RESEARCH REPORT:
EYE IRRITATION AND SURFACTANT PROPERTIES OF NONIONIC SURFACTANTS

Part 1. In Vitro Test Results

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Non-Animal Testing Research Subcommittee
Biomedical Research Committee
Soap and Detergent Association
475 Park Avenue South
New York, New York 10016

John E. Heinze, Ph.D.
Technical Consultant

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Summary

Research on nonionic surfactants was initiated by the Non-animal Testing Subcommittee of the Soap and Detergent Association as an extension of Phases I, II and III of the subcommittee's long term research program on in vitro tests. The goal of this program is to reduce the dependency on animal testing for the evaluation of the eye irritation potential of cleaning products and ingredients.

The results of the first three phases of this program demonstrated that a number of in vitro tests are suitable for screening materials for their eye irritation potential but none are accurate enough to replace the Draize eye test. This program also determined that alkaline materials are a category of cleaning products and ingredients which are not well predicted by at least some in vitro tests.

A logical next step for this program was to ask if there are other categories of cleaning products and materials which are not well predicted by in vitro tests. The results in Part I of this report describe a program of exploratory research conducted to determine if nonionic surfactants were, if fact, another such category.

The first section of this report is a review of the literature on nonionics surfactants. Analysis of the results in several papers suggested that the eye irritation potential of nonionic surfactants is not well predicted by some in vitro tests. This review also indicated that the quality of the in vivo eye irritation data is important in evaluating the ability of in vitro tests to predict the eye irritation potential of nonionics.

However, it is the unpublished data made available to the SDA that most clearly demonstrates that the eye irritation potential of nonionic surfactacts is not well predicted by a number of in vitro tests. For instance, in the first set of data examined, all five of the in vitro tests misclassified (false negative or false positive result) one or two of the seven nonionics tested. The results were even worse with the second set of data. One test (PGE2 release in the ZK1200 system) was found to give results (lowest release concentration) that varied by 100 fold or more. The four other in vitro tests examined misclassified two to four of the five nonionics examined.

Published structure-activity relationships were examined for their ability to explain in vivo eye irritation scores or in vitro test results on these nonionic surfactants. Since structure-activity relationships for eye irritation potential are not available, surfactant properties which have been used to predict aquatic toxicity or bioaccumulation were examined. There was no apparent relationship between either the in vivo or the in vitro results and surface tension, critical micelle concentration, octanol-water partitioning or hydrophilic-lipophilic balance. However, data on surfactant properties were not available for all the nonionics tested, a fact which limited the relationships that could be analyzed. Moreover, the surfactant data available was not determined on the actual samples tested in vivo or in vitro and was obtained by different laboratories at different times and perhaps by different test methods. These limitations and differences may have confounded the search for structure-activity relationships.

Nonionic Surfactants Report: Part I. In Vitro Test Results

This program of exploratory research has demonstrated that there is a need for high quality in vivo eye irritation results on a larger set of nonionics than is available today. To ensure consistency among the results, this data should be generated by simultaneously testing the nonionics in a single laboratory using an up-to-date test protocol. Furthermore, the relevant surfactant properties of the nonionics should be determined using the same materials. In this way a consistent and reliable database can be created to: 1) determine the eye irritation potential of a range of nonionic surfactants representative of those used in cleaning products; 2) determine if the eye irritation potential of these nonionics correlates with any surfactant properties or structure-activity relationship; and 3) provide a reference set of nonionics for use in evaluating in vitro test methods.

The results of tests designed to meet this data need are describe in Part II of this report.

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Introduction

The Soap and Detergent Association (SDA), under the direction of the Non-animal Testing Research Subcommittee of the Biomedical Research Committee, is evaluating the ability of in vitro assays to predict the eye irritation potential of products and ingredients important to the industry. The goal of this research program is to significantly reduce the dependency on animal testing for the evaluation of the eye irritation potential of cleaning products and ingredients.

The SDA evaluation of in vitro assays has consisted of a sequential or phased approach with the aim of validating one test or a small battery of tests to predict eye irritation potential. The plan was for each phase of the evaluation to focus on the best tests from the previous phase using a broader range of products until the best tests could be identified and their validity assured. Consequently, Phase I compared the results from the in vitro tests of 14 investigators with 8 test materials (Booman et al., 1988). Phase II examined 9 tests and 23 materials (Booman et al., 1989). The results of Phase II suggested that alkalinity was an additional parameter that had to be considered in the evaluation of in vitro test methods. Alkalinity is a property of many cleaning products and a potential source of eye irritation that is apparently not detected in some in vitro tests. Moreover, it was becoming clear that the universe of existing in vitro test methods is highly dynamic: some test methods continued to be improved while others were abandoned and new methods developed.

Consequently, Phase III consisted of two parts: one part focused on the effects of alkalinity and pH on eye irritation potential. By using aqueous solutions at a single pH but buffered to specific alkalinities, this study was able to demonstrate that both alkalinity and pH are important in determining eye irritation potential (Neun, 1993).

The second, and far more complex part, of Phase III was a refinement of Phase II in which ten in vitro tests were evaluated with 22 materials representing various classes of cleaning products and ingredients (McCormick, 1989). The in vitro tests included the best available tests from Phase II as well as several improved or new test methods. The refinements included: 1) the range of the types of cleaning products and ingredients was extended over that in Phase II; 2) cleaning product ingredients were tested at concentrations likely to be found in cleaning products; 3) test materials and test concentrations were selected to obtain a range of eye irritation responses; 4) the alkalinity of the test materials were measured and considered in the data analysis; 5) eye irritation responses of test materials were confirmed in a modified Draize eye irritation test; and 6) in vitro test results were compared to the maximum average score from the modified Draize eye irritation test and to classification of the eye irritation potential according to Federal Hazardous Substances Act (FHSA) criteria.

The results of this evaluation (Bagley et al., 1994) showed that some in vitro tests are better than others at predicting the eye irritation potential of alkaline materials. However, when the alkaline materials were excluded from the analysis, all of the in vitro tests performed equally well, with

correlation coefficients of 80 to 90%. Six of the 10 tests correctly identified the five nonirritants in the study although none of the tests were able to predict the relative irritation potential of all 17 irritants.

Based on these results, none of the evaluated in vitro tests is accurate enough to replace the Draize eye test so that further attempts at validation, using larger sets of test materials, would not be productive. However, one or more of the tests evaluated in Phase III may be useful for screening cleaning products and ingredients for eye irritation potential. Indeed, in vitro tests are being used as part of screening programs by member companies to reduce the dependency on animal testing.

The next logical step is to determine if there are other classes of cleaning products or ingredients, like alkaline materials, the eye irritation potential of which is not well predicted by in vitro tests. Based on the published literature and unpublished data made available to SDA, nonionic surfactants seem to be such materials.

Nonionic surfactants are a key class of surface active compounds having no ionic charge in aqueous solution. Nonionic surfactants are widely used in cleaning products because of their excellent detergent and emulsification properties, the variety of chemical structures and properties available and their insensitivity to water hardness.

Literature Review

Despite their widespread use in cleaning products, relatively few studies have focused on predicting the eye irritation potential of nonionic surfactants.

Shopsis et al. (1985) examined a group of 17 surfactants, including 6 nonionics, in a battery of in vitro tests for predicting eye irritation. The surfactant predicted to have the lowest eye irritation potential (based on inhibition of uridine uptake in Balb/c 3T3 mouse cells) was a nonionic surfactant, polyoxyethylene (20) sorbitan monooleate (TWEEN 80). Inhibition of uridine uptake required a lower concentration of TWEEN 80 than that required for a severely irritating material, allyl alcohol, suggesting that surfactants have greater in vitro potency relative to their eye irritation potential than other materials. However, Shopsis et al (1985) did not attempt a direct comparison of the in vitro potency of surfactants as measured in their battery of tests with in vivo eye irritation scores.

