Toxicity of Laundry Detergent Components to a Freshwater Cladoceran and Their Contribution to Detergent Toxicity

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The toxicity of 39 laundry detergent components including surfactants, enzymes, builders, fabric brighteners, fillers, and coloring agents to the cladoceran *Ceriodaphnia* cf. *dubia* was determined. The difference between the most and the least toxic components was approximately 17,000-fold and 1,000,000-fold for the mg/L and mmol/L EC_{50} data, respectively. Two of the components had high toxicity (EC_{50} values <1 mg/L), 11 moderate toxicity (EC_{50} values between 1 and 10 mg/L), and the remaining 26 components had low toxicity (EC_{50} values >10 mg/L). Analysis revealed that mixtures of the components interacted antagonistically, additively, and synergistically. On a molarity basis the most toxic group of compounds was the surfactants followed by the brighteners. The most toxic individual components included sodium carboxymethyl cellulose, sodium silicate solution, four brighteners, sodium perborate tetrahydrate, and the surfactants. Many of the most toxic components, however, contributed very little to the toxicity of the detergents due to being present in the detergents at low concentrations. The main contributors to the toxicity of detergents were the sodium silicate solution and the surfactants—with the remainder of the components contributing very little to detergent toxicity. The potential for acute aquatic toxic effects due to the release of secondary or tertiary sewage effluents containing the breakdown products of laundry detergents may frequently be low. However, untreated or primary treated effluents containing detergents may pose a problem. Chronic and/or other sublethal effects that were not examined in this study may also pose a problem. © 1999 Academic Press

Key Words: toxicity; laundry detergents; surfactants; builders; enzymes; brighteners; fillers; *Ceriodaphnia* cf. *dubia*.

INTRODUCTION

Detergents are an extensively used group of chemicals with the annual worldwide use of surfactants exceeding 2 billion kg (Ainsworth, 1992). Large quantities of detergents and their components enter the environment. For example, McAvoy *et al.* (1993) estimated that 5% of all linear alkylbenzene sulfonate (LAS) produced in the United States reaches the aquatic environment. Concern over the potential environmental impact of detergents and some of the components has led to extensive study of their environmental effects (e.g., Ainsworth, 1992; Hennes-Morgan and de Oude, 1994). This work, however, has concentrated largely on the toxicity, biodegradation, and removal by sewage treatment plants (STP) of LAS surfactants and to a lesser extent surfactants in general and builders. Research into the toxicity of commercial formulations is limited. Most of the research on commercial formulations that has been conducted is relatively old and hence largely irrelevant because of the constant change in detergent formulations (Lewis, 1992).

Over the last decade there has been a rapid growth in the use of phosphate-free laundry detergents. Despite this increased usage, relatively little work has focused on the environmental effects of these and concerns have been raised over phosphate-free formulations. These include the remobilization of metals by chelates (Horstmann and Gelpke, 1991), reduced rates of biodegradation (Dwyer *et al.*, 1990; Chambon, 1990), the effectiveness of sewage treatment in their degradation and removal (Dwyer *et al.*, 1990), and toxic effects on aquatic organisms (Chambon, 1990; Scholten *et al.*, 1994).

To investigate the concern that phosphate-free laundry detergents may be more toxic than phosphate-containing laundry detergents a study was conducted by Warne (1995), which compared the toxicity of 24 laundry detergents to the freshwater cladoceran *Ceriodaphnia* cf. *dubia*. The toxicity (48 h EC_{50} immobilization) of the detergents varied from 1.6 to 70.3 mg/L. The various statistical analyses conducted (Warne, 1995) revealed that the concentration of phosphorus in detergents had no statistically significant effect on detergent toxicity. The variation in detergent toxicity also could not be explained by the presence or absence of

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enzymes and/or zeolites. It was concluded that the variation in detergent toxicity was most likely due to other detergent components such as surfactants, enzymes, and chelates.

The aims of the present study were to determine the toxicity of a range of laundry detergent components that included surfactants, enzymes, builders, fabric brighteners, fillers, and coloring agents and to determine which components were the major contributors to the toxicity of Australian laundry detergents.

MATERIALS AND METHODS

Cladoceran Maintenance

The cladoceran used in this study was identified as *C. dubia* Richard (Julli *et al.*, 1990). However, while the cladoceran conforms (cf.) to the description of *C. dubia* it was not identical and to denote that it may be a different species it has been called *C.* cf. *dubia*.

C. cf. *dubia* were cultured and tested at $23 \pm 1°C$ in dechlorinated Sydney mains water which was filtered (1 μm), aged (1 month), and adjusted to 500 μS/cm with seawater. Cultures of *C.* cf. *dubia* were maintained in 2-L glass beakers and transferred to fresh water three times weekly. Food was provided after water renewal at a concentration of 25,000 cells/ml of each of the unicellular algae *Pseudokirchneriellia subcapitata* Printz (formerly named *Selenastrum capricornutum*) and *Ankistrodesmus* sp. All neonates used in the toxicity tests were less than 24 h old.

Detergent Component Selection and Preparation

The components tested (Table 1) were selected based on detergent chemical composition information provided by detergent manufacturers. All components were stored in the dark at $22 \pm 2°C$.

