yr, and for PCB and PCP 10 million to 50 million lb/yr. The distinction between production rates and environmental release rates is important, because rarely is all of the chemical produced released to the aquatic environment.

In order to assess the hazard of chemicals in the aquatic environment, certain environmental values must be measured or estimated. Table 1 shows the environmental information for the case study chemicals. This information includes the maximum concentrations of the case study chemicals which should not cause an observed adverse effect to fish-eaters, including man, i.e., acceptable daily intake (ADI) values combined with fish residue data. The ADI values were derived from long-term feeding studies

Table 1. Essential information for assessing the hazard of chemicals in the aquatic environment.

Chemical	Hazard to fish			Hazard to fish eaters		
	Ambient exposure concentration in water, mg/L (ppm)[a]	NOEC mg/L (ppm)[b]	Safety margin[c]	Ambient concentration in fish, µg/g (ppm)[a]	ADI residues for fish eaters[d]	Safety margins[c]
LAS	<0.05	0.5	>10	<0.5	750	1500
PCP	<0.000033	~0.005	150	0.01	9	900
DEHP	~0.0025	0.003	~1.2	~10	180	18
PCB	0.000010	0.005	500	>5	<5	<1

[a]Samples from areas outside the mixing zone of an effluent; large lakes, rivers, and estuaries; concentration of chemical in true solution.
[b]NOEC = no observable adverse effect concentration in chronic or sensitive life-stage tests.
[c]Safety margin = no observable adverse effect concentration/ambient exposure concentration.
[d]Fish residue = acceptable daily intake (ADI) µg/g/day × 60 kg fish eater/0.2 kg fish eaten per day.

in rodents and assumed a safety factor of 100. For the case study chemicals, the following ADI values were used: DEHP, 0.6 mg/kg-day (*4*); LAS, ~2.5 mg/kg-day (*5*); PCB, ~0.016 mg/kg-day (*6*); PCP, 0.03 mg/kg-day (*7*). It is appropriate to use long-term exposure data since the hazard assessment of the case study chemicals was based on ambient concenations of the chemicals.

If all the information in Table 1 is available, hazard assessment is an easy task, involving merely dividing the no-effect concentration by the exposure concentration, but rarely, if ever, is all the information available to everyone's satisfaction. Typically, the data least available or reliable are the ambient exposure concentrations. As an alternative to direct measurements of the exposure concentration, it seemed reasonable that rough estimates of exposure could be made for the purpose of prioritizing chemicals, by examining the quantities released to the aquatic environment, the resulting residues in fish, the bioconcentration potential, and the degradation potential.

Correlation Between Release Rates, Persistence/Accumulation, and Fish Residues

It was assumed in reviewing the available information for this paper that in the future more and better data will be available on the ambient concentrations of chemicals in fish tissue. It was further assumed that these concentrations were a direct function of the persistence/accumulation properties d environmental release rates.

The PCB residues in Table 1 of 5 ppm were for the ars 1972 and 1975 and were increasing with time (*6*). The linear alkylbenzene sulfonate residues in fish are estimated to be less than 0.5 ppm based on limited measurements of the concentrations in water and bioconcentration factors of approximately ten (*8*). Pentachlorophenol measurements are about 0.01 ppm and declining (D. Stallings, personal communication, 1979). The DEHP residues are not well documented. Characteristically, they are measured as by GC-MS *M/e* 149, which is the common mass ion for all phthalate esters. The limited monitoring data which are available suggest that the ambient levels in fish tissue of DEHP are at least 10 ppm (*9*).