Marinovich et al. (1990) examined the ability of in vitro assays to predict the eye irritation potential of 14 surfactants, including three nonionics. The assays used an established mouse epidermal cell line (HEL/30) to measure leakage of a cytoplasmic enzyme (lactate dehydrogenase), protein synthesis (using radiolabeled leucine) and total protein content (Lowry method) after 2 hours exposure to the surfactants. Surfactants were classified as to their eye irritation potential based on the NIOSH *Registry of Toxic Effects of Chemical Substances* (1977).

Inhibition of protein synthesis was found to be the most sensitive assay. However, the enzyme leakage assay and the protein content assay, which gave results which were highly correlated with each other, gave a somewhat better prediction of eye irritation classification than the protein synthesis assay. The difference in correlation was primarily due to the results with two nonionics, cocodiethanolamide (cocamide DEA) and polyoxyethylene (16) octylphenol ether (TRITON X-155), both of which were considered to be "mild." The classification of cocamide DEA is supported by the report of the Cosmetic Ingredient Review (1986). However, no reference was found to specific data on TRITON X-155 in the NIOSH database (NIOSH, 1980), and it is classified as a rabbit and human eye irritant by Grant (1986, p. 873). Based on the revised classification of TRITON X-155, the enzyme leakage and protein content assays misclassified this nonionic while the protein inhibition assay misclassified cocamide DEA.

Sina et al. (1992) assessed five in vitro cytotoxicity assays for their ability to predict the eye irritation potential of 27 commercially available compounds of diverse chemical structures, including three surfactants. The assays used either primary rabbit corneal cells or an established cell line (V79 Chinese hamster lung fibroblasts) and measured protein synthesis (using radiolabeled leucine), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] dye reduction, or neutral red uptake. Eye irritation potential of the compounds was classified as "mild," mild/moderate," "moderate," and "severe" based on literature results from in vivo testing. The only nonionic surfactant tested, polyoxyethylene (20) sorbitan monolaurate (TWEEN 20), was a false positive, showing more toxicity than the three other mild compounds. The other two surfactants tested, an anionic and a cationic, were the most cytotoxic of the 9 severe irritants tested, supporting the suggestion of Shopsis et al. (1985) that surfactants should be evaluated using a separate scale from other compounds. Sina et al. (1992) concluded from their analysis of the data that none of the assays examined were able to accurately predict the eye irritation potential of the compounds tested.

Gay et al. (1992a) studied a group of 35 chemicals, including an anionic, a cationic and four nonionic surfactants. The test system was a three dimensional tissue model (Living Dermal Equivalent, LDE) composed of human dermal fibroblasts in a collagen-containing matrix using MTT dye reduction to quantify viable cells. Published rabbit eye irritation scores from several sources were normalized to a scale of 1 to 5, where 1 is "nonirritating" and 5 is "severely irritating." The authors reported a generally good rank order correlation between cytotoxicity in the LDE and eye irritation classification. As with Shopsis et al. (1985), the main exceptions to this correlation were the two nonirritating nonionic surfactants, polysorbate 40 [polyoxyethylene (20) sorbitan monopalmitate] and polysorbate 80 [polyoxyethylene (20) sorbitan monooleate]. These nonionics were substantially more cytotoxic than the other nonirritating compounds tested (CARBOWAX 600, glycerin, mineral oil, palmitic acid, petrolatum and water). Since the other four surfactants were the most cytotoxic compounds tested, the results suggest that surfactants have greater in vitro potency relative to their eye irritation potential than other materials.

The results of SDA phase III testing, which included eight surfactants among the 16 compounds tested as well as 6 cleaning products (Bagley et al., 1994), and results from a number of other in vitro

systems (Sasaki et al., 1991; Rougier et al., 1992; Christian & Diener, 1996) contradict this claim. These studies show that the in vitro potency of surfactants and surfactant-based products is similar to that of non-surfactant organic compounds which have similar in vivo potency.

Moreover, several authors have reported success in the use of in vitro tests to distinguish the small differences in relative mildness between pairs of ethoxylated and non-ethoxylated (less mild) anionic surfactants. For instance, Watnabe et al. (1989) was able to distinguish between sodium lauryl sulfate and sodium laureth (polyoxyethylene 12) sulfate with a colony forming assay using primary rabbit corneal cells. Heinze et al. (1991) were able to distinguish among sodium C12-C14 alcohol sulfate, sodium C12-C14 alcohol ether (polyoxyethylene 3) sulfate and sodium C12-C14 alcohol ether (polyoxyethylene 7) sulfate with the Microtox™ luminescent bacteria test, the Tetrahymena motility assay and the the Testskin™ Living Dermal Equivalent test measuring inflammatory mediator (prostaglandin E2) release. In SDA phase III testing (Bagley et al., 1994), nine of the ten in vitro assays were able to distinguish between 10% sodium alkyl sulfate (Draize maximum average score (MAS) = 36.2) and 10% alkylethoxylsulfate (MAS = 18.8). Domsch et al. (1996) was able to distinguish between sodium lauryl sulfate and sodium laureth sulfate by measuring hemolysis of red blood cells and denaturation of hemoglobin.

One explanation for the discrepancy between the generally favorable results with surfactants on one hand and those of Shopsis et al (1985) and Gay et al. (1992a) on the other hand is that the difficulty is only with certain surfactants, namely nonionics.

Several authors have suggested that nonionic surfactants such as TRITON X-100 (polyoxyethylene (9) octylphenol ether) give false positive responses in various in vitro assays for predicting skin irritation potential (Cornelis et al., 1991; Gay et al., 1992b; Harvell et al., 1994). However, the detailed examination of test methods for predicting skin irritation of nonionic surfactants is beyond the scope of this report.

Based on this review, there are some indications in the published literature that nonionic surfactants cause difficulties for at least some in vitro tests. It seems clear that the quality of the in vivo eye irritation data and the types of compounds that are compared to nonionic surfactants are important in determining the ability of in vitro tests to predict the eye irritation potential of nonionic surfactants. Unpublished data made available to SDA by member companies has further illuminated the uncertainties in the testing of nonionic surfactants.

Unpublished Data From In Vitro Tests

Table 1 shows the nonionic surfactants examined in the first set of data and their eye irritation classification based on the published literature. This set consists of one anionic surfactant (sodium lauryl sulfate) and seven nonionics, including five that are classified as mild.

Table 1 also shows published and unpublished results with these surfactants in two cytotoxicity assays using established lines. Effective concentrations for 50% inhibition (EC-50) of uridine uptake after four hours exposure was determined in Balb/c 3T3 mouse cells (Shopsis et al., 1985) while EC-50's for inhibition of colony forming ability after one hour exposure were measured in SIRC rabbit corneal cells (North-Root et al., 1982). These results suggest that BRIJ-35 gave a false positive for irritation in the uridine uptake assay and that GLUCOPON 625CS and BRIJ-35 were false positives in the SIRC cell assay -- or that both nonionics are misclassified for eye irritation and should be considered "irritants."

