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TOXICITY OF SURFACTANT-HERBICIDE COMBINATIONS TO *LEMNA MINOR* L.

by

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INTRODUCTION

The use of surfactants to formulate pesticides in a range of wettable powders, solutions and emulsifiable concentrates is a routine practice in the modern pesticide industry. The effects of these surfactants in superior wetting, sticking or retention of spray solutions are most important to the insecticide and fungicide technologies. The use of surfactants to enhance herbicide toxicity has often resulted in reduced selectivity and increased crop damage.

Several investigators have suggested that interactions between surfactant, herbicide and plant surface are more important than are the alterations of the physical properties of herbicide solutions by surfactants. Surfactants have been phytotoxic in varying degrees when evaluated in a number of bioassay systems. Surfactant phytotoxicity is governed by the nature of the hydrophilic and hydrophobic portions of the surfactant molecule. Increasing evidence suggests that modification of cell permeability and possible disruption of cellular organization are consequences of surfactant toxicity.

In the present investigation a convenient, recognized herbicide-assay system employing Lemna minor L. was used to study the inherent phytotoxicity of surfactants and surfactant-herbicide combinations. "Probit analysis" was used to obtain dosage-response curves which allowed the comparison of the "relative potency" of the test chemicals at all levels of kill. The use of this assay system circumvented the problems of drying effects and well developed cuticles encountered in foliar application to terrestrial plants.

REVIEW OF LITERATURE

Surfactants are used extensively in the formulation of herbicides, and have been shown to increase the phytotoxicity of herbicides. It appears that surfactants may increase the activity of herbicidal solutions other than through effects on the physical properties of the solutions. The physicochemical properties of surfactants are such that surfactant molecules are highly polar and, therefore, surface active. Many surfactants are inherently phytotoxic and this phytotoxicity appears to be related to the polarity of the surfactants. It is considered, generally, that surfactants, in some way, regulate the penetration of chemicals into plant cells and thus enhance the phytotoxicity of herbicides. Whether the inherent phytotoxicity of surfactants plays a significant role in the penetration of chemicals into plant tissues and, consequently, plays a significant role in the enhancement of herbicidal activity is a question which must be answered.

Physicochemical Properties of Surfactants

Surfactants are chemical substances which possess surface active properties, and are, characteristically, long chain molecules possessing both a hydrophobic and hydrophilic end. The hydrophobic portion of the molecule imparts lipid solubility to the surfactant while the hydophilic portion of the molecule imparts water solubility.

Griffin (1949) has devised a system for classifying surfactants according to their hydrophilic and lipophilic characteristics. In this system, called the "HLB method", an HLB number is assigned to each surfactant in accordance with its hydrophilic and hydrophobic tendencies.

The letters HLB symbolize the "Hydrophile/lipophile balance". Surfactants with a high HLB are of chiefly a hydrophilic nature while surfactants with a low HLB are predominantly of a lipophilic nature. Since surfactants contain both hydrophobic and hydrophilic groups within the same molecule, they become oriented at interfaces and may alter energy relations at such interfaces and thus modify the physical properties of solutions.

Surfactants are characterized as anionic, cationic, nonionic or ampholytic, depending upon the nature of the hydrophilic portion of the surfactant molecule. A paraffin chain, an alkyl-substituted benzene ring or a napthalene ring, commonly serves as the hydrophobe of surfactant molecules. When these hydrophobes are balanced by negatively charged carboxyl, sulphate or phosphate hydrophiles the surfactant molecules are classified as anionic. When the hydrophobes are balanced by positively charged hydrophilic groups such as an amine or quarternary ammonium, sulfonium or phosphonium base the surfactants are classified as cationic. When the hydrophilic portion of the surfactant molecule is nonionized, the surfactants are termed nonionic. The hydrophilic groups of nonionic surfactants are usually hydoxyl groups of alcohols which may be polymerized with ethylene oxide to give ethoxylated ether and ester derivatives of alcohols. Ampholytic surfactants are cationic in acid media and anionic in basic media. The structure, properties and applications of surfactants have been reviewed ably by Schwartz and Perry (1949) and Schwartz et. al. (1958).

Surfactant Enhancement of Herbicides

Surfactants have been used for many years in the formulation of insecticidal and fungicidal sprays to improve wetting, spreading and sticking of spray materials on leaf surfaces of crop plants. When selective herbicides were developed, surfactants were explored as a possible means of increasing the effectiveness of herbicides. In general, results have been disappointing because reduced selectivity has accompanied herbicide enhancement. Zimmerman and Hitchcock (1942) found, in their early work with plant hormones, that hormone solutions containing a carbowax carrier were much more effective than water solutions of these same hormones. Mitchell and Hamner (1944) later showed that carbowax compounds (polyethylene glycols) increased the effectiveness of 2,4-D in promoting growth responses in tomato, soybean and kidney bean plants and suggested the possibility of using 2,4-D in combination with carbowax as a selective herbicide. This was largely corroborated by Ennis and Boyd (1946) who showed that 2,4-D, when sprayed in 0.5% solutions of various carbowax compounds, inhibited the growth of kidney bean plants but did not cause inhibition of the growth of soybean plants. This concentration of carbowax is somewhat lower than that used by Mitchell and Hamner (1944), thus it is suggested that a concentration effect may be involved in the differential responses obtained. A number of other carriers and emulsifying agents were examined by Withrow and Howlett (1946) for possible use with growth regulators, and they found that carbowax was highly toxic to tomatoes as well as to other plants; therefore, they were reluctant to use carbowax in their formulations. Hitchcock and Zimmerman (1948) reported that, in