The third part of this correlation deals with the persistence/accumulation. A composite of these properties was represented as a ratio of dissipation and bioconcentration potential; ratio DIS/BCF. Dissipation was assumed to be the sum of both the evaporative loss of the chemical from water and the rate of photochemical, hydrolytical, or biological degradation (Table 2). Since quantitative measurements of the rate of degradation were either not readily available or comparable, relative rates were assigned, where 100 was the maximum rate and a minimum rate of 1 was chosen for substances like metal ions which generally do not degrade or evaporate from water. For LAS, a relative degradation rate of 100 was assigned based on the first-order rate constant for CO_2 evolution, 0.10/day as reported by Larson (*10*). For PCP, a relative degradation rate of 50 was assigned based on 100% disappearance in various soils after 4 hr to 30 days (*11*). For DEHP, a relative rate of 10 was assigned based on 50% of the parent compound remaining in a hydrosoil test after 14 days (*12*). For PCP, a relative degradation rate of 1 was assigned based on the second-order rate constant for $^{14}CO_2$ evolution from ^{14}C-[2,5,2'-trichlorobiphenyl] in activated sludge, 0.009 g/g-hr (*13*). In the future, rate constants should replace these arbitrarily assigned relative rates.

The bioconcentration factors used to calculate the ratio of dissipation to bioconcentration potential were all measured values (Table 3) and relative dissipation rates were obtained from Table 2. What can be seen by this ratio of dissipation to bioconcentration potential is that the greater the dissipation and

Table 2. Dissipation of chemicals from water.

Chemical	Water solubility, mg/l.[a]	Evaporation LK × 100	Degradation biol/photo (relative rate)[b]	Relative dissipation rate (DIS)
LAS	>10,000	<0.1	100	100
PCP	8,000	<0.1	50	50
DEHP	6	1	10	11
PCB[c]	0.00025	4	1	5

[a]pH 7.5
[b]100 is maximum value combining both evaporation and degradation rates. Metal ion dissipation has minimum value of 1.
[c]Representative PCB isomer: 2,5,2'-trichlorobiphenyl (*13*).

Table 3. Ratio of dissipation to bioconcentration potential of chemicals in aquatic environment.

Chemical	Relative Dissipation (DIS)[a]	Factor (BCF)	Ratio DIS/BCF
LAS	100	10[b]	10
PCP	50	200[c]	0.25
DEHP	11	800[d]	0.014
PCB	5	50,000[e]	0.0001

[a]100 is maximum value combining both evaporation and degradation rates. Metal ion dissipation was a minimum value of 1.
[b]Data of Comotto et al. (*8*).
[c]Data of Dow Chemical Co. (*11*).
[d]Data of Branson (*15*).
[e]Data of Nisbet (6).

the lower the bioconcentration potential, the larger the value of the ratio. Chemicals like LAS receive a large number, DIS/BCF = 10, because of high degradation and low bioconcentration potential; chemicals like PCB's with high bioconcentration factors and low dissipation potential receive very small numbers, DIS/BCF = 0.0002. The nomogram for predicting the unknown ambient exposures (Fig. 2) illustrates an empirical relationship between fish residues, environmental release rates, and the ratio of dissipation to bioconcentration potential. Recognizing that the straight lines plotted on this graph are only made from two points each, the absolute position of the line is tentative at best. It seems reasonable, however, to assume that higher environmental release rates would result in a parallel line higher on this graph and lower release rates would result in a lower line. The graph does serve to illustrate the hypothesis that if any two of the three pieces of information are known, the third piece can be estimated. For example, if the DIS/BCF ratio a chemical is 0.001 and its release rate is about 10^7 lb/yr, then the predicted ambient residues in fish are about 1 ppm, but if the environmental release rate is about 10^8 lb/yr then the ambient residues in fish would be about 100 ppm. Also, given that fish residue data (C_f) are available, measured or estimated from Figure 2, the ambient exposure concentration in water (C_w) can be estimated by dividing by the bioconcentration factor: $C_w = C_f/\text{BCF}$. The shaded