Table 1
Comparison of Eye Irritation Potential with Two Cytotoxicity Assays

Surfactant Name[1]	Surfactant Brand name/ abbreviation	Eye Irritation[2]	Eye Irritation[3]	3T3 mouse cells Uridine uptake 4 hr. exposure EC-50, ppm[4,5]	SIRC corneal cells Colony formation 1 hr. exposure EC-50, ppm[4,6]
Sodium lauryl sulfate (anionic)	SLS	Irritant	Severe	85	28[7]
POE (9) octyl-phenol ether	TRITON X-100	--	Severe	--	25-50[8]
POE (16) octyl-phenol ether	TRITON X-155	Irritant	--	23	--
C12.8 alkylpoly-glucoside (1.6)	GLUCOPON 625CS[9]	--	--	--	15[8]
POE (23) lauryl ether	BRIJ-35	Mild	--	70	36
POE (20) sorbitan monolaurate	TWEEN 20	Mild	Mild	--	135[10]
POE (20) sorbitan monopalmitate	TWEEN 40	Mild	--	190	11500
POE (20) sorbitan monooleate	TWEEN 80	Mild	--	400	13300[7]

[1]POE = polyoxyethylene, C = carbon chain, (number) = number of oxyethylene or D-glucose units.
[2]Grant, 1986, pp. 871-3.
[3]Kennah et al., 1989.
[4]EC-50 = Effective concentration for 50% inhibition.
[5]Shopsis et al., 1985.
[6]North-Root et al., 1982.
[7]J. Demetrulias, personal communication.
[8]CONDEA-Vista unpublished data.
[9]Mild (Henkel unpublished data).
[10]Booman et al., 1988.

Table 2 shows the EC-50 results with this same set of compounds in the Microtox™ luminescent bacteria test (LBT) after 15 minutes of exposure. TRITON X-155 could not be assayed in this test

because aqueous solutions were cloudy and interfered with light transmission in this assay. Based on the results with the other nonionics, GLUCOPON 625CS appears to give a false positive result.

Table 2
Comparison of Eye Irritation Potential with Light Emission and MTT Uptake Assays

Surfactant brand name/ abbreviation[1]	Eye Irritation[2]	Eye Irritation[3]	MICROTOX LBT[4] Light emission 15 minute exposure EC-50, ppm[5] Exp. #1	Exp. #2	SKIN^2 ZK1200 MTT uptake 30 minute exposure EC-50, ppm[5] Exp. #2	Exp. #1[6]
SLS (anionic)	Irritant	Severe	1.7	1.3	2700	2700
TRITON X-100	--	Severe	--	1500	4200	--
TRITON X-155	Irritant	--	--	?	35400	--
GLUCOPON 625CS[7]	--	--	4	--	--	13100
BRIJ-35	Mild	--	5200	--	--	14100
TWEEN 20	Mild	Mild	--	6300	>>30000	--
TWEEN 40	Mild	--	--	20000	>>30000	--
TWEEN 80	Mild	--	--	17000	>>30000	--

[1]See Table 1 for full names.
[2]Grant, 1986, pp. 871-3.
[3]Kennah et al., 1989.
[4]LBT = luminescent bacteria test.
[5]CONDEA Vista unpublished data. EC-50 = effective concentration for 50% inhibition; Exp. = experiment. Results from two independent experiments are shown.
[6]Corrected for differences between exposure conditions in Exp. #1 (100 µl) and Exp. #2 (50 µl on applicator pad) by multiplying values in Exp. #1 times 2700/706.
[7]Mild (Henkel unpublished data)

Table 2 also shows the EC-50's for inhibition of MTT dye uptake in a three-dimensional tissue model, SKIN^2 ZK1200 from Advanced Tissue Sciences. EC-50's were estimated after 30 minutes topical exposure. A correction factor has been applied to the values observed in the first experiment to correct for apparent differences in response to the two methods of surfactant application used in the two experiments. The results indicate that TRITON X-155 gives a false negative result for eye irritation in this test.

Table 3 shows the results obtained using a modification of the MTT assay in the SKIN^2 ZK-1200 tissue model. In this assay, the exposure time required to produce a 50% reduction in MTT uptake (ET-50) is quantified. ET-50 scores are evaluated using the following scale: <1 minute = harsh; 1-10 minutes = mild to moderate; >10 minutes = innocuous. As in the previous MTT assay in ZK-1200, TRITON X-155 appears to give a false negative response for eye irritation in this test. Although less

dramatic than TRITON X-155, sodium lauryl sulfate (SLS) also gives a false negative response. The results from this assay have now been published (Rachui et al., 1994).

Table 3
Comparison of Eye Irritation Potential with SKIN[2] Topical Exposure Assay

Surfactant brand name/ abbreviation[1]	Eye Irritation[2]	Eye Irritation[3]	SKIN[2] ZK1200 MTT uptake Topical exposure[4] Conc.	ET-50 (min.)	Grade
SLS (anionic)	Irritant	Severe	10%	1.9	Mild-Mod.
TRITON X-100	--	Severe	10%	<1	Harsh
TRITON X-155	Irritant	--	10%	23	Innocuous
GLUCOPON 625CS[5]	--	--	15%	14	Innocuous
BRIJ-35	Mild	--	Undil.	21	Innocuous
TWEEN 20	Mild	Mild	Undil.	9.4	Mild-Mod.
TWEEN 40	Mild	--	Undil.	>15	Innocuous
TWEEN 80	Mild	--	Undil.	>30	Innocuous

[1]See Table 1 for full names. [2]Grant, 1986, pp. 871-3. [3]Kennah et al., 1989.
[4]Rachui et al., 1994: Conc. = concentration tested; ET-50 = effective time for 50% inhibition of MTT uptake; mod. = moderate; undil. = undiluted; 2% SLS control (n = 4) gave mean (standard deviation) = 1.1 (0.4) minutes.
[5]Mild (Henkel unpublished data)

Table 4 shows the nonionic surfactants examined in the second set of data, their maximum average scores (MAS) in the standard Draize eye test and their eye irritation classification.

Table 4 also shows the results observed with two in vitro tests previously considered, inhibition of colony formation in SIRC rabbit corneal cells and inhibition of light emission in the Microtox™ test. Using the value for sodium lauryl sulfate as a benchmark, then in the SIRC cell test, the C8-C10 alcohol ethoxylate with 7.8 oxyethylene units [AE 810(50:50)-7.8], which is a moderate irritant in the Draize eye test, appears to give a false negative response while the C12-C14 alcohol ethoxylate with 6.9 oxyethylene units [AE 1214(50:50)-6.9], the C12.8 alkylpolyglucoside with 1.6 D-glucose units [GLUCOPON 625CS], and the C12-C14 alcohol ethoxylate with 1.1 oxyethylene units [AE

1214(50:50)-1.1], all mild or nonirritating nonionics in the Draize eye test, appear to false positive responses. Depending on the interpretation of the results with polyoxyethylene (23) lauryl ether (BRIJ-35), the SIRC cell test misclassified four or five of the five nonionics tested.

Table 4
Comparison of Eye Irritation Potential with Colony Formation and Light Emission Assays

Surfactant Name[1]	Surfactant Brand name/ abbreviation	Draize Eye Test-- MAS[2]	Draize Eye Test-- Classification[2]	SIRC corneal cells Colony formation 1 hr. exposure EC-50, ppm[3]	MICROTOX LBT Light emission 15 min. exposure EC-50, ppm[3]
Sodium lauryl sulfate (anionic)	SLS	10%= 37.3	Irritating	28	1.7
C8-C10 alcohol ethoxylate (7.8)	AE 810 (50:50)-7.8	33.8	Moderate	100-200	10
C12-C14 alcohol ethoxylate (6.9)	AE 1214 (50:50)-6.9	19.7	Mild	7.6	1.5
POE (23) lauryl ether	BRIJ-35	<<20	Nonirritating	36	5200
C12.8 alkylpoly glucoside (1.6)	GLUCO-PON 625CS	15%=4.0	Nonirritating	15	4.0
C12-C14 alcohol ethoxylate (1.1)	AE 1214 (50:50)-1.1	4.3	Nonirritating	9.2	1.2

[1]POE = polyoxyethylene, C = carbon chain, (number) = number of oxyethylene or D-glucose units.
[2]CONDEA Vista unpublished data except for SLS (Tachon et al., 1989), BRIJ-35 (North-Root, et al., 1982) and GLUCOPON 625CS (Henkel Corporation, unpublished data). MAS = maximum average score.
[3]CONDEA Vista unpublished data: LBT = luminescent bacteria test.

