

primarily by way of the gastric mucosa. The ATA molecule is generally completely absorbed in the gastrointestinal tract. The accumulation data indicate that after absorption ATA is distributed through most body tissues. Highest radioactivity in all tissues is generally reached within 1 hour after dosing, maintaining the same level for 2 to 3 hours before the activity starts to decrease. The rate of elimination of ATA from all tissues is fast and requires only from 2 to 3 hours for the removal of one half of its concentration. ATA-5-C¹⁴ is not completely oxidized by rats, and the C¹⁴ does not appear in the breath. Elimination of this chemical from the body is chiefly from the kidney by way of urine excretion.

Liver is the site of ATA metabolism with the formation of a radioactive metabolite. Removal of this metabolite from the liver is much slower than ATA. In view of the similarity between the rate of elimination of this metabolite from liver or kidney and the rate of decrease on catalase inhibition as reported by Heim, Appleman, and Pyfrom (4) in the same organs, the ATA metabolite might be the responsible toxic agent. Also, no free ATA is present in the liver sample

after 24 hours following dosing, yet the inhibition of catalase activity still persists. On the other hand, the concentration of ATA in blood is reasonably high (next only to the concentration found in the liver), while blood catalase activity of the treated animals remained normal. However, Tephly *et al.* (10) concluded from their experiment that the failure of ATA to inhibit blood catalase is due to an insufficient amount of hydrogen peroxide in the erythrocytes. Price, Rechcigl, and Hartley (8) demonstrated that the inhibition of liver catalase activity by ATA is a result of catalase destruction. The rate constant, k_D , for catalase destruction is 0.027 which is reasonably close to the result for the removal of metabolite-1 from liver ($k_2 = -0.024$). Isolation and identification of metabolite-1 are now in progress. Whether or not this metabolite-1 is identical to ATA-catalase complex as reported by others (5-7) remains unanswered.

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ENHANCEMENT OF HERBICIDE ACTIVITY

Relation of Structure of Ethylene Oxide Ether-Type Nonionic Surfactants to Herbicidal Activity of Water-Soluble Herbicides

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SURFACTANTS have had a considerable impact on the development and use of herbicides in recent years. In agricultural applications, some of the potential of surfactants for lowering costs of weed control, increasing the efficiency of herbicides, and reducing residue hazards is already being realized. However, a fundamental technology of surfactant-herbicide systems is needed to promote further industrial and agricultural applications.

Previous reports demonstrated that toxicity of herbicides to intact plants could be greatly enhanced by some surfactants, unaffected by others, or significantly suppressed by a third group (4, 5). The effects of a surfactant were related to its concentration but not to its ionogenic class. Phytotoxicities of surfactants themselves to two species (2) and toxicity of one herbicide to crabgrass (5) were influenced by structure of surfactants.

Nonionic surfactants derive their hydrophilic characteristics from non-ionizable groups, such as phenolic and alcoholic hydroxyls, carbonyl oxygens of esters and amides, ether oxygens, and analogous sulfur-containing configurations. Water solubility is a function of the number of hydrophilic configurations in the molecule. Nonionic surfactants are little influenced by the degree of water hardness and are compatible with most organic ions, characteristics which account for their widespread use in pesticide formulations.

Because of their low production cost, the ethylene oxide adducts of long-chain alcohols and phenols (the ether-linked nonionics) are probably more frequently encountered as emulsifiers and wetting agents than any other type of nonionic surfactants. Ethylene oxide can be added to the hydrophobic moieties in an essentially step-wise fashion, but the reaction products represent a range of

adducted species. The average mole ratio of ethylene oxide (EO) per hydrophobe is used to characterize the mixture (6).

Objectives of the current investigations were to evaluate critically the relations of hydrophile-lipophile structural configurations of related surfactants to their effects on herbicidal activity and to study the correlation of these relations with the physical-chemical properties of the spray solutions. This paper characterizes some of the activity-structural relations in the ether-linked polyoxyethylene nonionic surfactants and uses the EO mole ratio as an experimental variable.