Stock solutions of each component, except the enzymes, were made by dissolving the appropriate amount in 1 or 2 L of the water described previously and then gently stirred for 12 h in the dark at $23 \pm 1°C$ using Teflon magnetic stirrers. Stock solutions of the enzymes were made immediately prior to commencing the bioassays and were stirred for a maximum of 15 min. Stirring was conducted so that no bubbles were formed when the surfactants were dissolved, as the formation of bubbles leads to depletion of surfactants in the solution (Aboul-Kassim and Simoneit, 1993). Light was excluded during the stirring to minimize photodegradation of the chemicals. Stock solutions were diluted to the appropriate concentrations immediately prior to the commencement of a test.

Bioassay Procedure

The acute 48-h immobilization tests were conducted using methods developed by the NSW Environment Protection Authority (Warne and Julli, in press). The test vessels were 250-ml glass beakers which held 200 ml of the test solution or control solution (water as described previously). Each bioassay consisted of five concentrations of a detergent component arranged in a geometric series, plus a control. Five cladocera were randomly allocated to each test beaker and each treatment in a test was triplicated. Beakers were then randomly positioned in constant temperature cabinets and maintained at $23 \pm 1°C$ with a 16 : 8-h light to dark regime and light intensity below 1000 lx at the surface of the solutions. Animals were not fed during the tests. The temperature, dissolved oxygen, pH, and conductivity of the test solutions were measured immediately prior to the addition of the cladocera and on completion of each bioassay. The tests were terminated after 48 h and the numbers of immobile cladocera counted. Immobilization was defined as the absence of visible movement by the cladocera within 15 s of gentle agitation of the test solution (ASTM, 1988). Tests were considered invalid and the data not included in the study if more than 10% of the control neonates were immobilized. Each detergent component was tested in a range-finder and definitive test over the period of the study. If the resulting EC_{50} values were markedly different, a second definitive test was conducted. In such cases the EC_{50} values were averaged and the 95% confidence intervals for both definitive tests are presented in Table 1.

Data Analysis

The 48-h EC_{50} (immobilization) values and 95% confidence intervals were based on nominal concentrations and were determined by the trimmed Spearman–Karber method (Hamilton *et al.*, 1977).

The toxicity of the components was expressed in three different units—mg/L, mmol/L, and toxic units. The molarity was calculated using molecular weights supplied by the manufacturers except for zeolite where the molecular weight was calculated by assuming it had the molecular formula $Na_{12} \cdot (AlO_2)_{12} \cdot (SiO_2)_{12} \cdot 27H_2O$. The toxicity (mg/L and mmol/L) of the surfactants solutions and formaldehyde solutions was expressed in terms of active constituent. This was not necessary for any other of the chemicals, as they were essentially pure. The toxicity in mmol/L of the sodium silicate solution and sodium silicate was expressed in terms of SiO_2. The toxicity of the enzymes was converted to kiloprotease units (KPU), kilolipase units (KLU), kilo-α-amylase units (KAAU), and cellulase viscosity units (CEVU) for the protease, lipase, α-amylase, and cellulase enzymes, respectively.

The toxicity of the components expressed in toxic units was calculated by the equation

$$TU = EC_{50comp}/x, \tag{1}$$

where EC_{50comp} is the toxicity (measured in mg/L) of each component measured individually and x is the percentage

TABLE 1
Component Name and Number, CAS Number, Molecular Weight, Toxicity Values (EC_{50}) Expressed in mg/L and mmol/L and the Percentage of Toxicity the Component Contributes to the Toxicity of the Detergent