The Microtox™ luminescent bacteria test (LBT) gave somewhat better results than the SIRC cell test with this series of surfactants, correctly identifying BRIJ-35 as milder than SLS. However, as in the SIRC cell test, the LBT gave false positive results for AE 1214(50:50)-6.9 and AE 1214(50:50)-1.1, based on the benchmark value for SLS. Compared to the value for BRIJ-35, the LBT also appears to give a false positive result for GLUCOPON 625CS. Thus the Microtox™ test misclassifies three of the five nonionics tested.

Table 5 shows the results with this set of surfactants using a three-dimensional tissue model, SKIN^2 ZK1200. Two assay methods were used, inhibition of neutral red uptake and release of prostaglandin E2 (PGE2). With the neutral red assay, AE 810(50:50)-7.8 appears to give a false

negative response when compared to the results with GLUCOPON 625CS and AE 1214(50:50)-1.1 while AE 1214(50:50)-6.9 appears to give a false positive response compared to the results with SLS.

Table 5
Comparison of Eye Irritation Potential with Neutral Red Uptake and PGE2 Release Assays

Surfactant brand name/ abbreviation[1]	Draize Eye Test-- MAS[2]	Draize Eye Test-- Classification[2]	SKIN[2] ZK1200 Neutral red uptake 4 hrs. exposure? EC-50, ppm[3]	SKIN[2] ZK1200 PGE2 release 4 hrs. exposure LRC, ppm[3]
SLS (anionic)	10%= 37.3	irritating	29	98
AE 810 (50:50)-7.8	33.8	moderate	330	100
AE 1214 (50:50)-6.9	19.7	mild	30	100
BRIJ-35	<<20	nonirritating	60	10000
GLUCOPON 625CS	15%=4.0	nonirritating	250	>50000
AE 1214 (50:50)-1.1	4.3	nonirritating	320	1000

[1]See Table 4 for full names.
[2]See Table 4, footnote 2, for source of data.
[3]CONDEA Vista unpublished data: PGE2 = prostaglandin E2; LRC = "lowest release concentration," the lowest concentration of test material producing a significant release of PGE2.

PGE2 release was measured as the lowest release concentration (LRC), the lowest concentration of test material producing a significant release of PGE2. PGE2 release was considered significant when it was greater than the 95% confident interval for PGE2 release in the solvent control. The LRC is measured since PGE2 release occurs only at sublethal concentrations and the amount of PGE2 release does not increase proportionally to higher doses of test material applied due to toxicity to cells (Issekutz & Movat, 1982; Arturson, 1983). As with EC-50 values, the higher the LRC, the less irritating the test compound.

As shown in Table 5, the results measuring PGE2 release, measured as the LRC, distinguish between the irritating and moderate surfactants (SLS, AE 810(50:50)-7.8) and the nonirritating nonionics (BRIJ-35, GLUCOPON 625CS and AE 1214(50:50)-1.1). The mild nonionic, AE 1214(50:50)-6.9 seems to give a false positive response compared to the other materials.

However, attempts to repeat this favorable pattern of results with SLS, BRIJ-35 and GLUCOPON 625CS were unsuccessful (Table 6). In the second experiment, BRIJ-35 gave a lower LRC than SLS and showed greater release of PGE2 than SLS at all concentrations tested. Both of these measurements would indicate that BRIJ-35 was more irritating than SLS, contrary to the Draize eye test results on the two materials.

In the third experiment shown in Table 6, GLUCOPON 625CS had the same lowest release concentration as SLS and showed greater release of PGE2 at the LRC. However, when tested at ten-fold higher concentrations, GLUCOPON released no detectable PGE2, similar to the pattern seen in the first experiment where the PGE2 release at 0.1% just missed meeting the criterion for the LRC.

This pattern for GLUCOPON 625CS was confirmed in a repeat assay of the growth medium supernatants from experiment #3. The supernatants had been stored frozen since the initial assay and were re-assayed after 5 weeks. As might be expected, the PGE2 values in the repeat assay were generally lower than those in the original test of experiment #3. However, the same pattern of PGE2 values for SLS and GLUCOPON 625CS was observed in the re-assay as in the initial assay for these surfactants.

Decreased release of PGE2 at higher concentrations were also observed with SLS in the first experiment and with BRIJ-35 in the second experiment. The pattern of PGE2 release for SLS and BRIJ-35 suggests that decreased release was due to cell toxicity at the higher surfactant concentrations. However, the pattern observed in both experiments with GLUCOPON 625CS is more extreme than that observed with SLS or BRIJ-35 and suggests that GLUCOPON, at concentrations at and above those producing PGE2 release, interferes with the assay of PGE2. This assay depends on antibody binding and enzyme activity to detection PGE2. Apparently, GLUCOPON 625CS interferes with one or more steps in the PGE2 assay.

It is possible that the other surfactants tested (Tables 5 - 6) also interfere with the PGE2 assay. In any case, the examination of the data in Table 6 indicates that the LRC measured for surfactants by this test method can vary by one order of magnitude (SLS Exp. #1 vs. #2), two orders of magnitude (BRIJ-35 Exp. #1 vs. #2) or greater than two orders of magnitude (GLUCOPON 625CS Exp. #1 vs. #3). This lack of reproducibility between experiments makes this assay unsuitable for predicting the eye irritation potential of nonionic surfactants.

Table 7 shows the results obtained with the final in vitro tested examined, the chorioallantoic membrane vascular assay (CAMVA). The test uses fertilized hen eggs to measures vascular responses, such as bleeding and empty blood vessels, in the chorioallantoic membrane. This test was used in Phase III of the SDA testing program and found to give the highest correlation coefficient to the Draize eye test (91%) of the in vitro tests examined (Bagley et al., 1994).

Table 6
PGE2 Release Data (SKIN[2] ZK1200, 4 hrs. exposure)[1]

Surfactant, test concentration[2]	Experiment #1	Experiment #2	Experiment #3	Experiment #3 -Repeat Assay[3]
Untreated control, mean value	1.0	20.6	3.3	1
Untreated control, upper 95% confidence limit	2.7	60.8	6.3	--[4]
SLS (anionic), 10%	510, 1000	7800, 10000	>2000, >2000	>2000
SLS (anionic), 1%	>2000, >2000	570, 2100	96, 180	1700
SLS (anionic), 0.1%	1300, 1500	**280, 520**[5]	**15, 48**[5]	**6**[5]
SLS (anionic), 0.01%	**36, 69**[5]	52, 56	1, 2	0
SLS (anionic), 0.001%	0, 0	1, 6	2, 3	0
BRIJ-35, 10%	560, > 2000	1800, 2300		
BRIJ-35, 1%	**390, 590**[5]	7600, 8600		
BRIJ-35, 0.1%	0, 12	2800, 5800		
BRIJ-35, 0.01%	0, 2	**550, 710**[5]		
BRIJ-35, 0.001%	0, 1	19, 25		
GLUCOPON 625CS, 10%	0, 0		0,0	0
GLUCOPON 625CS, 1%	0, 0		0, 0	0
GLUCOPON 625CS, 0.1%	1, 13		**72, 110**[5]	**5**[5]
GLUCOPON 625CS, 0.01%	0, 0		0,1	0
GLUCOPON 625CS, 0.001%	0, 0		1, 3	0

[1]CONDEA Vista unpublished data. Duplicate values for PGE2 release are reported where available. PEG2 units are picograms per 0.1 ml of growth media supernatant.
[2]See Table 4 for full names.
[3]Frozen supernatants from experiment #3 were re-assayed for PGE2 five weeks later. Only single values were measured.
[4]Control values were 1, 1; confidence limits cannot be calculated but the upper confidence limits was assumed to = 3 (See Exp. #1).
[5]**LRC**, see Table 5 for definition. Note that, in the case of duplicate values, both values must be greater than the control upper 95% confidence limit to be considered the LRC.