Materials and Methods

Thirty-four nonionic surfactants (Table I), representing ether-ethoxylates of 15 hydrophobes belonging to four major hydrophobe groups, were selected

Thirty-four ethoxylated nonionic surfactants differentially enhanced the toxicities of four herbicides to corn and soybeans. Previous work was confirmed, for enhancement increased as surfactant concentration increased up to 1.0 gram per 100 ml. (1.0%). At high surfactant concentrations, differences in the toxicity of a herbicide were associated with variations in hydrophile-hydrophobe structure of the surfactants. With DNBP, toxicity was maximal when hydrophiles consisted of 3- to 5-mole conjugates of ethylene oxide (EO), unless the hydrophobe possessed an unsaturated carbon ring structure. Surfactants of the latter type failed to enhance DNBP activity on corn and gave only limited enhancement on soybeans. On soybeans, amitrole activity was slightly increased if the EO ratio exceeded 4 moles but was maximal on corn at 5 to 10 moles EO, except for sorbitan surfactants which were less effective than other types. Surfactants that had some phytotoxicity by themselves enhanced the toxicities of 2,4-D and dalapon somewhat more than did nontoxic surfactants, but not in proportion to their relative toxicities.

Table I. Structural Variations and Sources of Nonionic Surfactants Evaluated

Group and Base Hydrophobe	Code	Source ^a	Mole Ratios of Ethylene Oxide/Hydrophobe
MONOHYDRIC ALCOHOLS			
Isooctyl alcohol	M1	3	2.4, 8.9
Decyl alcohol	M2	3	2.9, 10.8
Lauryl alcohol	M3	2	4, 7, 23
Tridecyl alcohol	M4	3	1, 3, 4.5, 6, 9, 12
C ₁₆ -Aldox alcohol	M5	4	7.9
C ₂₀ -Aldox alcohol	M6	4	7.8
DIHYDRIC ALCOHOLS			
2,4,7,9-Tetramethyl-5-decyne-4,7-diol	D1	1	3.5, 8, 30
POLYHYDRIC ALCOHOLS			
Sorbitan monolaurate	P1	2	0, 10, 20
Sorbitan monostearate	P2	2	20
Sorbitan monooleate	P3	2	20
Sorbitan monotall oil esters	P4	2	4, 12, 20
Glycerol mannitan monolaurate	P5	2	0
ALKYLPHENOLS			
Nonylphenol	A1	3	1, 3, 6
Nonyl- <i>o</i> -cresol	A2	3	2, 7.4
Dodecylphenol	A3	3	5, 18.5

^a 1 = Air Reduction Chemical Co., 150 E. 42nd St., New York 17, N. Y.; 2 = Atlas Chemical Industries, Inc., Wilmington 99, Dela.; 3 = Enjay Laboratories, P. O. Box 45, Linden, N. J.; 4 = Esso Research & Engineering Co., P. O. Box 51, Linden, N. J.

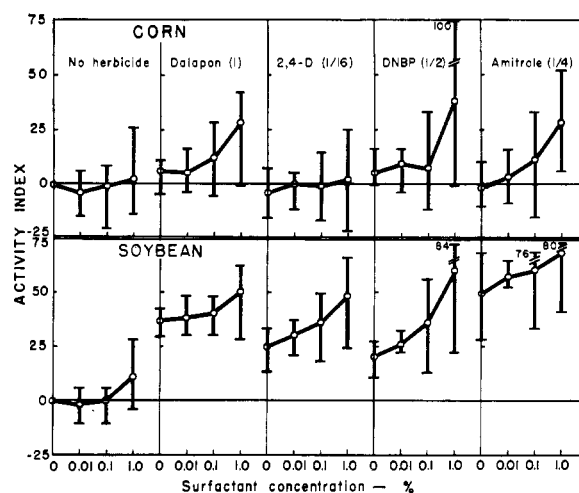


Figure 1. Relation of surfactant concentration to the effects of ethoxylated nonionic surfactants on the activity of several water-soluble herbicides applied to corn and soybean