Component number and name	CAS No.[a]	MW[b]	EC_{50} (mg/l)[c] (95% CL)	EC_{50} (mmol/L)[c,d] (95% CL)	Mean % detergent toxicity (range)
1. Sodium carboxymethyl cellulose	9004-32-4	700,000	87.26 (46.04–165.37)	0.0001 (0.00007–0.00024)	0.09 (0.01–0.41)
2. Ethoxylated C12-15 EO8 alcohol	68131-39-5	551.00	0.39 (0.37–0.43)	0.0007 (0.0007–0.0008)	56.08 (30.80–71.21)
3. Sodium C10-16 alkyl sulfate	68585-47-7	298.00	1.37 (1.18–2.21)	0.0046 (0.0040–0.0074)	10.51
4. Sodium silicate (40%) as SiO_2	1344-09-8	60.09	0.40 (0.28–0.57)	0.0067 (0.0005–0.0095)	66.56 (51.34–78.78)
5. Dimorpholino stilbene derivative	16090-02-1	924.00	6.85 (3.17–11.92) (6.17–8.03)	0.0074 (0.0034–0.0129) (0.0067–0.0087)	0.22 (0.14–0.31)
6. Sodium C12-14 EO2 lauryl ether sulfate	9004-82-4	421.00	3.12 (2.43–4.01)	0.0074 (0.0058–0.0095)	19.17
7. Sodium C12-16 EO2.7 alkyl ethoxysulfate	68585-34-2	423.00	3.43 (2.33–4.81) (3.09–4.00)	0.0081 (0.0055–0.0114) (0.0073–0.0095)	6.05
8. Ethoxylated C9-11 EO3 alcohol	68551-13-3	600.00	5.36 (4.61–6.25)	0.0089 (0.0077–0.0104)	nd[e]
9. Sodium C12-13 alkyl benzene sulfonate	26248-24-8	358	3.24 (2.85–3.68)	0.0091 (0.0080–0.0103)	27.22 (18.67–41.79)
10. Sodium C14-16 alpha olefin sulfonate	68439-57-6	324.00	4.53 (4.14–4.95)	0.0140 (0.013–0.015)	80.48
11. Sodium C10-13 dodecylbenzene sulfonate	68018-81-2	345.00	5.96 (4.89–7.26)	0.0173 (0.014–0.021)	8.22
12. C10-13 Dodecylbenzene sulfonic acid	68584-22-5	323.00	5.65 (4.66–6.83)	0.0175 (0.014–0.021)	25.99 (15.53–33.37)
13. Sodium dodecylbenzene sulfonate	25155-30-0	348.48	7.81 (3.26–8.07) (7.28–14.51)	0.0224 (0.009–0.025) (0.021–0.042)	50.41 (24.09–76.73)
14. Sodium perborate tetrahydrate	7632-040-4	153.86	6.98 (4.57–10.68)	0.0454 (0.03–0.069)	3.12 (2.92–3.33)
15. Bis-(triazinylamino)-stilbene disulfonic acid derivative	24565-13-7	840.40	38.35 (30.01–48.99)	0.0456 (0.036–0.058)	0.58
16. Bis-(triazinylamino)-stilbene sulfonic acid derivative	13863-31-5	900.40	42.51 (31.91–45.69) (39.69–55.24)	0.0472 (0.035–0.051) (0.044–0.061)	0.06 (0.03–0.19)
17. Biphenyl distyryl derivative	27344-41-8	562.50	40.33 (34.00–47.84)	0.0717 (0.060–0.085)	0.03 (0.02–0.05)
18. Zeolite	68989-22-0	2190.72	425.2 (357.5–528.9) (328.8–525.2)	0.19 (0.16–0.24) (0.15–0.24)	0.30 (0.01–1.07)
19. Formaldehyde	50-00-0	30.03	12.98 (12.10–13.92)	0.4322 (0.403–0.464)	0.24 (0.16–0.33)
20. Sodium silicate (as SiO_2)	6834-92-0	60.09	33.53 (22.94–49.01)	0.1822 (0.125–0.266)	3.03 (1.30–5.44)
21. Diethanolamine	111-42-2	105.14	72.92 (61.80–86.04)	0.6936 (0.588–0.818)	nd
22. STPP	7758-29-4	367.86	276.61 (238.35–321.01)	0.7519 (0.648–0.873)	0.93 (0.07–2.96)
23. Sodium hydroxide	1310-873-2	40.00	40.38 (34.59–47.13)	1.0095 (0.865–1.178)	0.61
24. Sodium carbonate	497-19-8	105.99	199.82 (166.9–298.9) (156.6–198.3)	1.8853 (1.58–2.82) (1.48–1.87)	1.99 (0.16–12.61)
25. Soda ash	497-19-8	105.99	226.79 (192.4–267.4)	2.1397 (1.82–2.52)	6.33 (1.59–10.19)

TABLE 1—*Continued*

Component number and name	CAS No.[a]	MW[b]	EC$_{50}$ (mg/l)[c] (95% CL)	EC$_{50}$ (mmol/L)[c,d] (95% CL)	Mean % detergent toxicity (range)
26. Sodium citrate		294.10	735.54 (655.0–825.9)	2.5010 (2.23–2.81)	nd
27. Triethanolamine	102-71-6	149.19	609.98 (565.2–658.3)	4.0886 (3.79–4.41)	0.02
28. Tartrazine	1934-21-0	534.37	5706.55 (4932–6602)	10.6790 (9.23–12.35)	nd
29. Sodium sulfate decahydrate	7727-73-3	322.20	6129.96 (5634–6993) (5515–6491)	19.0253 (17.49–21.70) (17.12–20.14)	0.09 (0.02–0.28)
30. Sodium sulfate anhydrous	7757-82-6	142.04	3150.21 (2807–3535)	22.1783 (19.76–24.89)	0.09 (0.00–0.31)
31. Sodium chloride	7647-14-5	58.44	2122.55 (1493–2434) (2068–2644)	36.3202 (25.55–41.66) (35.39–45.25)	0.02
32. Urea	57-13-6	60.06	6573.10 (6119–7061)	109.4422 (101.9–117.6)	nd
33. Protease liquid	9014-01-1 EC 3.4.21.62	16 KPU/g	23.78 (17.05–58.66) (11.61–21.88)	0.380 KPU	0.35
34. Protease powder	9014-01-1 EC 3.4.21.62	6 KPU/g	24.83 (16.87–36.53)	0.149 KPU	0.19 (0.11–0.31)
35. Lipase liquid	9001-62-1 EC 3.1.1.3	100 KLU/g	>1500	>150 KLU	nd
36. Lipase powder	9001-62-1 EC 3.1.1.3	100 KLU/g	307.96 (293.8-371.3) (225.3–362.0)	30.80 KLU	0.02 (0.01–0.03)
37. Amalase liquid	9000-90-2 EC 3.2.1.1	300 KAAU/g	>10,000	3000 KAAU	nd
38. Amalase powder	9000-90-2 EC 3.2.1.1	60 KAAU/g	3865 (2047–6688) (2447–6667)	231.9 KAAU	nd
39. Cellulase powder	901254-8 EC3.2.1.4	0.7 CEVU/g	793.28 (455.6–1381)	0.560 CEVU	0.02 (0.00–0.03)