Table 7
Comparison of Eye Irritation Potential with CAMVA Assay

Surfactant brand name/ abbreviation[1]	Draize Eye Test--MAS[2]	Draize Eye Test-- Classification[2]	CAMVA hen egg Vascular changes RC-50, ppm[3]	CAMVA hen egg Vascular changes Corrected RC-50, ppm[3,4]
SLS	10%= 37.3	irritating	120	120
AE 810 (50:50)-7.8	33.8	moderate	1100	1100
AE 1214 (50:50)-6.9	19.7	mild	1100	1100
BRIJ-35	<<20	nonirritating	3200	3200
GLUCOPON 625CS	15%=4.0	nonirritating	250	1670
AE 1214 (50:50)-1.1	4.3	nonirritating	526000	526000

[1]See Table 4 for full names.
[2]CONDEA Vista unpublished data except for SLS (Tachon et al., 1989), BRIJ-35 (North-Root et al., 1983) and GLUCOPON 625CS (Henkel Coporation, unpublished data). MAS = maximum average score.
[3]CONDEA Vista unpublished data: CAMVA = chorioallantoic membrane vascular assay; RC-50 = concentration causing a response (vascular changes such as bleeding or empty blood vessels) in 50% of the membranes.
[4]GLUCOPON 625CS results extrapolated to an RC-50 value for 15% active material (to match that tested in the Draize eye test), i.e., 250/.15 = 1670. Draize eye test results with the other nonionics were based on testing 100% active materials, and the RC-50 values for these nonionics were not corrected. Note that the RC-50 result for SLS was not corrected since SDA Phase I and Phase III testing data showed similar Draize eye irritation scores with 10% and 92.3% SLS (Booman et al., 1988; Bagley et al., 1993).

As shown in Table 7, CAMVA is not able to distinguish between the moderately irritating nonionic, AE 810(50:50)-7.8, and the mild nonionic, AE 1214(50:50)-6.9. Moreover, GLUCOPON 625CS appears to give a false positive response.

Also as shown in Table 7, the false positive response can be eliminated by realizing that the Draize eye test results and classification of GLUCOPON 625CS are based on testing of a 15% active solution whereas the results with the other nonionics are based on testing 100% active solutions.

RC-50 values, on the other hand, are calculated from the per cent active of the test material and in effect are based on 100% active material. For GLUCOPON 625CS, it reasonable to assume that the measured eye irritation potential would increase proportionately if more concentration samples were tested. Since Draize eye test data on such samples are not available, a logical alternative is to correct

the RC-50 value to a solution concentration like that used to generate the Draize eye data. This has been done in Table 7. Using the corrected RC-50 value, GLUCOPON 625CS no longer gives a false positive response in the CAMVA.

It should be noted that a similar correction was not applied to the RC-50 value for SLS in Table 7 because there are data available showing that the maximum average score for SLS in the Draize eye test is similar whether testing a 10% aqueous solution or a 92.3% active powder (Booman et al., 1988; Bagley et al., 1994).

It should further be noted that the SDA Phase III testing (Bagley et al., 1994) included an alcohol ethoxylate (sample F) similar to AE 1214(50:50)-6.9 in Table 7. In the Phase III testing the alcohol ethoxylate was evaluated in a 10% aqueous solution and gave a maximum average score in the Draize eye test of 14.7, very similar to the MAS of 19.7 observed for the 100% active (nonaqueous) sample reported in Table 7. The similarity of these results with a nonionic surfactant suggest that the correction applied to the RC-50 results with GLUCOPON 625CS may not be justified and GLUCOPON should be considered a false positive in CAMVA. If so, then the CAMVA, the best in vitro test in SDA Phase III testing, misclassifies two of the five nonionic surfactants tested.

Based on this review of unpublished data made available to SDA, several in vitro tests seem to have difficulty predicting the eye irritation potential of nonionic surfactants. This analysis also suggests that some of this difficulty may be in the quality of the Draize eye test data available on nonionic surfactants. This limitation would seem to apply to most of the published data and to the first set of seven nonionics for which unpublished data was provided (Tables 1 - 3). Another source of difficulty may be the relatively small number of nonionic surfactants examined in most published studies and in the second set of unpublished data (Tables 4 - 7).

Structure-Activity Relationships

One approach to evaluating eye irritation data on nonionic surfactants is to determine structure-activity relationships. Such a relationship, if found, could be used to evaluate the quality of the data on individual compounds by examining how well the data fits a structure-activity curve. A structure-activity relationship may allow testing of additional compounds based on their structure and predicted activity, and may identify structures which give unique activity values.

There is no published data on structure-activity relationships among nonionics (or surfactants) for eye irritation. However, such relationships have been studied for other properties of surfactants such as surface tension, octanol-water partitioning and hydrophobicity.

Surface Tension

Bode et al. (1978) reported that the surfactant concentrations for 50% lethality (LC-50) for a freshwater multicellular organism, Hydra attenuate, coincide with a surface tension of 49 ± 4 dynes/cm (= N/m). It was hypothesized that this was the surface tension required to disrupt the cell membranes in this organism.

For Hela cells and B16 melanoma cells, lethal concentrations of surfactants, including a series of alcohol ethoxylates, coincided with a surface tension of 45 dynes/cm or below (Ernst and Arditti, 1980; Partearroyo et al., 1990). For the bacterial cells used in the Microtox™ test (Photobacterium phosphoreum), the lethal concentrations for three nonionic surfactants (a polyoxyethylene nonylphenol ether, a C-9 alcohol ethoxylate (6 oxyethylene units) and a secondary alcohol ethoxylate) coincided with a surface tension of 32-34 dynes/cm (Sherrard et al., 1996), suggesting that bacterial cells can withstand lower surface tensions that animal cells, possibly due to the protective nature of the bacterial cell wall.

Unfortunately, surface tension does not appear to explain eye irritation potential or test results with in vitro tests. First, consider how the surface tension of surfactants changes with concentration. All surfactants, by the nature of their surface-active properties, tend to accumulate at surfaces and interfaces, and to form complexes (micelles) above a certain concentration, specific for each surfactant, commonly known as the critical micelle concentration (CMC). The CMC may be determined by measuring the surface tension of the surfactants as a function of its concentration in water. As surfactant is added to water, the surface tension is reduced until the critical micelle concentration is reached. Once the CMC is obtained, no further reduction in surface tension is produced with further addition of surfactant. According to surfactant theory, the addition of surfactant above the CMC results only in an increase in the size and/or number of micelles present and the concentration of soluble (nonmicellar) surfactant, which is dynamic equilibrium with the micellar molecules, remains constant above the CMC.

With this theory in mind, consider the results obtained with the first group of nonionic surfactants examined (Table 1). Of these, CMC data is available for only one compound, GLUCOPON 625CS (Henkel Corporation, 1992). However, one can assume that the CMC for TRITON X-100 [polyoxyethylene (9) octylphenol] is similar to that for TRITON N-101 [polyoxyethylene (9) nonylphenol]. Based on the data given in the product brochure for the latter nonionic (Union Carbide Chemicals and Plastics Company, 1991), the CMC is approximately 30 ppm and the surface tension at the CMC is 29.4 dynes/cm.

As shown in Table 8, the surface tensions of the two nonionics at their respective CMCs, i.e. the minimum surface tension values, are very similar and their CMCs are identical. However, GLUCOPON 625CS is considered a mild surfactant while TRITON X-100 is classified as a severe irritant. The lack of correlation of eye irritation potential with surface tension or CMC is perhaps

not surprising since the eye irritation of TRITON X-100 was determined on the neat (100% active) material and that of GLUCOPON 625CS on a 15% active solution, both concentrations considerably higher than the CMCs.