Mean values (circles) for concentrations of 0, 0.01, 0.1, and 1.0% were derived from 12, 11, 30, and 30 determinations, respectively. Vertical bars indicate range of values from which means were derived

to compare several ethoxylated adducts of individual hydrophobes or a number of hydrophobes with comparable EO ratios. Techniques described previously for the evaluation of surfactants in aqueous herbicide sprays were employed (4). Surfactant concentrations varied from 0.01 to 1.0% (weight by volume, or grams active surfactant per 100 ml.). Herbicides studied were the sodium salt of 2,2-dichloropropionic acid (dalapon), the triethanolamine salt of 2,4-dichlorophenoxyacetic acid (2,4-D), the triethanolamine salt of 2,4-dinitro-6-*sec*-butylphenol (DNBP), and 3-amino-1,2,4-triazole (amitrole). Sprays were applied at 40 gallons per acre (g.p.a.) to give acid-equivalent or active-ingredient herbicide rates (pounds per

acre) as follows: dalapon, 1; 2,4-D, 1/16; DNBP, 1/2; and amitrole, 1/4. Plants of Lee soybean [*Glycine max* (L.) Merr.] were treated at the first-trifoliolate-leaf stage and U. S. No. 13 corn [*Zea mays* L.] at the 4- to 5-leaf stage. Plants were cultured in the greenhouse for 2 weeks after treatment. The experimental design was a randomized block with three replications. The numerical average of four evaluations of treatment effectiveness, expressed on a 0-100 basis, constituted an activity index for each treatment. An index of 100 represented maximum herbicidal effectiveness. Criteria used to evaluate effects were contact injury score, general injury score, height reduction percentage, and weight reduction percentage.

Experimental Results

Influence of Surfactant Concentration. Data for 30 surfactants were pooled for each herbicide and species, and the means and ranges of herbicidal activity were plotted as a function of surfactant concentration (Figure 1). Surfactant enhancement of herbicide activity on both corn and soybeans was greatest with DNBP. The activity of 2,4-D sprays on corn reflected only the toxicity of surfactant components. Statistical analyses indicated that only the data for the 1.0% concentration of most surfactants merited detailed discussion. Unless otherwise specified, the 1.0% concentration is referred to in the remainder of this paper.

Influences of Hydrophile Structure

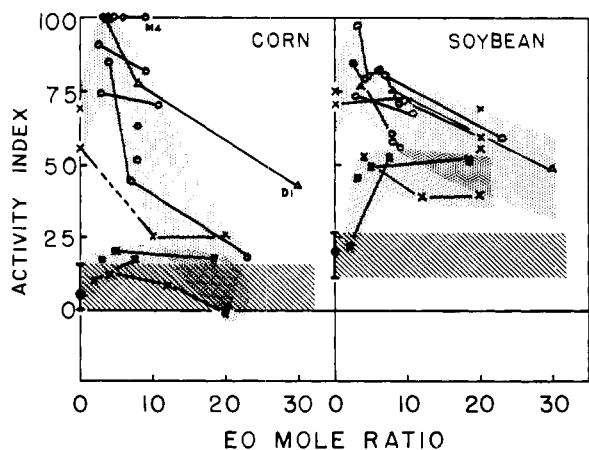


Figure 2. Influence of ethylene oxide (EO) mole ratio in a number of hydrophobe series of ethoxylated nonionic surfactants on the activity of DNBP

Data for 30 surfactants at 1.0% concentration. Solid lines connect members of individual series of monohydric (○), dihydric (△), and polyhydric (X) alcohols, and alkylphenols (■). Shaded curves show best grouping of data by hydrophobe classifications. Specifically designated points refer to individual hydrophobes listed in Table I. The polyhydric alcohol series in the lower curves is for a sorbitan mono-tall oil base hydrophobe. Vertical bars on ordinates indicate range of values obtained for activity of DNBP without surfactant in six experiments, and cross-hatched areas indicate horizontal projection of each range; solid circles show mean activity of the herbicide alone