addition to carbaryl, other adjuvants including polyethylene glycol monooleate and similar materials enhanced the activity of 2,4-D. They suggested that such adjuvants increase the penetration of 2,4-D into plant tissues. Stanifarth and Loomis (1949) found that commercial soapless powders with a base of sodium lauryl sulphate increased the toxicity of 2,4-D sprays to corn and soybeans. Since increases in the concentration of the wetting agents beyond the point of maximum surface tension depression resulted in increases in toxicity of the herbicide solutions, they considered that effects other than those of surface tension reduction were involved in the increased toxicity of 2,4-D.

Since the early work with 2,4-D, a number of investigators have demonstrated the modifying effects of surfactants on the toxicity of many herbicides to a variety of crop and weed species. Research in the field has been reviewed by Currier and Dybing (1959), Jansen et al. (1961), Buchanan (1965) and Parr and Norman (1965). Surfactants have been shown to modify the herbicidal activity of: a) the phenylureas, McWhorter and Sheets (1961), McWhorter (1963b), Will et al. (1964) and Bayer and Drever (1965); b) the triazines, Klnicki et al. (1965), and Dexter et al. (1966); c) the amino triazoles, Jansen (1964), (1965) and Smith et al. (1966); and e) dalapon, Jansen et al. (1961), Jansen (1964), (1965), McWhorter (1963a), Jordan et al. (1963), Lange et al. (1964), Foy and Smith (1965), and Smith et al. (1966).

Currier and Dybing (1959) have summarized a number of possible ways by which surfactants may enhance herbicidal effectiveness. They include "a) improving coverage; b) removing air films between spray and

leaf surface; c) reducing interfacial tension between relatively polar and apolar submicrosopic regions of the cuticle; d) inducing stomatal entry; e) increasing the permeability of the plasma membrane, through incipient toxicity; f) facilitating cell wall movement in the region of the wall-cytoplasm interface; g) acting as cosolvents; h) interacting directly with the herbicide in some manner; i) acting as humectants secondarily."

Temple and Hilton (1963) have discussed a number of theories regarding the activation of herbicides by surfactants. They suggest that surfactants may regulate spray retention; herbicide penetration or plant cuticle solubilization, and that surfactants may act also as herbicide co-solvents, chemical reactants or complexants and aid in water retention as humectants or hydroscopic agents. These investigators suggest further that surfactants, in acting as phytotoxic entities, may enhance the penetration of herbicides into plant tissues.

Although a completely satisfactory explanation of the action of surfactants on herbicide effectiveness has not been advanced, it has been repeatedly shown that surfactants increase the absorption of a number of herbicides by plant tissues. These include the following: a) phenoxy herbicides, Bryan, Loomis and Staniforth (1950), Mitchell and Linder (1950), Hauser (1955), Englund (1955), Mitchell and Linder (1957), Norris and Freed (1962); b) dalapon, Foy (1962) and Prasad et al. (1962); c) triazines, Biswas (1964) and Foy (1964); d) amino triazoles, Freed and Montgomery (1958). In contrast, Zukel et al. (1956) reported that the addition of surfactants did not generally improve the absorption

rate of maleic hydrazide.

Currier (1959) considered that the pathway of foliar penetration of aqueous herbicides may be both cuticular and stomatal. For the stomatal penetration of aqueous herbicides, he found that it was necessary to have an efficient surfactant in the herbicide solution and concluded that this was the main effect of surfactants. In their review of the topic of foliar penetration of herbicides, Currier and Dybing (1959) submit that entry of chemicals into the leaf is sometimes cuticular and stomatal. They point out that, although the intact cuticle presents a formidable barrier to the penetration of chemicals, herbicides apparently are sometimes able to penetrate such barriers. The work of Orgell (1957) suggests that cracks and perforations in the cuticle may increase its permeability, and that hydrated cuticles may be more readily permeable to polar compounds than are less hydrated ones. Orgell also has shown that surfactants may influence the sorption of herbicide molecules by plant cuticles. More recently, Dybing and Currier (1961) have studied the uptake of radioactively labelled herbicides and flourescent dyes as influenced by surfactants. Although they were able to show that the penetration of these materials occurred both by stomatal and cuticular penetration, cuticular penetration was very slow and was only slightly increased by added surfactants. Stomatal penetration was greatly increased by added surfactants.