[a]The enzymes have their CAS No. and also their International Union of Biochemistry enzyme classification number.

[b]Rather than presenting the molecular weight of the enzymes the activity is given where KPU is kiloprotease units, KLU is kilolipase units, KAAU is kilo-α-amylase units, and KCEVU is kilocellulase viscosity units.

[c]The EC$_{50}$ values are either the definitive test result when it was not significantly different from the range finder result or the mean of two definitive tests when the EC$_{50}$ from the first definitive test differed from the range finder. In the latter case the 95% CIs for each test are presented.

[d]The EC$_{50}$ values for the enzymes are not expressed in terms of mmol/L but rather in terms of enzymes acitivity.

[e]nd: there were no detergents that contained these components and for which there was toxicity data; therefore a percentage contribution could not be determined.

composition (mass/mass) of the component in the detergent multiplied by the toxicity (mg/L) of the detergent. The EC$_{50comp}$ values were not expressed in terms of active ingredient unless the chemical composition supplied by the manufacturers was expressed that way. The toxicity data for the detergents were obtained from Warne (1995) and Warne *et al.* (1996). Equation (1) is expressed in slightly different terms but is consistent with the usual means of converting toxicity data to toxic units (Calabrese, 1991).

It is perhaps noteworthy that some of the components (e.g., Component Nos. 12, 21, 24, 25, and 27) tested in this study may not be present in the laundry detergents, as they were reactants in the manufacture of the detergents. These components were not included in the following calculations.

The TU values for all components present in each detergent were summed to give a total TU value which was termed S and then converted to the corrected toxicity enhancement index (cTEI) (Warne and Hawker, 1995). This index unlike other indices of mixture toxicity has a linear scale and does not lead to visual underestimation of antagonistic interactions (Warne and Hawker, 1995). The cTEI

values were calculated by

$$cTEI = (1/S) - 1, \tag{2}$$

when S is less than 1, and by

$$cTEI = (-1 \times S) + 1, \tag{3}$$

when S is greater than 1. Corrected toxicity enhancement index values of -2, 0, and 2 indicate the toxicity of the mixtures is one-half as toxic, as toxic, and two times more toxic than the same mixture if the toxicity was additive. A cTEI value of zero means that the components in the mixture have interacted in an additive manner (i.e., the toxic effect of the mixture is equal to the sum of the toxic effects of the components measured individually). However, due to experimental errors, mixtures with cTEI values between -0.5 and 0.5 are defined as having additive toxicity, while mixtures whose components interact in an antagonistic and synergistic manner have cTEI values less than -0.5 and greater than 0.5, respectively (Marking, 1985). Antagonism and synergism are defined as occurring when the effect of the mixture is less than or greater than the sum of the toxic effects of the components measured individually.

The percentage of the toxicity of a detergent that is attributable to any component or group of components in the detergent was determined by

$$\text{Percentage of toxicity} = 100 \ (TU_{comp}/S), \tag{4}$$

where TU_{comp} is the toxic unit for either an individual component or a group of components that belong to a detergent.

RESULTS

The toxicities of the 39 laundry detergent components expressed in both mg/L and mmol/L to *C. cf. dubia* are presented in Table 1. The variation in EC_{50} (mg/L) and EC_{50} (mmol/L) values of the components was very large, approximately 17,000- and 1,000,000-fold, respectively. Based on the Worksafe Australia and NICNAS (1991) scheme for toxicant classification, the ethoxylated C12-15 alcohol (CAS No. 68131-39-5) and the 40% sodium silicate solution (CAS No. 1344-09-8), had high toxicity (EC_{50} values <1 mg/L). Eleven compounds had moderate toxicity (EC_{50} values between 1 and 10 mg/L), and the remaining 26 components had low toxicity (EC_{50} values > 10 mg/L).

DISCUSSION

The toxicity expressed in milligrams per liter does not take into consideration the molecular weight of the chemical. To compare the relative toxicities of chemicals, toxicity data are best expressed in terms of the number of molecules that cause a particular toxic effect (i.e., molar units). Thus comparisons of the toxicity of the components in this study were conducted using millimole per liter toxicity data. However, this was not possible when comparing the data from this study with findings from other research on detergents, as most of these are only expressed in milligrams per liter.

While EC_{50} (mg/L and mmol/L) data provide information on the absolute and relative toxicities of the components acting by themselves, they do not indicate the contribution of a component to the toxicity of a detergent. This form of analysis requires the toxicity data to be expressed in terms of toxic units (TU).