Table 8
Comparison of Surface Tension Data with Eye Irritation Potential and In Vitro Tests

Surfactant brand name/ abbre-viation[1]	Surface Tension, dynes/ cm (at CMC[2], ppm)	Eye Irritancy[3]	MICROTOX LBT[4], Light emission, 15 minute exposure, EC-50, ppm[5]	SKIN[2] ZK1200, MTT uptake, 30 minute exposure, EC-50, ppm[5]	SKIN[2] ZK1200, MTT uptake, Topical exposure, Grade[6]
TRITON X-100	30 (30)[7]	Severe	1500	4200	Harsh
GLUCO-PON 625CS[8]	29.4 (30)[9]	--	4	13100	Innocuous

[1]See Table 1 for full names.
[2]CMC = critical micelle concentration (values in parenthesis). Surface tension measured at 25° C.
[3]Kennah et al., 1989. [4]LBT = luminescent bacteria test.
[5]CONDEA Vista unpublished data. EC-50 = effective concentration for 50% inhibition. Results from two independent experiments are shown. See Table 2 for details.
[6]Rachui et al., 1994. See Table 3 for details.
[7]Value estimated for TRITON N-101 from Union Carbide Chemicals and Plastics Company (1991) data.
[8]Mild (Henkel unpublished data). [9]Henkel data.

The results from the in vitro tests also show no apparent correlation to surface tension or CMC. Only GLUCOPON in the MICROTOX test produced an effect at a concentration (4 ppm) below the CMCs for these materials and this result is considered a false positive response (see above). The results in the topical exposure SKIN[2] test, like those in the Draize eye test, were determined at concentrations (10-15%) considerable above the CMCs and yet large differences in response were observed.

For two of the alcohol ethoxylates tested in the second group of surfactants (Table 4), the CMCs and the surface tensions at the CMCs can be extrapolated from published data (Cox, 1989), as shown in Table 9. In this table the alcohol ethoxylates are listed by their weight percent polyoxyethylene, rather than the average number of moles, since that is the way the alcohol ethoxylates are described in this data.

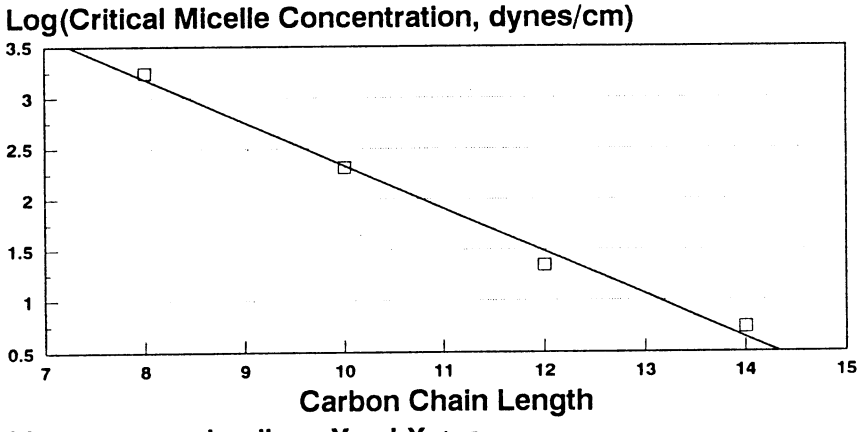
As shown in Table 9, surface tensions of the alcohol ethoxylates at the CMCs vary only slightly while the CMCs show a large variation. In fact, the CMCs appear to increase as a logarithmic

Table 9[1]
Comparison of CMC and Surface Tension Values of Alcohol Ethoxylates

Alcohol Ethoxylate[2]	Critical Micelle Concentration (CMC), ppm	Surface Tension at CMC, dynes/cm
8-60	1739	24.9
10-60	205.6	24.8
12-60	22.1	25.7
14-60	5.5	27.1
12-70	43.1	27.0
Extrapolated:		
810-70[3]	1166[4]	26.1[5]
1214-60[6]	11[7]	26.4[8]

[1]Data for first five alcohol ethoxylates from table 3 of Cox (1989). Surface tensions measured at 38°C.
[2]The number before the hyphen is the alkyl chain length and the number following is the weight percent polyoxyethylene.
[3] = AE 810(50:50)-7.8.
[4]Calculated as: (log average of CMCs of 8-60 and 10-60) times CMC for 12-70 divided by CMC for 12-60.
[5]Calculated as: [mean of surface tensions (at CMCs) for 8-60 and 10-60] times surface tension for 12-70 divided by the surface tension for 12-60.
[6] = AE 1214(50:50)-6.9.
[7]Calculated as: log average of CMCs of 12-60 and 14-60.
[8]Calculated as: mean of surface tensions of 12-60 and 14-60

Figure 1
Alcohol Ethoxylate C-Chain (60% EO) vs. Log(CMC)
Data of Cox (1989).



Linear regression line: Y = bX + a
R-square=.99, b=-0.423, a=6.567, n=4

function of decreasing alkyl chain length when the weight percent of polyoxyethylene is held constant. This is illustrated in Fig. 1, which shows an excellent linear regression fit ($R^2 = .99$) of a plot of the alcohol ethoxylate carbon chain length vs. the logarithm of the CMC.

Increasing the weight percent polyoxyethylene from 60 to 70% also has a dramatic effect, approximately doubling the CMC. Both patterns are understandable since decreasing the alkyl chain length or increasing the polyoxyethylene content increases the solubility of the alcohol ethoxylate and hence the CMC. What is interesting is that the surface tensions at the CMCs vary so little, suggesting that this property is largely determined by the overall chemical structure of the surfactant and is less influenced by the size of the lipophilic (alkyl chain) or hydrophilic (polyoxyethylene) portions of the molecule.

Based on this CMC and surface tension data, logarithmic averages were used to calculate the CMCs for the C8-C10 and C12-C14 alcohol ethoxylates as shown in Table 9. All other extrapolated values were calculated by simple proportion.

Note that the CMC and surface tension values from Table 9 were determined at 38°C while those in Table 8 were determined at 25°C. Since temperature has a major effect on these values, the CMC and surface tension values for GLUCOPON 625CS from Table 8 cannot be compared to the values in Table 9 even though GLUCOPON 625CS was among the surfactants examined with the alcohol ethoxylates (Tables 4 - 7).

As shown in Table 10, the Draize Eye Test classification does not correspond to the surface tension or CMCs for these two alcohol ethoxylates. This is not surprising since these were tested as neat (100% active) materials, nonaqueous liquids in which aqueous surface tension and CMC have little meaning.

There is some suggestion from this data that the SIRC cell test and the MICROTOX test are responding to the surface tension. The EC-50 values vary in the same direction, almost to the same magnitude as the CMCs. However, reduction in surface tension cannot explain the greater sensitivity (lower EC-50s) of the bacterial cells in the MICROTOX test compared to the rabbit corneal cells in the SIRC test. Whatever the explanation for the mechanism of response, both in vitro tests gave false positive results for AE 1214(50:50)-6.9 and the SIRC cell test gave a false negative response for AE 810(50:50)-7.8 based on eye irritation potential (see discussion on Table 4 above).

The results with the three-dimensional skin model (ZK1200) do not seem to be based entirely on surface tension since the EC-50 response for AE 1214(50:50)-6.9 occurs at a concentration three fold higher than the CMC. Whatever the mechanism of response, the test gave a false positive result for AE1214(50:50)-6.9 and a false negative result for AE810(50:50)-7.8 based on eye irritation potential (see discussion on Table 5 above).