Jansen (1964) attempted to correlate the hydrophile-hydrophobe structure of nonionic surfactants with herbicide enhancement by these surfactants. In studying the effects of ethoxylated nonionic surfactants, it was shown that the length of the ethylene oxide chain in the

hydrophilic portion of the surfactant molecules strongly influenced the enhancement of herbicides by a surfactant with a given type of hydrophobe. Within various hydrophobe series, enhancement generally increased up to a maximal ethylene oxide content of the hydrophile and then decreased. It was shown also that surfactants, which were phytotoxic in themselves, enhanced the toxicity of 2,4-D and dalapon somewhat more than did non-toxic surfactants, but not in proportion to their relative toxicities. Jansen (1965), subsequently, expanded the study of herbicide structure relationships to include ionic surfactants of the alkyl-benzene sulfonate class. Using a series of isomers of n-dodecyl benzene sulfonate surfactants, he related the effectiveness of surfactants in enhancing herbicide toxicity to the lipophilic structural configuration of the surfactant molecule. These surfactants also caused a marked modification of the physical-chemical properties of sprays in relation to their structure and concentration. However, Jansen (1965) was unable to correlate these properties, of the sprays studied, with herbicidal effectiveness. Smith et al. (1966) reported a similar relationship between structure and activity of homologous series of nonionic polyethoxylated ether surfactants of the octyl-, nonyl- and laurylphenol types. These authors showed, as did Jansen, that when these surfactants were added to aqueous solutions of dalapon, amitrole and paraquat herbicides which were subsequently applied as foliar sprays to corn, enhancement of herbicide activity was correlated with the ethylene oxide (EO) content of the surfactant molecules. Maximum toxicity of the surfactant-herbicide mixtures occurred when surfactants of intermediate EO content were used. Herbicide enhancement by surfactants with EO content greater or less

than optimum was less than maximum. As the concentration of the surfactant was increased, the EO content which gave maximum toxicity shifted to a lower value.

Phytotoxicity of Surfactants

In addition to enhancing the activity of the primary toxicants in herbicide solutions, surfactants have been shown to be inherently toxic to a large number of plant tissues. Several aspects of surfactant phytotoxicity have been reviewed comprehensively by Buchanan (1965). Surfactant toxicity has been reflected by reduced seed germination, suppression of root and shoot growth, and chlorosis of foliage. The patterns of surfactant phytotoxicity to these plant systems suggest that strong structure-activity relationships exist within surfactant series.

Traube and Marusawa (1915) reported that "capillary-active", higher fatty acids decidedly inhibited the rate of seed germination. These fatty acids, among other types of added substances, usually were considered to inhibit the imbibition phenomenon. Peas and other seeds containing relatively larger quantities of protein were more affected by the added substances than were starchy seeds such as barley. In later work, Traube and Rosentein (1919) investigated the influence of a large number of other "capillary-active" substances on plant seeds and found that many of the chemicals tested were strongly toxic to seed germination.

In their assessment of the toxicity of N-n- octadecylethylenethioureas, by diverse criteria, Ross and Ludwig (1957) were able to show that these

materials inhibited germination of wheat, barley and radish seeds. In a similar study involving several criteria of phytotoxicity, Buchanan (1965) found that the seeds of oats, corn and radish as well as seeds of the weed, giant foxtail, were inhibited in their germination by a wide variety of surfactants. In these tests oat seeds were by far the most sensitive to surfactants.

Alcohols are reportedly toxic to a number of organisms, and Eisenmenger (1930) showed that a number of different alcohols supressed the rate of root elongation of soybeans. Alcohol concentration was a determining factor; inhibition of root growth by a given alcohol increased as the concentration of the alcohol was increased. Stiles and Stirk (1932) also showed that a number of alcohols were also toxic to potato tuber tissue.

Prill et al. (1949) examined the effects of thirteen surface-active materials for effects on the growth of wheat roots grown in nutrient solutions. Surfactants of cationic, anionic and nonionic types were evaluated. The nonionic surfactants, except for saponin, which was highly inhibitory, generally did not inhibit root growth, but the three cationic and four anionic surfactants were strong inhibitors of wheat root growth.

Anionic and cationic surfactants, in general, have been shown to be quite toxic to root growth. For example, Jones et al. (1950) showed that a number of quarternary ammonium surfactants were toxic to the growth of rape and oats. Likewise, Allen and Skoog (1951) found that a number of imidazolines, oxazolines and related compounds inhibited wheat and radish roots. Buchanan (1965) was also able to show that a number of cationic

and anionic surfactants strongly inhibit the rate of elongation of corn roots.

It is generally suggested that nonionic surfactants are less toxic to roots than are the ionized type of surfactants. Parr and Norman (1964) point out that polyethylene sorbitan fatty acid esters are less inhibitory to roots than are other nonionic surfactants of either the ether or ether-alcohol types. These suggestions are borne out by Buchanan's work (1965).

Dills and Hemusan (1935) evaluated the toxicity of a number of aliphatic fatty acids and their potassium soaps to the foliage of a number of plant species including tomato, tobacco, potato, bean, cabbage and nasturtium. Many of these fatty acids and their soaps were highly toxic. An interesting aspect of the toxicity of aliphatic acids is brought out in the work of van Overbeek and Blondeau (1954). They showed that placement of beet tissue discs in three per cent solutions of straight-chain fatty acids resulted in the release of the red anthocyanin pigment from the beet tissue. This, they suggest, indicates that fatty acids alter the permeability of cell membranes.

Cory and Langford (1935) observed that sulfated fatty alcohols are also phytotoxic to foliage. Chrysanthemums were more susceptible to these surfactants than were snapdragons. Sodium oleyl sulfate was shown to be more toxic to foliage than was sodium lauryl sulfate.