Table II. Pooled Activity Indexes for Four Grouped Hydrophobe Series of Ethoxylated Surfactants in Sprays of 2,4-D and Dalapon Applied to Corn (C) and Soybeans (S)

Surfactant Group	Concentration, %	n ^a	No Herbicide		2,4-D (1/16 lb./A.)		Dalapon (1 lb./A.)	
			C	S	C	S	C	S
No surfactant	0	10	0	0	-4	25	6	37
Monohydric alcohols	0.1	13	1	-2	-2	35	17	39
	1.0	13	14	21	16	54	38	50
Dihydric alcohols	0.1	3	0	4	1	39	16	46
	1.0	3	7	19	7	46	32	54
Polyhydric alcohols	0.1	13 ^b	-3	0	-4	40	9	41
	1.0	13 ^b	-6	3	-7	45	23	48
Alkylphenols	0.1	5	3	3	-3	29	11	37
	1.0	5	9	11	13	48	30	49

^a n = number of evaluations of individual surfactants pooled at indicated concentrations.

^b Number includes five repetitions of a standard determination for a polyoxyethylene sorbitan monolaurate (Tween 20).

of Various Surfactants on Individual Herbicides. By scatter-diagram plotting of EO content against herbicidal activity, some of the broader activity relations of hydrophile structure in different surfactant families could be shown comparatively for each test species. Minor variations within a family were de-emphasized, and exceptional behavior of individual surfactants became more apparent.

DNBP. The ability of a surfactant to enhance DNBP activity was influenced by both hydrophilic and hydrophobic structure (Figure 2). The various surfactants fell roughly into two major groups, distinguished by the nature of their hydrophobes. One group, the mono- and dihydric alcohols and most of the polyhydric alcohols, was characterized by a pronounced EO effect,

which maximized at EO mole ratios of 3 to 5. The tridecyl alcohol hydrophobe (M4) at EO ratios up to 9 and the dihydric alcohol (D1) at 30 moles enhanced DNBP activity on corn more than was anticipated on the basis of EO content. A second group of surfactants, consisting of the alkylphenols and a series of sorbitan monotall oil esters, failed to increase DNBP toxicity to corn and showed no apparent relation to EO content. The unusual behavior of the tall oil surfactants in comparison to other sorbitan ester surfactants may have resided in the nature of the resin acids of tall oil. Abietic acid, the major resin acid, consists of three conjugated 6-carbon rings, two of which contain one unsaturation each. On this basis, one may postulate that an unsaturated carbon ring structure, such as is found in

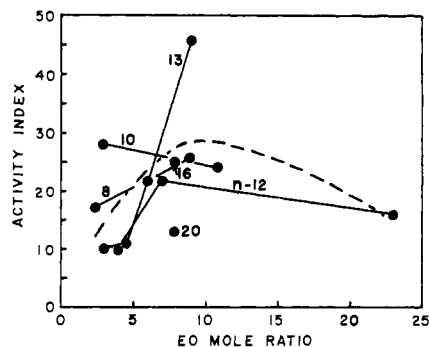


Figure 3. Effect of EO content on toxicity to soybean of 13 monohydric alcohol surfactants at 1.0% concentration

Figures indicate number of carbon atoms in base hydrophobes; n indicates normal or straight carbon chain; all others probably branched. Solid lines connect different members of individual hydrophobe series; broken line represents curve of best fit for all data

the alkylphenols and the sorbitan mono-tall oil surfactants, prevents or reduces DNBP toxicity to corn. Other sorbitan ester surfactants possessed straight-chain aliphatic acid substituents.

On soybeans also, DNBP activity was separately influenced by the two groups of surfactants (Figure 2). Both groups showed peak DNBP activity at 3 to 5 moles EO, but the phenolic and tall oil surfactants provided only limited enhancement. At high EO ratios, both groups enhanced DNBP toxicity to soybeans much more than to corn. Distinction between the two groups was less apparent as EO ratios approached 20.