Table 10
Comparison of AE Surface Tension Data with Eye Irritation Potential and In Vitro Tests

Alcohol ethoxylate abbre-viation[1]	Surface Tension, dynes/ cm (at CMC[2], ppm)	Draize Eye Test-- Classifi-cation[3]	SIRC Cell Test[3], Colony formation, EC-50, ppm[4]	MICROTOX LBT[5], Light emission, EC-50, ppm[4]	SKIN[2] ZK1200, Neutral Red Uptake[6], EC-50, ppm[4]
810(50: 50)-7.8	26.1 (1166)	Moder-ate	100-200	10	330
1214(50: 50)-6.9	26.4 (11)	Mild	7.6	1.5	30

[1]See Table 4 for full names.
[2]CMC = critical micelle concentration (values in parenthesis). Extrapolated values (Table 9) based on surface tension measured at 38° C.
[3]CONDEA Vista unpublished data. See Table 4 for details.
[4]EC-50 = effective concentration for 50% inhibition.
[5]LBT = luminescent bacteria test.
[6]See Table 5 for details.

Octanol-Water Partitioning

A quantitative structure-activity relationship has been developed by Roberts (1991) for predicting acute aquatic toxicity of nonionic surfactants. This QSAR is based on the logarithm of the octanol-water partitioning values (logP), that is, the log of the molar ratio (at equilibrium) of the amount of nonionic which dissolves in 1-octanol versus the amount which dissolves in pure water. This model seems to predict the generalized toxicity produced by nonionic, nonreactive molecules which is caused by a perturbation of cellular membranes and hence the correlation to logP.

Unfortunately, the relationship between logP and eye irritation potential or in vitro tests results have not been examined. However, the logP QSAR for nonionics has been used to normalize chronic toxicity (no observed effect concentration, NOEC) values measured with various alcohol ethoxylates in different aquatic species to that which represents the NOEC for the average alkyl and polyoxyethylene chain length found in sewage treatment plant effluent (Feijtel, 1994). This normalization procedure can be used to compare eye irritation potential and in vitro test results with alcohol ethoxylates and alkylphenol ethoxylates.

The procedure used is as follows. EC-50 values are converted from ppm to moles/L and log(1/EC-50) calculated. The predicted log(1/EC-50) value for a structurally similar nonionic can be calculated by adding 0.54 log units for each additional alkyl carbon and subtracting 0.1 for each additional polyoxyethylene unit. The predicted EC-50 value (in ppm) can then be calculated from the log(1/EC-50) value.

Of the first group of nonionic surfactants tested (Table 1) this method is applicable only to the two alkylphenol ethoxylates (TRITON X-100 and TRITON X-155). Of the in vitro assays examined, only measurement of MTT uptake in the SKIN^2 ZK1200 system gave qualitative EC-50 data on both compounds. Calculation of the predicted EC-50 for TRITON X-155, based on the measured value for TRITON X-100, is shown in Figure 2. The predicted value (31,800 ppm) is within 12% of the measured value (35,400 ppm, Table 2).

Figure 2
Predicted EC-50 Value for TRITON X-155
LogP method (Feijtel, 1994)

Using TRITON X-100 (Mol. Wt. = 602 g/mole):

- **EC-50 = 4200 ppm = 4200 mg/L = 4.2 g/L**
- **EC-50 = 0.006977 moles/L**
- **1/EC-50 = 143.3**
- **Log(1/EC-50) = 2.1563**

For TRITON X-155 (Mol. Wt. = 910):

- **Difference in POE chain = 16 - 9 = 7**
- **7 x (-0.1000) = -0.7000**
- **Predicted Log(1/EC-50) = 2.1563 - 0.7000 = 1.4563**
- **1/EC-50 = 28.6**
- **EC-50 = 0.03497 moles/L**
- **EC-50 = 31800 ppm**

The predicted ET-50 value, the time in minutes for 50% inhibition of MTT uptake in the topical exposure test (Table 3) can be predicted for TRITON X-100 based on the quantitative results in this test with TRITON X-155. In this case it is necessary to assume that logP function is in terms of dose, where dose = concentration (moles/L) x time (minutes). Using the ET-50 value for TRITON X-155 (23 minutes) and the tested concentration tested (10%), the predicted ET-50 for TRITON X-100 at the tested concentration of 10% would be 3.0 minutes versus the ET-50 of less that 1 minute observed.

The second group of nonionics tested (Table 4) includes four alcohol ethoxylates. For these compounds, there is variation in both polyoxyethylene and alkyl chain lengths. The relevant data for the four alcohol ethoxylates is shown in Table 11. To simplify the presentation of the data, only the relative logP values are shown. These were calculated as described above assuming an EC-50 value of 1.0 ppm for AE 1214(50:50)-1.1, the compound predicted to have the lowest EC-50 value.

Table 11
Comparison of LogP Values with Eye Irritation Potential and In Vitro Tests

Surfactant abbre-viation[1]	Relative LogP Value[2]	Draize Eye Test--Classifi-cation[3]	SIRC Cell Test[2], Colony formation, EC-50, ppm[4]	MICROTOX LBT[3,5], Light emission, EC-50, ppm[4]	SKIN[2] ZK1200, Neutral Red Uptake[3,6], EC-50, ppm[4]
AE 1214 (50:50)-1.1	1	Non-irritating	9.2	1.2	320
AE 1214 (50:50)-6.9	8.5	Mild	7.6	1.5	30
AE 810 (50:50)-7.8	1470	Moder-ate	100-200	10	330
BRIJ-35[7]	2820	Non-irritating	36	5200	60

[1]See Table 4 for full names.
[2]Calculated as described in text and in Fig. 2, assuming a value of 1 ppm for AE 1214(50:50)-1.1. A value of -0.1000 was used for each one unit increase in polyoxyethylene chain length and -0.5400 was used for each one carbon decrease in alkyl chain length.
[3]CONDEA Vista unpublished data. See Table 4 for details.
[4]EC-50 = effective concentration for 50% inhibition.
[5]LBT = luminescent bacteria test.
[6]See Table 5 for details.
[7]= AE 12-23

As shown in Table 11, there is no apparent relationship between relative logP values and eye irritation potential. There is also no apparent correlation between logP and the results from the SIRC cell test or neutral red uptake in SKIN[2] ZK1200. The MICROTOX test results show an apparent trend of increasing values (lower toxicity) with increasing relative logP values. However, the quantitative correlation between the two sets of values is poor. For instance, the predicted EC-50 for AE 1214(50:50)-6.9 is 1.2 ppm x 8.5 = 10.2 ppm versus an observed value of 1.5 ppm. For AE 810(50:50)-7.8 the agreement is even worse, with a predicted EC-50 of 1.2 ppm x 1470 = 1760 ppm versus an observed value of only 10 ppm.

Hydrophobicity

Other structure-activity relationships for surfactants have been proposed. For instance, bioaccumulation or bioconcentration has been studied for a number of surfactants (Tolls et al., 1994). There is a suggestion that the first order rate constant K_1 for uptake of certain cationic surfactants increases with increasing length of the alkyl chain (Versteeg & Shorter, 1992), and for C-12 alcohol ethoxylates decreases with increasing length of the polyoxyethylene chain (Wakabayashi, 1987). Increasing the alkyl chain or decreasing the polyoxyethylene chain would be expected to increase the hydrophobicity of a surfactant.

A similar pattern has been observed in a comprehensive review of the aquatic toxicity of alcohol ethoxylates (BKH Consulting Engineers, 1994). Three dimensional plots were made of alkyl chain length (C9-C18) versus average polyoxyethylene chain length (POE1-POE20) for three data sets: acute toxicity results with single invertebrate species, Daphnia magna (n = 70); with all fish species (n = 137) and with all aquatic species available (n = 248). Rather complex plots were produced which nevertheless allowed the following patterns to be observed: 1) the more hydrophilic combinations of short alkyl chains with longer polyoxyethylene chains were less toxic; 2) the more lipophilic longer alkyl chain molecules were more toxic; and 3) the latter trend was especially clear for alcohol ethoxylates with medium and longer polyoxyethylene chains where water solubility did not limit the concentrations of longer alkyl chain molecules that could be tested. The overall conclusion of this review is that "the balance between the two parts of the molecule that determine the hydrophilic-lipophilic balance correlates with toxicity."