In evaluating the phytotoxic effects of many solvents and emulsifiers used in the formulation of insecticide sprays, Gast and Early (1956) observed that many of these materials, which are decidedly surface-active, were toxic to the foliage of a number of species of test

plants. Commercial trade names were used in this paper to describe the emulsifiers used, consequently their purity and chemical structures were not defined, thus little knowledge can be derived from this data as to the effects of specific chemical types of surfactants on foliage. It should be pointed out, however, that the foliar toxicity of alcohols and ethoxylated alcohols is apparent in this study. Interestingly, Bourget and Parups (1963) found that the long chain fatty alcohols, hexadecanol and docosanol, when applied to the soil at rates greater than 0.002 per cent, reduced the growth of tobacco. In contrast Roberts (1961) observed no adverse effects on corn grown in soils containing 0.002 per cent hexadecanol.

The addition of surfactants to insecticide and fungicide formulations can present a problem in that the surfactants are inherently phytotoxic and may induce spray injury in the crop being protected. Swales and Williams (1956) encountered this problem with wetting agents used in fungicidal sprays for apples. Nonionic surfactants were observed, by Daines et al. (1957), to increase the phytotoxicity of captan sprays. Consequently, Furmidge (1959a) made a comprehensive evaluation of the phytotoxicity of a number of surfactants on leaves of apple and plum. He observed that leaf damage was largely dependent upon the concentration and chemical nature of the surfactant used. Ionic surfactants were quite phytotoxic while nonionic surfactants were considered to be relatively safe. It was concluded that damage to leaf tissue was the result of a disorganization of the cell permeability barriers resulting in the entry of surfactants through the leaf cuticle.

Stowe (1960) showed that a number of the Pluronic and Tetronic

surfactants were markedly phytotoxic. These surfactants are formed by the attachment of polyoxyethylene chains onto lipid-soluble polyoxypropylenes. Recently, Jansen et al. (1961), Temple and Hilton (1963) and Buchanan (1965) have evaluated a wide variety of surfactants of a wide variety of the major classes of surfactants. In these studies it was observed that chemical structure, ionization and concentration of the surfactant played significant roles in determining the relative phytotoxicity of these materials. As a rule, ionic surfactants were more phytotoxic than were nonionic surfactants. The phytotoxicity of active surfactants generally increased with increasing surfactant concentration.

Within homologous series of chemical substances it has often been observed that biological activity increases as the series is ascended. This has often been the case with the phytotoxicity of such series. That such a relationship exists is evident in the work of Stiles and Stirk (1932). They found that the relative phytotoxicity of n-alkyl alcohols increased as carbon chain length increased. N-octyl alcohol was 2139 times as toxic to potato tissue as was methyl alcohol. Similarly, Eisenmenger (1930) found that the inhibition of root growth by straight-chain, alkyl alcohols increased in direct relation to chain length. Dills and Menusan (1935), also were able to show that the phytotoxicity of aliphatic acids and their potassium soaps increased as carbon chain length increased up to a certain length but decreased with longer-chained members of the series. Capric and lauric acids were more toxic than either shorter or longer-chained acids. In a series of n-alkyltrimethylammonium salts it was observed by Jones et al. (1950) that phytotoxicity increased as the series was ascended. Maximum phytotoxicity to

rape occured at C_{14}, however, in the case of oats the maximum appeared to be higher. A similar relationship was evident in the results of Allen and Skoog (1951) for imidazoline and oxazoline derivatives. Ross and Ludwig (1957) examined the phytotoxicity of a homologous series of ^{N}-n-alkylethylenethioureas ranging from ethyl to dodecyl. In this series of compounds, peak phytotoxicity was shown at or near the amyl homologue.

Furmidge (1959a) also found that, within homologous series of ionic surfactants, alkyl sulfates, alkyl sulfonates and quartenary ammonium and pyridium salts, there was a definite relationship between the number of carbons in the alkyl chain and phytotoxicity to apple and plum. In the case of nonionic surfactants, of the ethylene oxide-ether type, Furmidge (1959b) found that not only does the length of the alkyl chain in the hydrophobic portion of a homologous series influence phytotoxicity, but also that the number of ethylene oxide molecules present in the hydrophilic group, apparently, may play an important role. He states that "phytotoxicity is reduced as the length of the ethylene oxide chain is increased". Buchanan (1965) also observed this relationship between ethylene oxide content, of a number of homologous series of surfactants containing ethylene oxide condensates, and phytotoxicity.

Veldstra and Booj as quoted by Allen and Skoog (1951, p. 622) "have emphasized that surface activity is an important property contributing to the activity of plant growth substances. They point out that all active compounds contain both hydrophilic and lipophilic groups and are, therefore, active at water-lipoid interphases. They claim that the physiological activity, especially the toxicity of compounds which

affect permeability, is dependent upon a delicate balance between hydrophilic and lipophilic linkages, even if other more subtle properties of the molecules are responsible for their specific growth-regulatory functions within the cell".