The 15 most toxic laundry components, on a molarity basis, will be examined in detail. Thereafter groups of components will be examined.

The most toxic component was sodium carboxymethyl cellulose, with an EC_{50} of 0.0001 mmol/L. However, this value was so high because of the very large molecular weight of the chemical, i.e., 700,000. When expressed in mg/L (Table 1) carboxymethyl cellulose is considered to have low toxicity (Worksafe Australia and NICNAS, 1991). While this chemical was present in 16 of the 23 detergents studied, it was present in low concentrations. In all cases the TU of the carboxymethyl cellulose was less than 0.004 and therefore it never contributed more than 0.41% to the total identified toxicity of the detergents in which it was present (Table 1). A search of the USEPA Aquire database (Pilli *et al.*, 1989) found no toxicity data for this chemical.

The surfactants had either high or moderate toxicity and formed 9 of the 12 most toxic (mmol/L) components. They will therefore be dealt with as a single group rather than individually. They contributed between 10.4 and 98.8% of the toxicity of the detergents with a mean contribution of 40.7%.

The toxicity of surfactants has been found to vary with various chemical characteristics of the surfactants. For example, Holman and Macek (1980), Woltering and Ritchie (1984), Kimerle (1989), and Prats *et al.* (1993) found the toxicity of LAS homologues increased with increasing chain length and/or molecular weight. This trend was not observed in this study (compare components 9, 10, 11, and 13, Table 1). This could be due to the fact that this study used commercial products that contained surfactants of different alkyl chain lengths, whereas previous researchers used surfactants of a single chain length. The mixed alkyl chain lengths of the LAS surfactants may have masked any difference in toxicity.

A difference in toxicity was found between the two nonionic alkyl ethoxylate (AE) surfactants. The AE containing eight ethoxylated (EO) groups (component 2) with an EC_{50} of 0.0007 mmol/L was notably more toxic

than the AE containing three ethoxylated groups (component 8) with an EC_{50} of 0.0089 mmol/L (Table 1). As there were only two AE surfactants, which had different alkyl chain lengths, it is difficult to state, with any degree of confidence, that there was a trend of increased toxicity with increased ethoxylation. No such trend has been observed in the literature. For example, Feijtel and Van de Plassche (1995) stated that there was no relationship between the carbon chain length or the number of ethoxylated groups and toxicity for AE surfactants.

Painter (1992) noted that the toxicity of alkyl ether sulfates (AES) with a chain length of less than 16 carbon atoms tended to decrease with increasing numbers of EO groups. The EC_{50} values for the two AES surfactants (components 6 and 7) appeared to conform to this trend. However, as the 95% confidence intervals overlapped, it was not possible to determine whether the toxicity of the two components was statistically different (Nelson, 1989; Lo, 1994). Thus it is also possible, as stated by Feijtel and Van de Plassche (1995), that there is no clear relationship between alkyl chain length, number of ethoxylated groups, and acute toxicity for AES surfactants. Direct comparison of the EC_{50} values for the AE and AES toxicity data determined in this study with that of other researchers was not possible, as they did not report the extent of surfactant ethoxylation.

The ranges of toxicity values (mg/L) for the LAS, AES, and AS surfactants to *Daphnia magna* in the literature were found to be broad and largely overlapping (Hennes-Morgan and de Oude, 1994). Despite this, the LAS surfactants tended to be the most toxic followed by the AES surfactants that were either more toxic than or as toxic as the AS surfactants (Hennes-Morgan and de Oude, 1994). In contrast, the present study found that the toxicity (mg/L) of the surfactants increased from LAS, to AES, to AS. It should however, be noted that only one AS component was tested in this study (component 3) and therefore the relative toxicity of this group was not definite. The EC_{50} values of LAS and AES surfactants determined in this study were in the middle and low end of their respective ranges of EC_{50} values to *D. magna*. Thus, the apparent change in the order of surfactant toxicity could be an artifact of the limited number of surfactants tested rather than a real difference in relative toxicity.

The literature also indicated that, in general, nonionic surfactants (e.g., AE surfactants) were more toxic, in terms of milligrams per liter, to aquatic organisms than anionic surfactants (Hennes-Morgan and de Oude, 1994). Two AE surfactants were tested in the present study. One (component 2) was more toxic than the anionics while the other (component 8) was less toxic than the AS and AES surfactants but with toxicity very similar to that of the LAS surfactants (Table 1). Thus, from the present study, no conclusion could be reached regarding whether the anionic or the nonionic surfactants were more toxic.