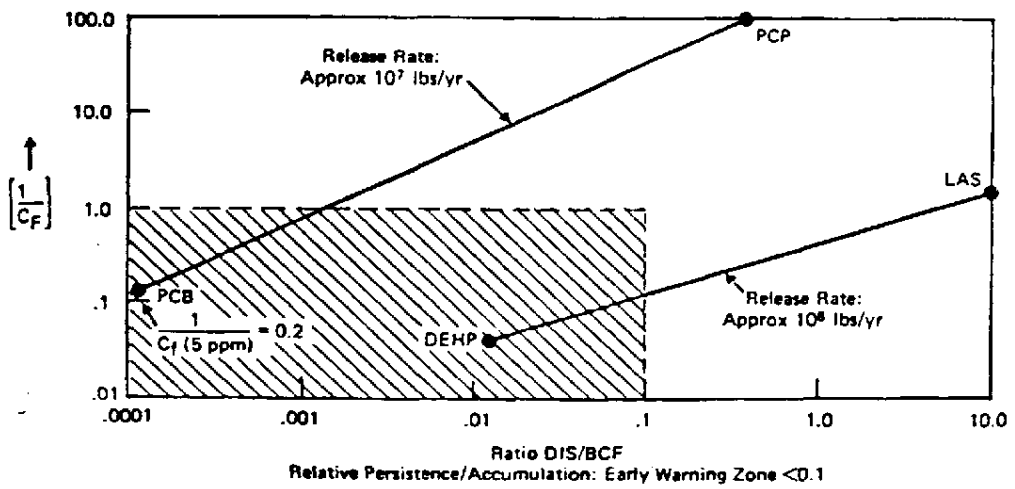

FIGURE 2. Preliminary nomogram for predicting the unknown ambient exposures. The ambient concentration of a chemical in water, C_w, can be roughly estimated from the ambient concentration in fish, C_f, and the bioconcentration factor at steady state, BCF, i.e., $C_w = C_f/\text{BCF}$.

area illustrates qualitatively that chemicals with high environmental release rates and/or persistence/accumulation properties will end up in this zone. Most of the chemicals, on the other hand, will end up outside this zone, since these curves were drawn with chemicals with known fish residues and fairly high environmental release rates.

Correlations of environmental properties of chemicals derived from large data bases confirm that most of the chemicals are outside this zone of concern. Vieth and Konasewich (personal communications, 1979) have shown that only about two-thirds of the 2,100 chemicals manufactured or used around the Great Lakes have octanol-water partition coefficients suggestive of low concern for fish residues. Also Bailey (personal communications, 1979), indicated approximately two-thirds of 600 chemicals screened for biodegradability in a simple biochemical oxygen demand test yielded 30% or more BOD in 20 days, indicating a low degree of persistence in the aquatic environment. Both of these properties, accumulation and persistence, appear to be related to low water solubility (Table 2). In general, the environmental fate of the majority of chemicals currently manufactured and used by society is characterized by a low degree of persistence and accumulation and will, therefore, be of low hazard because the ambient exposure concentrations are low.

Hazard Assessment

Figure 3 compares the margin between the no effect concentrations and the ambient exposure concentrations for the case study chemicals. A reciprocal of hazard was used to show hazard by the height of the bar graph, i.e., narrow margins of safety yield tall bar graphs and vice versa. The PCB example illustrates a case in which residues in fish would cause concern to fish eaters but the ambient levels of PCBs in water would cause little or no concern to aquatic life. On the other hand, DEHP illustrates a case in which the aquatic organisms could be at risk but not the fish eaters. Both of these materials fall across an arbitrary line where the margin between effect and exposure is equal to or less than five. A safety margin of five appears to be a reasonable criterion for distinguishing between chemicals that should and should not be classified as "priority." Neither LAS nor PCP appears to be reasonably classified as a priority chemical.

The safety margin concept was also employed by Klapow and Lewis (*14*) in selecting water quality standards in the marine environment. More importantly, the safety margin of the typical standard was less than a factor of five above the ambient exposure concentration, e.g., for copper, ambient 0.002 mg/l., standard 0.005 mg/l., margin of safety 2.5; and for