The Overton-Meyer lipoid theory implied such a relationship between toxicity and hydrophile-lipophile balance. Danielli as quoted by Ross and Ludwig (1957, p. 66) stated that "Overton found that substances penetrate cells in the same relative order of their oil/water partition coefficients. Since this work of Overton it has been generally assumed that such a correlation indicates a large proportion of lipoid material in the plasma membrane and that substances penetrate rapidly because they are more soluble in the lipid layer". Allen and Skoog (1951) concluded that "the correlation between lipoid solubility, as indicated by the oil/water partition coefficient, and toxicity have been attempted primarily to relate the latter to the rate of penetration into cells". Davies (1957) has shown that hydrophile-lipophile balance may be closely related to oil/water partition coefficients. It seems evident, then, that the hydrophile-lipophile theory of Veldstra and Booj is simply a restatement of the Overton-Meyer theory.

Ferguson (1939) discussed the nature of equilibrium relations within homologous series based on phase distributions. He concluded that the effect of phase distributions could be eliminated from comparisons of toxicity if the chemical potentials of physically toxic substances, rather than their concentrations, were measured in the external solution. Since, at equilibrium, the chemical potential is equal in all phases, the chemical potential at the site of action within the organism will be

identical with that in the external solution.

Hansch et al. (1965) have recently used octanol/water partition coefficients to determine the effects of substituents on chemical molecules in relation to the biological activity of such molecules. They derived a substituent constant, π , which is defined as π = log (P_X/P_H) where P_H is the partition coefficient of a parent compound and P_X that of a derivative. These authors have hypothesized that, in general, the biological activity of a series of drugs shows a parabolic relationship to their respective π constants.

In summarizing the literature dealing with the relationship of the chemical structure of molecules to their biological activity, it is apparent that the relative hydrophile-lipophile tendencies of such molecules are strong determinants of the degree of biological response encountered. Similarly, it appears likely that the hydrophile-lipophile balance of surfactant molecules is a major factor in surfactant phytotoxicity. The literature also suggests that the hydrophile-lipophile balance of surfactants may play a significant role in the enhancement of herbicide activity by surfactants.

MATERIALS AND METHODS

Plant Material

Duckweed, Lemna minor L., was used in this study as a test organism for the bioassay of the phytotoxicity of surfactants and surfactant-herbicide combinations. This plant has a number of important characteristics which make it suitable for physiological studies of plant behavior: 1) it can be readily grown in nutrient culture on a simple, inorganic, mineral solution, 2) its small size requires a minimum of space for growing cultures, 3) it can be sterilized easily and, hence, can be grown in sterile culture, 4) it may be rapidly propagated by vegetative means, and 5) genetically homogenous clones are easily obtained. These factors, coupled with the fact that Lemna minor is a higher plant, make it an ideal plant material for physiological studies. A summary of some of the work which has been done with Lemna minor is discussed in a recent review published by Hillman (1961).

Lemna minor is a monocotyledonous plant species belonging to the Lemnaceae family. Four genera: Lemna, Spirodela, Wolffiella, and Wolfia, are found in this family. The members of this family are commonly known as the duckweeds. The taxonomic features of the family have been discussed by Hegelmaier (1868).

Close examination of a culture of Lemna minor reveals that the plants are made up of small, floating bodies. These floating bodies, or "fronds", are variously regarded to be stems or leaves by morphologists, as is apparent in the following statement of Ashby et al. (1949):

> Morphologists do not agree about the way to interpret the frond of Lemna, but for the purpose of this study it is

> unnecessary to hold an opinion as to what the Lemna frond is. Whether fronds are regarded as leaves (Goebel, 1891-3), or phyllodes (Arber, 1919), or cladodes (Hegelmaier, 1868), or shoots (Caldwell, 1899), they are morphological units of the same organism separated by intervals of time...

Blodgett (1915) has studied the several characteristics of the Lemna frond discussed below. The adult frond is a propagative, pear-shaped, sporophyte, plant organ. At the base, this frond has a "pouch" on each side which is formed by outgrowths of the upper and lower surfaces of the frond. The next vegetative generation, daughter fronds, arise within these two pouches. The daughter fronds eventually separate from the parent and form new plants which in turn produce daughter fronds. From the ventral surface of the young daughter fronds, a single root is initiated from the fourth layer of cells. The outer layer which pushes through the epidermis forms a cap which is clearly visible as a "root sheath" in the mature frond. The cells of the young fronds divide rapidly, but this rapid division terminates before the frond has matured and cell enlargement follows. During this enlargement stage the anterior portion of the frond elongates rapidly, and the walls between cells separate forming air cavities. These air cavities, which provide bouyancy, are separated from one another by a single layer of cells. Another layer of cells, through which the vascular strands of the fronds pass, separates the dorsal and ventral regions of air cavities.

From the same pouches which produce daughter fronds, flowers also arise. According to Hillman (1961), "As far as is known, a frond produces only one flower in a lifetime; the primordia are usually well-differentiated while the mother frond itself is still young, even entirely enclosed within the mother frond." These flowers consist of a

single pistil and two stamens surrounded by a one cell thick spathe which extends beyond the edge of the frond.

Caldwell (1899) has discussed the following anatomical features. Vascular strands may be observed in the mature frond halfway between the dorsal and ventral surfaces; these strands appear as a main branch with two side branches diverging from the node into the foliar portion of the frond. Only a single row of small tracheids occurs in these strands along with two or three layers of phloem cells. The tracheal elements have spiral or ring thickenings and are surrounded by parenchymatous tissue.