2,4-D. Within the limits of experimental error, all sprays containing 2,4-D on corn had the same activity as corresponding sprays containing surfactant alone (Table II)—i.e., they showed only the toxicity of surfactants.

On soybeans, toxic and nontoxic surfactants differentially affected 2,4-D activity. Among the monohydric alcohols, toxicity at 1.0% peaked at about 9 moles of EO (Figure 3). The 9-mole adduct of tridecyl alcohol was exceptionally toxic. Enhancement of 2,4-D activity was somewhat greater with the more toxic surfactants but not proportionally so. None of the surfactants showed appreciable toxicity at 0.1%, but several enhanced 2,4-D activity to some extent. Within the various hydrophobe series, enhancement of 2,4-D generally increased as the EO mole ratio increased. Alkylphenols were less effective than the alcohol-derived surfactants.

DALAPON. Dalapon toxicity to corn was enhanced less by the polyhydric alcohols than by other groups of surfactants (Table II). On soybeans, all groups were essentially equivalent. Within individual hydrophobe series, dalapon toxicity to corn was generally maximal in the range of 5 to 10 moles EO and to soybeans from 5 to 20 moles. In some instances, the index value for a

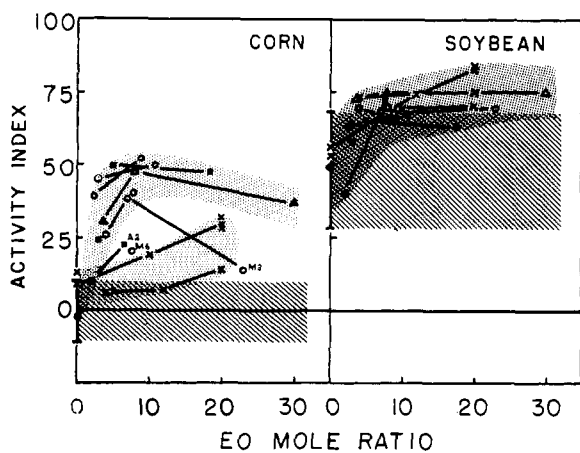


Figure 4. Influence of EO mole ratio on activity of amitrole

For explanation see Figure 2

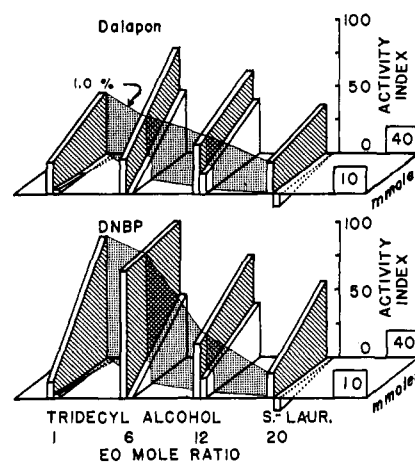


Figure 5. Toxicity responses of corn to sprays containing surfactants alone (clear blocks) and in combination with dalapon and DNBP (cross-hatched blocks) and their relationship to surfactant concentrations and structures

Shaded planes show extrapolated relation of 1.0% standard concentration to concentrations on a molar basis. S. LAUR. = sorbitan monolaurate

Table III. Derivation of Activity Indexes of Sprays Containing a Dodecylphenol Surfactant (1.0%) and Dalapon (1 Pound Per Acre) Applied to Corn (C) and Soybeans (S)

Type of Activity ^a	Surfactant ^b		Dalapon		Dalapon + Surfactant	
	C	S	C	S	C	S
Contact injury	20	23	0	17	20	30
General injury	20	17	33	57	60	67
Height reduction	2	10	2	48	32	68
Weight reduction	1	1	-2	13	12	32
Activity index (av.)	11	13	8	34	31	49

^a Values for each type of activity expressed on a scale of 0 to 100; 0, no activity; 100, maximal activity.

^b Dodecylphenyl polyoxyethylene glycol, 18.5 moles EO.

combination spray appeared to be the sum of the individual values for dalapon and the surfactant alone. Calculation of the activity index, however, often masked true surfactant enhancement, which could be detected by one or more of the methods of evaluation (Table III).