It has been known for some time that hydrophobicity can be quantified by calculating the hydrophile-lipophile balance or HLB (Griffin, 1954). The HLB is simply the ratio of the hydrophile and lipophile portions of the surfactant molecule, on a 0 to 20 scale, where a higher number indicates a higher proportion of the molecule is hydrophilic and a lower number indicates than a higher proportion of the molecule is lipophilic.

The calculations are quite straightforward for surfactants containing an ether bond between the polyoxyethylene (hydrophilic) and the alcohol or alkylphenol (lipophilic) portions of the molecule: the HLB is equal to 1/5 of the weight percentage of the polyoxyethylene chain. Thus an alcohol ethoxylate which is 50% by weight polyoxyethylene would have an HLB of 10.

HLB values for the first group of surfactants tested are shown in Tables 12 and 13. There is no apparent correlation between the HLBs and eye irritation potential or test results in any of the in vitro tested used.

HLB values for the second set of nonionic surfactants tested are shown in Table 14. Again there is no apparent relationship between the degree of hydrophobicity (HLB number) and the eye irritation potential. There is also no apparent correlation between HLB and the results from the SIRC cell test or neutral red uptake in SKIN^2 ZK1200. The MICROTOX test results show an apparent trend of

increasing values (lower toxicity) with increasing HLB. However, it is difficult to have much confidence in the validity of this pattern since it was not observed in Table 12 with a somewhat different set of nonionics but covering the same range of HLB values.

Table 12
Comparison of HLB Values with Eye Irritation Potential and Two In Vitro Tests

Surfactant brand name/ abbreviation[1]	Hydrophilic Lipophilic Balance (HLB)	Eye Irritancy[2]	Eye Irritancy[3]	3T3 mouse cells Uridine uptake 4 hr. exposure EC-50, ppm[4,5]	SIRC corneal cells Colony formation 1 hr. exposure EC-50, ppm[4,6]
GLUCOPON 625CS[7]	12.1[8]	--	--	--	15[9]
TRITON X-100	13.7[10]	--	Severe	--	25-50[9]
TWEEN 80	15[11]	Mild	--	400	13300[12]
TWEEN 40	15.6[11]	Mild	--	190	11500
TRITON X-155	15.8[10]	Irritant	--	23	--
TWEEN 20	16.7[11]	Mild	Mild	--	135[13]
BRIJ-35	16.9[10]	Mild	--	70	36

[1]See Table 1 for details.
[2]Grant, 1986.
[3]Kennah et al., 1989.
[4]EC-50 = Effective concentration for 50% inhibition.
[5]Shopsis et al., 1985.
[6]North-Root et al., 1982.
[7]Mild (Henkel unpublished data).
[8]Henkel Corp., 1992.
[9]CONDEA-Vista unpublished data.
[10]Calculated from weight percent polyoxyethylene.
[11]Rabaron et al., 1993.
[12]J. Demetrulias, personal communication.
[13]Booman et al., 1988.

Conclusions

1. The research on nonionic surfactants initiated by the Non-animal Testing Subcommittee of the Soap and Detergent Association was a logical extension of earlier phases of the subcommittee's long term research program on in vitro tests.

2. The program described in this report consisted of exploratory research to determine if nonionic surfactants were, if fact, a category of cleaning products ingredients which were not well predicted by some in vitro tests.

3. An analysis of the results in several published papers suggests that the eye irritation potential of nonionic surfactants is not well predicted by some in vitro tests.

Table 13
Comparison of HLB Values with Three In Vitro Tests

Surfactant brand name/ abbreviation[1]	Hydrophilic Lipophilic Balance (HLB)[2]	MICROTOX LBT[3], Light emission, 15 minute exposure, EC-50, ppm[4]	SKIN[2] ZK1200, MTT uptake, 30 minute exposure, EC-50, ppm[4]	SKIN[2] ZK1200, MTT uptake, Topical exposure, Grade[5]
GLUCOPON 625CS	12.1	4	13100	Innocuous
TRITON X-100	13.7	1500	4200	Harsh
TWEEN 80	15	17000	>>30000	Innocuous
TWEEN 40	15.6	20000	>>30000	Innocuous
TRITON X-155	15.8	?	35400	Innocuous
TWEEN 20	16.7	6300	>>30000	Mild-Mod.
BRIJ-35	16.9	5200	14100	Innocuous

[1]See Table 1 for full names. [2]See Table 12 for details. [3]LBT = luminescent bacteria test.
[4]CONDEA Vista unpublished data. EC-50 = effective concentration for 50% inhibition.
Results from two independent experiments are shown. See Table 2 for details.
[5]Rachui et al., 1994. See Table 3 for details.

4. The literature review also indicates that the quality of the in vivo eye irritation data is important in evaluating the ability of in vitro tests to predict the eye irritation potential of nonionics.

5. Two sets of unpublished data were made available to the SDA. In the first set, all five of the in vitro tests misclassified (false negative or false positive result) one or two of the seven nonionics tested.

6. In the second set, one test (PGE2 release in the ZK1200 system) was found to be unsuitable for testing nonionics because the results (lowest release concentration) varied by a larger factor (10 fold or more) than the differences between mild and irritating surfactants. The four other in vitro tests examined misclassified two to four of the five nonionics examined.

7. This unpublished data demonstrates that the eye irritation potential of nonionic surfactants is not well predicted by a number of in vitro tests.

Table 14
Comparison of HLB Values with Eye Irritation Potential and Three In Vitro Tests

Surfactant abbre-viation[1]	Hydro-philic Lipo-philic Balance	Draize Eye Test--Classifi-cation[2]	SIRC Cell Test[2], Colony formation, EC-50, ppm[3]	MICROTOX LBT[2,4], Light emission, EC-50, ppm[3]	SKIN[2] ZK1200, Neutral Red Uptake[2,5], EC-50, ppm[3]
AE 1214 (50:50)-1.1	4[6]	Non-irritating	9.2	1.2	320
AE 1214 (50:50)-6.9	12[6]	Mild	7.6	1.5	30
GLUCO-PON 625CS	12.1[7]	Non-irritating	15	4	250
AE 810 (50:50)-7.8	14[6]	Moder-ate	100-200	10	330
BRIJ-35	16.9[6]	Non-irritating	36	5200	60

[1]See Table 4 for full names. [2]CONDEA Vista unpublished data. See Table 4 for details.
[3]EC-50 = effective concentration for 50% inhibition.
[4]LBT = luminescent bacteria test. [5]See Table 5 for details.
[6]Calculated from weight percent polyoxyethylene.
[7]Henkel Corp., 1992.

8. Structure-activity relationships to explain eye irritation scores or in vitro test results on surfactants were not identified in the published literature.

9. Use of surfactant properties which have been used to predict aquatic toxicity or bioaccumulation (surface tension, critical micelle concentration, octanol-water partitioning or hydrophilic-lipophilic balance) showed no apparent relationship to either the in vivo eye irritation or the in vitro test results.

10. The following limitations may have confounded the search for structure-activity relationships: 1) data on surfactant properties were not available for all the nonionics tested, limiting the relationships that could be analyzed; 2) the surfactant data available was not determined on the actual samples tested in vivo or in vitro; and 3) the surfactant data was obtained by different laboratories at different times and perhaps by different test methods.

11. The exploratory research described in this report has demonstrated that there is a need for high quality in vivo eye irritation and surfactancy results on a larger set of nonionics than is available today. Specifically, 1) the nonionics to be tested should be representative of those used in cleaning products and selected to facilitate the search for structure activity relationships; 2) eye irritation and all relevant surfactant properties of the nonionics should be determined using the same test materials; and 3) all data should be generated by simultaneously testing the entire set of nonionics in well qualified laboratories using up-to-date test protocols.

12. The results of tests designed to meet this data need are describe in Part II of this report.

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