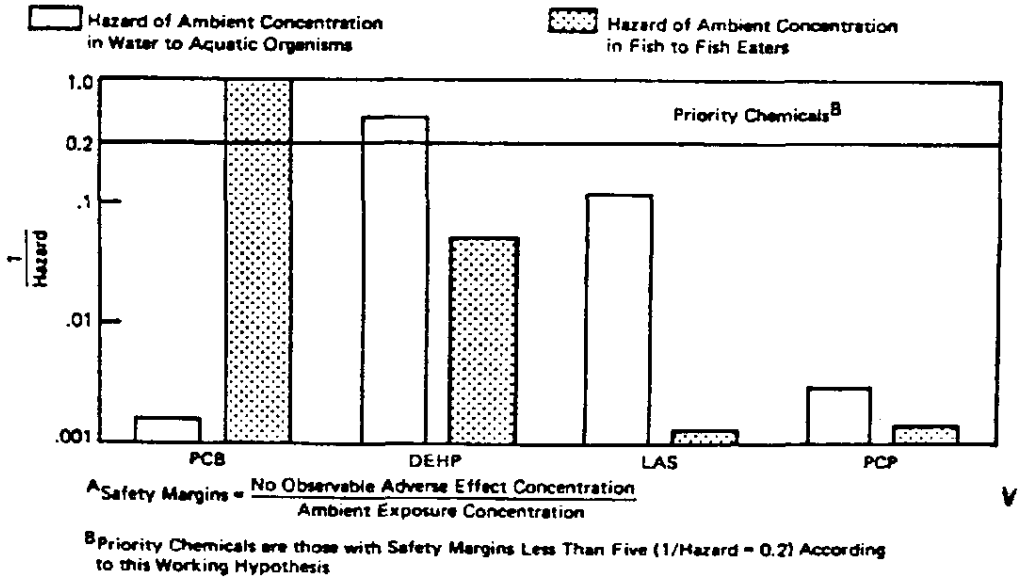

FIGURE 3. Safety margins of chemicals in the aquatic environment. The numerical values associated with each chemical are only meant to illustrate the concept of prioritization of chemicals according to safety margins, i.e., degree of hazard. Confidence limits and difference in geographical sites for ambient measurements should be considered before using these values for more than illustrating the concept.

zinc, ambient 0.008 mg/L., standard 0.02 mg/L., margin of safety 1.5. Therefore the margin of safety factor of five used here to select priority chemicals is conservative.

Research Needs

The basic premise for suggesting areas of research to improve hazard assessments of any chemical in the aquatic environment for any decision-making values is that exposure and effect concentrations must be available for both and that the biggest research need is estimating exposure concentrations. The following areas of research are very important.

It is necessary to develop and validate test methods for measuring rates of degradation, evaporation, and bioconcentration of chemicals in water. These test methods must accommodate chemicals with low water solubility (less than 1 ppm), and the results should be reported as measured rate constants. With more experience in generating rate constant data, it is hoped that some preliminary estimates of their value can be based on structure through various regression correlations.

The lack of reliable information on the amount of chemicals released to the environment is the single largest source of error in environmental hazard assessment (Baughman, personal communications, 1978). The approach used by the 1975 NSF Workshop seems promising and should be updated. It takes the use patterns of industrial chemicals into account.

There is a glaring lack of field studies to show that laboratory data correctly forecast the fate and effects of chemicals in the real world. Predictive models need to be validated for several different aquatic environments based on time-concentration data with adequate material balance accountability.

It would be valuable to add more data points (case study chemicals) to Figure 2, as data are generated from more and better analysis of fish and water samples, DIS/BCF, ratios and environmental release rates. These data should be used in prioritizing and deprioritizing chemicals.

Conclusions

Principles of hazard assessment can be used as a prescreen in selecting priority chemicals. These principles involve estimates or measurements of both the exposure and the no effect concentrations. In addition to narrow safety margins, chemicals should not be classified as priority chemicals unless there is evidence of (a) potential to cause significant human toxicity including carcinogenicity, mutagenicity and teratogenicity; or (b) analytical detection at toxicologically significant concentrations in five or more controllable point sources.

Rough estimates of ambient exposure concentrations can be made if environmental release rate and the ratio of dissipation (DIS) to bioconcentration potential (BCF) are known. Also, the ratio DIS/BCF can be used as an early warning index for chemicals or used for predicting fish residues or environmental release rates.

Under the principles of environmental hazard assessment, both PCB and DEHP would be candidate for a list of priority chemicals. Neither LAS or PC would be priority chemicals.

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