AMITROLE. Amitrole by itself was highly toxic to soybeans, but the activity index for this treatment varied considerably from experiment to experiment (Figure 4). Any surfactant having an EO ratio of four or more increased amitrole activity to a remarkably uniform level, characterized by a narrow range of variation. In other words, amitrole-surfactant sprays on soybeans were more consistently active than were sprays containing amitrole alone.

Corn was almost completely tolerant to amitrole by itself (Figure 4). However, most surfactants of the monhydric alcohol, dihydric alcohol, or alkyphenol groups caused marked increases in amitrole toxicity. Within these groups, maximal toxicity was obtained at EO ratios between 5 and 10. Nonyl-*o*-cresol (A2) with 7.4 moles EO, the

C₂₀-aldox alcohol (M6) with 7.8 moles, and the lauryl alcohol (M3) with 23 moles provided less enhancement than related surfactants. The polyhydric alcohol surfactants were appreciably less effective than were other groups. In the polyhydric alcohol group, amitrole toxicity was progressively increased with increases in EO up to 20 moles per hydrophobe.

Comparative Evaluation of Surfactants at Equimolar Concentrations.

The results just described established that herbicidal activity is influenced by both hydrophilic and hydrophobic moieties of surfactants; however, the data did not permit critical distinction between the relative importance of the two moieties. Molecular weights of the surfactants varied from approximately 235 to 1546. Equal-weight comparisons, therefore, represented up to a sixfold difference in molar concentrations. The effects of several surfactants were studied at concentrations of 10 and 40 mmoles per liter. Surfactants included sorbitan monolaurate with 20 moles EO, tridecyl alcohols with 1, 6, and 12 moles EO,

and nonylphenols with 1 and 6 moles EO.

Surfactant toxicity appeared to be a function of the entire molecule, not of specific hydrophiles or hydrophobes. At comparable concentration levels, all sprays contained the same number of milliequivalents of surfactant hydrophobes. Among the tridecyl surfactants, however, the 1-mole EO adduct showed none of the toxicity to corn found with the 6- and 12-mole adducts (Figure 5). A specific hydrophobe effect was thereby eliminated. In the sorbitan surfactant, the 20-mole EO's are probably arranged in four chains of approximately 5 EO's each. Based on EO chain lengths, toxicity of this surfactant should be equal to or greater than that of the 6-mole tridecyl surfactant. However, the performance characteristics of the sorbitan surfactant closely paralleled those of the 1-mole tridecyl adduct. Thus, a specific ethylene oxide effect was also ruled out. In other words, ethylene oxide by itself does not determine whether a surfactant will be phytotoxic to a given species, nor does the addition of ethylene oxide necessarily render a hydrophobe toxic.

In the equimolar study, neither dalapon nor DNBP alone was measurably toxic to corn. With nontoxic surfactants, dalapon activity was progressively enhanced as surfactant concentration increased; but with toxic surfactants, activity appeared to be related to the toxicity of the surfactant. Maximal dalapon toxicity in the tridecyl alcohol series was obtained with the 6-mole EO adduct, a relation which would not have been as apparent in a 1.0% comparison. Data for DNBP treatments supported the previous observation that the tridecyl hydrophobe provided exceptional behavior at mole

Table IV. Relation between Structure and Activity of Several Ethoxylated Nonionic Surfactants and DNBP at a Selected HLB Value

Hydrophobe	Number of Hydrophobe Carbons	Moles of EO for HLB 10 ^b	Activity Index ^a	
			Without DNBP	With DNBP
None	0	6
Isooctyl alcohol	8	3.0	28	90
Decyl alcohol	10	3.6	25	74
Tridecyl alcohol	13	4.5	52	100
Dodecylphenol	12 + 6	6.0	12	20

^a Extrapolated from data for 1.0% concentration of surfactants on corn.