The sodium silicate solution (40%) was the fourth most toxic component tested, with an EC_{50} of 0.0067 mmol/L. It was present in 18 of the detergents studied and unlike the sodium carboxymethyl cellulose, it was present in relatively high concentrations. The sodium silicate solution contributed at least 50% and on average 66.6% of the identified toxicity in all detergents in which it was present (Table 1). The sodium silicate solution was, in fact, the largest single contributor to the toxicity of all detergents in which it was present. The solution form of sodium silicate was markedly more toxic than the solid form, which had an EC_{50} of 0.1822 mmol/L. The difference in toxicity of these two forms is possibly due to the presence of NaOH in the solution (14% mass/mass). Dowden (1961) reported the 48-h EC_{50} of the sodium silicate solution (40%) to *D. magna* to be 494 mg $NaSiO_2$ solution per liter. When the toxicity to *C.* cf. *dubia* was converted to the same units the EC_{50} was 1.47 mg $NaSiO_2$ solution per liter, indicating that the *C.* cf. *dubia* was markedly more sensitive than *D. magna*. The toxicities of the sodium silicate solution to a range of other organisms were one to two orders of magnitude larger than to the cladoceran used in this study (Dowden and Bennett, 1965; Wallen *et al.*, 1957).

The dimorpholino stilbene derivative was the fifth most toxic component, with an EC_{50} of 0.0074 mmol/L. However, it was only present in two detergents and at low concentrations. It had TU values of 0.005 and 0.0005 and never contributed more than 0.3% of the toxicity for either detergent.

After the surfactants the next most toxic component was sodium perborate tetrahydrate, which is used as a bleaching agent. This component was only present in two of the detergents and at such low concentrations that the TU values were 0.058 and 0.042. The perborate therefore accounted for only 2.9 and 3.3% of the toxicity of the detergents in which it was present. EC_{50} values for sodium perborate to *D. magna* ranged from 1900 to 3200 mg/L (Maier and Knight, 1991; Hennes-Morgan and de Oude, 1994), which indicated that it was more tolerant than *C.* cf. *dubia*, which had an EC_{50} of 6.98 mg/L.

The brighteners were the second most toxic group of components with EC_{50} values 0.0074, 0.046, 0.047, and 0.72 mmol/L. They were present in 21 of the detergents but at such low concentrations that the combined TU for all brighteners in a detergent never exceeded 0.003 and were never responsible for more than 0.6% of the measured toxicity of a detergent. No toxicity data for other crustaceans were available in the literature; however, data were available for channel catfish and rainbow trout (Keplinger *et al.*, 1974). For the biphenyl distyryl derivative and the bis-(triazinylamino)-stilbene sulfonic acid derivative, *C.* cf. *dubia* was approximately two to four times more sensitive than fish, while for the dimorpholino stilbene derivative *C.* cf. *dubia* was 100 to 200 times more sensitive than fish.

Eight builders and cobuilders were tested in this study (components 4, 18, 20, 22, 23, 24, 25, and 26, Table 1). The ranking of these components, in order of decreasing toxicity (mmol/L), was sodium silicate solution, zeolite, sodium silicate, sodium tripolyphosphate (STPP), sodium carbonate, soda ash, and sodium citrate. The toxicities of the sodium carbonate and soda ash were very similar. This was not unexpected, as they are essentially the same chemical. The STPP was more toxic (mmol/L) than most of the alternative builders—sodium carbonate, soda ash, and sodium citrate. The literature (United Nations, 1992; Dowden and Bennett, 1965; Dowden, 1961; Freeman and Fowler, 1953; Hennes-Morgan and de Oude, 1994; Canton and Slooff, 1982; Morse *et al.*, 1994) indicated that STPP, soda ash, and possibly zeolite had similar toxicities and were all more toxic to *D. magna* than the sodium silicate solution. These differences in relative toxicity occurred because the toxicity of STPP, zeolite, sodium silicate solution, and sodium citrate to *C.* cf. *dubia* was noticeably greater than that reported for *D. magna* (Freeman and Fowler, 1953; Canton and Slooff, 1982; United Nations, 1992; Hennes-Morgan and de Oude, 1994; Morse *et al.*, 1994) while the sodium carbonate EC_{50} values for *D. magna* and *C.* cf. *dubia* were similar (Dowden, 1961; Dowden and Bennett, 1965).

Four different types of enzymes were tested: a protease, a cellulase, a lipase, and an α-amylase. Each enzyme, except the cellulase, was tested in both liquid and encapsulated forms—thus a total of seven enzymes were tested. Based on the Worksafe Australia and NICNAS (1991) toxicant classification scheme the enzymes were of low toxicity, having EC_{50} values ranging from 15 to greater than 10,000 mg/L. The protease, lipase, α-amylase, and cellulase enzymes hydrolyze proteins, triglycerides, polysaccharides, and cellulose respectively. Proteins and triglycerides are both key components in cellular membranes such as gills. The exoskeleton of *C.* cf. *dubia* is composed predominantly of chitin, a nitrogen containing polysaccharide (Hackman, 1987). This exoskeleton could potentially be hydrolyzed by the α-amylase. However, this enzyme hydrolyzes 1,4-α-glucosidic bonds while chitin only contains 1,4-β-glucosidic bonds (Hackman, 1987). It might therefore be expected that the protease and lipase enzymes would be more toxic than the α-amylase and cellulase enzymes. The activity of enzymes are expressed using units such as KNPU/g of formulation, where 1 KNPU is the amount of enzyme needed to metabolize a substrate at a given rate, under specific conditions. However, the activity of different enzymes is expressed using different units (Table 1) and therefore, the toxicities are not directly comparable.