In examining the root, the outer layer is the root cap which is not an intimate contact with the cells of the main body of the root, but is closely appressed to the root tip. The main body of the root is composed of a region of cortical cells separated by air spaces, from an inner cylinder of elongated cells. This inner cylinder represents the reduced vascular system of the root. Typically, the root may be several centimeters long but is less than a millimeter in diameter.

According to Hillman (1961), stomata are located on the upper surface of the frond with the lower surface being free of stomata. The upper epidermal layer of the frond also differs from the lower epidermis in that it is highly outinized.

Culture Techniques

A culture of the duckweed, Lemna minor L., was collected from Lake Anabesacook at Winthrop, Maine in September 1963. Approximately fifty

plants were surface sterilized by a brief immersion in a ten per cent solution of "Chlorox" and placed in sterile nutrient solution containing two per cent sucrose. The sucrose supplied a carbohydrate source which enhanced the growth of bacteria and fungi remaining in the culture, and revealed contamination of cultures by the resulting turbidity of the nutrient media. Contaminated cultures were discarded, and a vigorous plant was selected for the propagation of a sterile stock culture. Stock cultures were maintained in two hundred and fifty mililiter flasks, which were plugged with cotton and contained a modified Hoagland's solution as described by Allen (1960). Each culture was transferred weekly to fresh media, and sub-cultures were initiated weekly also. By these methods, a continous supply of fresh cultures of Lemna minor was maintained.

Phytotoxicity Assay

Lemna minor was used, for several reasons, in assaying the phytotoxicity of the test solutions evaluated in this study. The chief reasons are pointed out by Offord (1946) who called attention to "...the convenience and economy in the use of Lemna minor for estimating the phytocidal action of chemicals where a large number of tests are needed and where costs and availability of chemicals are important considerations...". The techniques used in assaying the toxicity of surfactants in these experiments were essentially those outlined by Blackman (1955). A sterile stock culture of Lemna minor was maintained in a growth chamber at 25° C. under a light intensity of 1200 foot candles on a photoperiod of 16 hours. From this stock culture plants were randomly selected and placed in 50 milliliters of distilled water in 125 mililiter flasks to

which the surfactants or herbicides being tested were added at various concentrations. These treated plants were placed in the growth chamber and maintained at the conditions mentioned above for a 48 hour period after which they were transfered to flasks containing 50 mililiters of fresh, dilute Hoagland's solution. These fresh flasks were then returned to the growth chamber for an additional twenty-four hour period. The criteria of phytotoxicity were discussed by Blackman (1952) as follows: "At the end of this time those fronds which were chlorotic over half of the surface, for the purpose of statistical analysis, were counted as 'dead' and the concentration which caused a 50% mortality (LD_{50}) was taken as the standard measure of biological activity."

Surfactants and Herbicides Evaluated

In these experiments, the surfactants evaluated consisted of members of the nonionic, n-alkyl alcohol, alkyl polyether alcohol and alkyl aryl polyether alcohol types. Combinations of certain of these surfactants with the herbicides 2,4-D and paraquat were also evaluated. The chemical characteristics of these materials are summarized in table 1.

Statistical Analysis

Dose-response relationships for each of the surfactants and surfactant-herbicide combinations tested in these experiments were determined from the raw data by the methods of "Probit analysis" as outlined by Finney (1952). In essence, probit analysis entails the transformation of per cent kill data to "probits" by means of the "probit transformation". Transformation of the percentage-kill response data to probits converts the typical sigmoid curve observed, when percentage-kill

Table 1. Chemical nature, sources and trade names of the surfactants and herbicides used

Material	Moles of Ethylene oxide	Common or Trade name
n-alkyl alcohols		
ethyl alcohol[1]	0	Ethanol
n-butyl alcohol[1]	0	n-Butanol
n-hexyl alcohol[2]	0	n-Hexanol
n-octyl alcohol[2]	0	n-Octanol
Alkyl polyether alcohols		
octoxyethoxyethanol[3]	1	
octoxypolyethoxyethanol[3]	5	
octoxypolyethoxyethanol[3]	9	
alkyl aryl polyether alcohols		
octyl phenoxyethanol[4]	5	Triton X-45
octyl phenoxyethanol[4]	7-8	Triton X-114
octyl phenoxyethanol[4]	9-10	Triton X-100
Octyl phenoxyethanol[4]	12-13	Triton X-102
2,4-Dichlorophenoxy acetic acid[5]	0	2,4-D
1,1-dimethyl-4,4-bipyridilium chloride[6]	0	paraquat

[1]Fisher Scientific Co., Chicago, Illinois.

[2]Mateson Coleman & Bell, East Rutherford, New Jersey.

[3]Enjay Laboratories, Linden, New Jersey.

[4]Rohm & Haas Co., Philadelphia, Pennsylvania.

[5]Eastman Organic Chemicals, Rochester, New York.

[6]Chevron Chemical Co. (Ortho division), Richmond, California.

data is plotted against the logarithm of the applied dosage, to a straight line. This "probit regression line" is obtained by computing the linear regression of the probit response data on the logarithm of the applied dosage.

The computations necessary for this study were made on an IBM 360 computer, using the "probit analysis" and "potency probit analysis" programs of Daum and Killcreas (1966a), (1966b). The first of these programs obtains the weighted linear regression of probit-response on log-dose, and the second program obtains the relative potency (the ratio of equally effective doses) for two or more parallel dose-response lines by weighted linear regression of probit-response on log-dose. Both these programs employ the maximum likelihood procedure outlined by Finney (1952).