^b Calculated from the formula $HLB = \frac{E}{5}$, in which E is the weight per cent of ethylene oxide (EO) in the molecule (3).

ratios up to 9. The combination of DNBP and the 6-mole adduct suggested that some sort of synergistic action existed even at the 10 mmolar concentration. The 1-mole EO adduct of nonylphenol was essentially insoluble in water and provided no enhancement of either herbicide.

Since molecular weights of many surfactants are at best rough approximations, equimolar comparisons of surfactants offer no particular advantage over weight comparisons in systematic evaluations. Results obtained by either the equimolar or the equal-weight method of evaluation lead to the same general interpretations.

Discussion

Surfactant relations in herbicide sprays are complex. The present work confirmed the activity-concentration relations (4) and showed additionally that much of the variability obtained at a given concentration (Figure 1) could be effectively related to differences in chemical structure of surfactant hydrophiles. The EO-type of hydrophile effect, nevertheless, was influenced by the hydrophobic moiety and varied with different herbicides and species (Figures 2 and 4).

Freed and Montgomery (7), working with a limited number of surfactants and a single herbicide, concluded that a specific intermolecular relationship between herbicide and surfactant was responsible for increased amitrole absorption, and that this interaction was probably more important than surfactant influences on surface activity of the sprays. Data in the present paper support their conclusions and extend the interactions to include the species also. Strong herbicide-surfactant-species interactions were evident for DNBP and amitrole on both species and for 2,4-D on soybeans.

Although surfactants and herbicides contributed separately to the total toxicity of sprays, surfactant toxicities were reflected primarily in ratings for

contact injury and general injury, which are qualitative measurements of plant growth and response. Herbicide effects were both qualitative and quantitative in nature (Table III). The apparent additive nature of herbicide and surfactant toxicities in some sprays containing dalapon and 2,4-D was probably fortuitous.

Explanations for the various surfactant structural effects on spray activity remain obscure. Surfactant structure can influence a number of physical and chemical properties of solutions, and many of these properties may be involved in the biological activity of herbicide sprays.

Several conventions are utilized to characterize surfactants and facilitate their selection for specific purposes. One of these conventions, the hydrophilic-lipophile balance (HLB-system), has been widely used for numerical coding of ethoxylated nonionic surfactants. The HLB value can be calculated from the structural composition of a surfactant or can be determined experimentally (3). In the present work, DNBP activity was greatest with the tridecyl alcohol surfactant having 4.5 moles EO. The HLB value for this surfactant is 10. EO contents for other hydrophobes at HLB 10 were calculated, and the DNBP activity values at the required HLB were extrapolated from data for the EO series of each hydrophobe.

Comparisons of performance characteristics on an HLB basis show that "chemical type" of the surfactant, discussed by Griffin (3), is probably more important to herbicide activity and surfactant toxicity than HLB itself (Table IV). The optimal HLB for enhancing activity of a herbicide on a single species could serve as an initial index for evaluating a large number of chemical types of surfactants systematically. However, the obvious interactions between herbicide, surfactant, and species suggest that the required HLB should vary with different herbicide-species combinations.

The cuticle, as the receiving surface of

the plant and as a primary barrier to absorption of herbicide sprays, is unquestionably involved in spray activity. The wetting action of surfactant solutions and differential wetting and retention as a function of surface activity are well known. Surfactant enhancement of herbicide toxicity, however, takes place at much higher concentrations than are needed for wetting (4, 5). Also, at high concentrations, surfactants may exhibit specific toxicities of their own. These high concentration effects could be related to the relative chemical affinities of surfactants and herbicides for adsorption sites in or on specific cuticles.

Surfactant penetration of cuticle by absorption channels or dissolution of cuticular components was suggested by the toxic responses of underlying leaf-tissues to certain surfactants. Whether nontoxic surfactants similarly penetrated the cuticle could not be ascertained from the data. However, differences obtained in the activity of a given amount of herbicide in mixtures with many surfactants indicated that cuticle absorption pathways, and possibly the translocation channels, may have been modified by the surfactants. Information is needed as to whether surfactants differentially partition herbicides into barrier membranes or whether herbicide persistence on a surface is in any way affected by surfactants.

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