Despite this, comparison of the toxicities of the liquid and encapsulated forms of the same enzyme was possible. As the enzyme activities are measured under specified conditions they are directly related to the amount of enzymes present.

In order to compare the toxicities the EC_{50} values must be expressed in terms of the relative amount of enzyme, e.g., KNPU/L. This was done by converting the EC_{50} (mg/L) data to grams per liter and then multiplying by the activity (e.g., KNPU/g) (Column 5, Table 1).

In all cases the encapsulated form of the enzyme was markedly more toxic than the liquid form (compare components 33 and 34, 35 and 36, 37 and 38 in Table 1). Theoretically, as the enzymes in both forms are identical the EC_{50} values (KNPU/L) for both the encapsulated and liquid forms should be the same when tested under the same experimental conditions. In all the bioassays using the encapsulated enzymes there was a marked decrease in the dissolved oxygen content of the test solutions to levels as low as 5% saturation. Thus, the increased toxicity of the encapsulated form appeared to be due to the oxygen demand of the chemicals that formed the capsule.

The remaining laundry components were fillers, dyes, and miscellaneous additives, which included formaldehyde, diethanolamine, sodium hydroxide, triethanolamine, tartrazine, sodium sulfate decahydrate, anhydrous sodium sulfate, sodium chloride, and urea. All these components were of low toxicity (Worksafe Australia and NICNAS, 1991), having EC_{50} values that ranged from 12.98 to 6573 mg/L. They did not contribute significantly to the overall toxicity of the detergents, as the maximum percentage of the toxicity that could be attributed to these components in any one detergent was 0.6% (Table 1).

The TU values for all components in each detergent were summed (*S*) and converted to the corrected toxicity enhancement index values (cTEI), which are presented in Table 2. There were seven detergents that had cTEI values less than -0.5 and therefore the combined toxicity of the components in these detergents was antagonistic (Table 2). There were nine detergents that had cTEI values between -0.5 and 0.5 and therefore these detergents had additive toxicity. The remaining seven detergents had cTEI values of greater than 0.5 and thus were synergistic (Table 2).

These results should be interpreted cautiously, as the TU values for all components were not available for any of the detergents. As TU values of the missing components must be greater than zero, the inclusion of the missing data could only increase the *S* values and decrease the cTEI values [refer to Eqs. (2) and (3)]. Thus, while the detergents that were classified above as having antagonistic toxicity would remain antagonistic if the missing TU data became available, the cTEI values of the detergents classed as additive and synergistic would decrease. So it could not be definitely stated that these detergents had additive or synergistic toxicity. The types of detergent components that were missing toxicity and TU values for each detergent are indicated in Table 2. Based on the number of components missing TU data, as well as the closeness of the current cTEI values to

TABLE 2
Number of Components, Corrected Toxicity Enhancement Index (cTEI) Values, and Type of Components That Are Missing Toxicity and Toxic Unit Data for Each Detergent Studied

Detergent No.	cTEI	Missing components
1	4.21	P, S
2	4.00	P
3	1.78	P, S, Su, Salt, Polymer
4	1.33	P, D, EDTA
5	1.22	P, D
6	0.69	P, D, Buil, Sil
7	0.57	P
8	0.28	P, Salts
9	0.21	P, Bl, A, D, Su
10	0.10	P, D, Su
11	− 0.02	P, Buil
12	− 0.05	P, D, Su
13	− 0.19	P, D, Su
14	− 0.26	P, Sil, A, Polymer
15	− 0.29	P
16	− 0.35	P
17	− 0.59	P
18	− 0.61	P, Sil, D
19	− 0.69	Su
20	− 0.79	P, D
21	− 0.80	P, D, Su
22	− 0.81	P, D
23	− 1.00	P, Sil, A

Note. P, perfume; S, soap; Su, surfactant; D, dye; Buil, builder; Sil, silicon antifoam; Bl, bleach; A, amine.

those separating the different types of toxic interaction, it was estimated that only three detergents, Nos. 6, 7, and 14, were likely to change their type of interaction by the inclusion of the missing TU data. Detergents 5 and 6 may become additive while detergent 14 may become antagonistic.

The finding in this study that mixtures of detergent components had antagonistic, additive, and synergistic interactions was consistent with Lewis's (1992) findings on the toxicity of mixtures containing surfactants. It is also consistent with the study by Ross and Warne (in preparation), who found that 973 mixtures of organics, inorganics, metals, and pesticides had antagonistic, additive, and synergistic toxicities. That a large percentage of the detergents had additive toxicity was also not surprising. A number of reviews have concluded that the majority of mixtures are additive (e.g., EIFAC, 1987; Van Leeuwen et al., 1996; Warne and Hawker, 1995; Ross and Warne, in preparation).

Theoretically, chemicals should interact with other chemicals that have the same mechanism of action in an additive fashion (Hewlett and Plackett, 1959; Könemann, 1980). Evidence supporting this has been reported (Könemann, 1980; Broderius and Kahl, 1985). Thus, the antagonistic and

synergistic toxicity of detergent components found in this study indicate that some of the components have different mechanisms of action.