The "probit analysis" program described above was used to determine the dosage-response curve for each of the individual surfactants, herbicides and surfactant-herbicide combinations evaluated in this study. If the regression of probit-response on log-dose, computed for a specific set of data, proved to be statistically significant, the computer calculated and printed out the LD_{30}, LD_{50}, LD_{70}, LD_{90} and the 95 per cent confidence limits of these values for that particular set of data. These values, which are the dosages required to kill thirty, forty, seventy, and ninety per cent of test plants, respectively, give four coordaintes which when plotted on logarithmic-probability paper and connected by a line yield the dosage-response curve for the set of data being analyzed. In contrast, if the regression of probit-response on log-dose proved to be not statistically significant the computer simply printed NON

SIGNIFICANT REGRESSION.

The "potency probit analysis" described above was used in certain experiments of this study to determine the relative potencies of the individual treatments in a series tested simultaneously in the same experiment. Using this program, the computer tested for parallelism among the individual dose-response regressions, and, if they were parallel, calculated and printed out the relative potencies as well as their 95 per cent confidence limits. The relative potencies were computed by dividing the LD_{50} of the first line into the LD_{50} for each successive line. If the lines were not parallel then the computer simply printed out LINES NOT PARALLEL.

Determination of HLB Numbers

Griffin (1949) developed a system for describing the relative hydrophilic-lipophilic tendencies of surfactants. In this system an HLB number is assigned to each surfactant. The letters HLB are an abreviation for "Hydrophile-Lipophile Balance". Since the experimental determination of HLB numbers is complex and quite involved, Griffin (1955) has also derived equations which enable the calculation of HLB numbers for certain nonionic surfactants which closely approximate the experimentally determined values. Such an equation was used for the determination of the HLB numbers of the ethoxylated alkyl alcohols and ethoxylated alkyl phenols used in the present study. This equation is as follows:

$$HLB = E/5,$$

where E is the weight percentage of the ethylene oxide content in the

surfactant molecule.

The HLB numbers of the n-alkyl alcohols used in this study were the values calculated by Davies (1957) who employed the following equation:

HLB = (hydrophilic group numbers)

- n (group number per CH_2 group + 7

Davies (1957) assigned experimentally determined "group numbers" to the various hydrophilic and hydrophobic groups found in surface-active molecules. The group numbers for the CH_2- and -OH groups found in the n-alkyl alcohols are -0.475 and 1.9 respectively.

RESULTS

The phytotoxicity of nonionic surfactants and their interactions with herbicides appear to be related to the chemical structure of the surfactant molecule. These relationships of the phytotoxicity of surfactants to the physicochemical properties of the surfactants were studied by evaluating the responses of Lemna minor to several surfactants and surfactant-herbicide combinations. An understanding of such relationships is essential to the successful use of surfactants in herbicide technology.

The standard measure or index of phytotoxicity used in this study was the "LD_{50}", a value which designates the dosage of a toxicant required to cause a fifty per cent mortality of test plants. To obtain the LD_{50} of a surfactant or surfactant-herbicide mixture to Lemna minor, plants of this species were treated with a range of dosages of the test solution and the percentage of the plants killed at each dosage was observed and recorded. These data were then used to construct dosage-response curves by plotting the percentage kill versus surfactant dosage. LD_{50}'s were computed by probit analysis and can be extrapolated from the dosage-response curves fitted to the data by the methods of probit analysis. Relative potencies of the individual surfactants within homologous series were computed in the probit analysis of the data, by comparisons of the LD_{50}'s of the individual surfactants.

Correlations of the phytotoxicity of surfactants, against Lemna minor, to certain physicochemical properties of the surfactants were observed. The LD_{50}'s of the surfactants within homologous series were

correlated with hydrophile-lipophile balance numbers, oil/water distribution coefficients, and pi constants. Correlations were observed also between physico-chemical properties and the enhancement of herbicidal activity by surfactants.

Phytotoxicity of Surfactants

In this series of experiments, the phytocidal effects of certain nonionic surfactants to Lemna minor were evaluated. The phytotoxicity to Lemna minor, expressed as an LD_{50}, was determined for each of several, selected members of homologous series of normal alkyl alcohols, ethoxylated alkyl alcohols and ethoxylated alkyl phenols. The relative potencies of the individual members within homologous series of these surfactants were derived from the ratios of the LD_{50}'s of the individual members within the series. Within each homologous series, phytotoxicity was correlated with the hydrophile-lipophile balance of the surfactants within the series. Within the n-alkyl alcohol and ethoxylated alkyl phenol series, phytotoxicity was shown also to be correlated with oil/water distribution coefficients and Hansch's (1965) pi constants.