While many of the components were of moderate or high toxicity when examined individually, this does not automatically mean that aqueous solutions of detergents will have a major environmental impact. A number of factors need to be considered. First, the actual concentration at which the components are present in the detergents needs to be considered, as this can greatly reduce likely exposure concentrations. Second, the combined toxicity of the detergents must be assessed because antagonistic, additive, and synergistic toxic interactions occur. Third, under normal conditions detergent solutions are disposed of via the sewage system where they should undergo significant dilution. For example, Feijtal and Van de Plassche (1995) expect that aqueous solutions of detergent would be diluted 10-fold by the time they reached a STP. Fourth, detergent solutions generally only enter waterways after passing through a STP where many of the components, particularly the surfactants, are removed or biodegraded. A number of studies have found that upward of 90% of LAS, AE, AES, and AS surfactants are removed during passage through a STP with secondary treatment (Turner et al., 1985; McAvoy et al., 1993; Feijtel and Van de Plassche, 1995). However, primary treatment does not remove surfactants to the same extent and they have been identified as toxicants in primary effluent (Ankley and Burkhard, 1992). Finally, the effect of dissolved organic matter, suspended particulate matter, and sediments on the fate and biological availability of the detergent components once they reach waterways should be considered. For example, Traina et al. (1996) found that dissolved humic substances reduced the aqueous bioavailability of LAS and Cano et al. (1996) found that organic carbon in sediments reduced the toxicity to *Hyalella azteca*. Such factors could lead to the environmental impact of aqueous solutions of detergents being less than predicted from laboratory toxicity test results.

On the other hand, a factor that could increase the environmental impact of laundry detergent solutions is that surfactants can form micelles. Once micelles form they increase the apparent solubility (Valsaraj and Thibodeux, 1989), bioavailability (Guha and Jaffé, 1996), and toxicity (Poremba, 1993) of hydrophobic organic compounds present in the same solutions. As sewage receives a large mass of industrial effluent, there is considerable potential for this to occur.

The possibility that detergent solutions may exert an inhibitory effect on the sewage sludge microorganisms, too, should not be discounted. Any such effect could have serious consequences, as the effectiveness with which the sewage treatment removes pollutants and breaks down sewage might be compromised. This may warrant further research.

Further, this project assessed only acute toxic effects of components of laundry detergents. It is most likely that chronic effects would also occur and at lower concentrations. Recently Jobling *et al.* (1998) found sexual disruption of fish in rivers throughout the United Kingdom, which was positively linked to sewage effluent. This, combined with their earlier work (Jobling and Sumpter, 1993) that found that some detergent components are weakly estrogenic, highlights another potential problem associated with detergents. It is not known, at this stage, whether such effects are due to acute or chronic exposure. In rivers where a significant proportion of the flow is sewage effluent, the potential for sexual disruption should be considered.

Surfactants and the sodium silicate solution were the two main contributors to the toxicity of detergents, accounting for a mean of 40.7 and 66.6% of the toxicity of detergents they were in, respectively. Studies such as those mentioned above show that more than 90% of surfactants can be removed during secondary sewage treatment and that organic matter and sediments can affect the bioavailability of these detergent components. Thus, where substantial removal of surfactants occurs during sewage treatment, the potential environmental impact of surfactants in detergents can be substantially reduced before they enter an aquatic system. Such information, however, is not available for the sodium silicate solution component. It is not known what type of toxic interaction may occur in a mixture as a result of this component or if it is removed or degraded during sewage treatment processes. The effects of organic matter and sediments on the bioavailability of a sodium silicate solution are also not known. However, while it is present in relatively high concentrations in detergents, the concentration resulting from a 10-fold dilution of the EC_{50} would have no acute toxic effect on *C*. cf. *dubia*. As more dilution would take place after entering an aquatic system, the presence of the sodium silicate solution in detergents is unlikely to cause acute toxic effects on *C*. cf. *dubia* even if no removal or breakdown of this component occurs during passage through a STP.

CONCLUSIONS

The majority (56%) of the 39 components tested had low toxicity. Mixtures of the components interacted antagonistically, synergistically, and additively. On a molar basis the most toxic group of components was the surfactants followed by the brighteners. When examined individually the most toxic (mmol/L) components included sodium carboxymethyl cellulose, sodium silicate solution, a brightener, sodium perborate tetrahydrate, and the surfactants. However, many of the most toxic components, particularly the brighteners, contributed very little to the toxicity of the detergents, as they were present in low concentrations. The

sodium silicate solution and the surfactants were the two main contributors to the toxicity of detergents, accounting for a mean of 66.6 and 40.7% of the toxicity of detergents they were in, respectively. The remainder of the components individually contributed very little to detergent toxicity. As sewage treatment plants with at least secondary treatment can remove up to 90% of surfactants, such treatment may remove a substantial proportion of the toxicity identified in this study. Dilution in sewers may reduce toxicity from sodium silicate. Thus, the potential acute environmental hazard posed by effluent containing the breakdown products of laundry detergents may frequently be low. The discharge to waters, however, of untreated or primary treated effluent containing detergents may be more problematic as may be the chronic and/or other sublethal effects that were not evaluated in this study.

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