Phytotoxicity of n-alkyl alcohols

The dosage-response curves obtained for the phytotoxicity of normal alkyl alcohols to Lemna minor are shown in figure 1. From these dosage-response curves, the LD_{50}'s for each alcohol shown in table 2, was obtained. Since only two points were available for determining the slope of the dosage-response curve for n-octyl alcohol, a line having a slope equivalent to the common slope of the dosage-response curves of

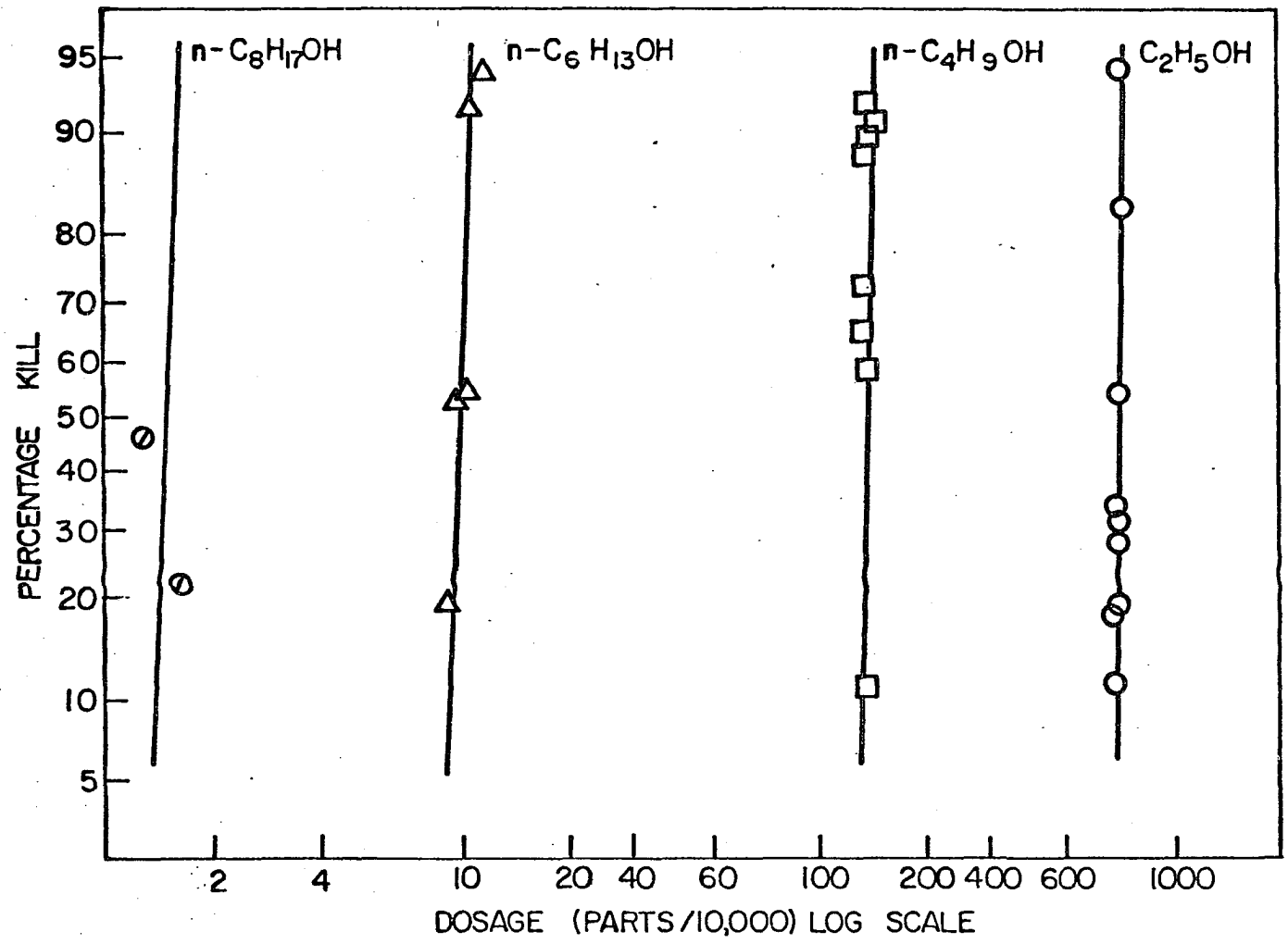

Fig. 1. Dosage-response curves of four n-alkyl alcohols against Lemna minor

Table 2. Phytotoxicity of homologous series of normal alkyl alcohols to Lemna minor

Alcohol	HLB No.[a]	LD_{50}[b]	Slope[c]
Ethyl	7.9	726.0	68.6
n-Butyl	7.0	136.7	108.1
n-Hexyl	6.1	10.2	29.4
n-Octyl	5.1	1.5	--

[a]Hydrophile-lipophile balance numbers derived by the method of Davies (1957).

[b]Median Lethal Dose or the concentration (parts/10,000) of toxicant required to kill fifty per-cent of the plants.

[c]Slope of the dosage-response curve of the alcohol against Lemna minor.

the other alcohols tested was fitted to the two points representing octyl alcohol. Thus, the LD_{50} for n-octyl alcohol contained in table 2 is an estimate.

The correlation between the LD_{50} and the hydrophile-lipophile balance of the n-alkyl alcohols tested is shown in figure 2. The phytotoxicity of these alcohols to Lemna minor, as measured by the LD_{50}, decreased as the HLB number of the individual alcohols increased, with those members of the homologous series tested. The phytotoxicity of these alcohols to Lemna minor appeared to be related linearly to their hydrophile-lipophile balance. However, had higher members of the series been included the relationship may have deviated from linearity.

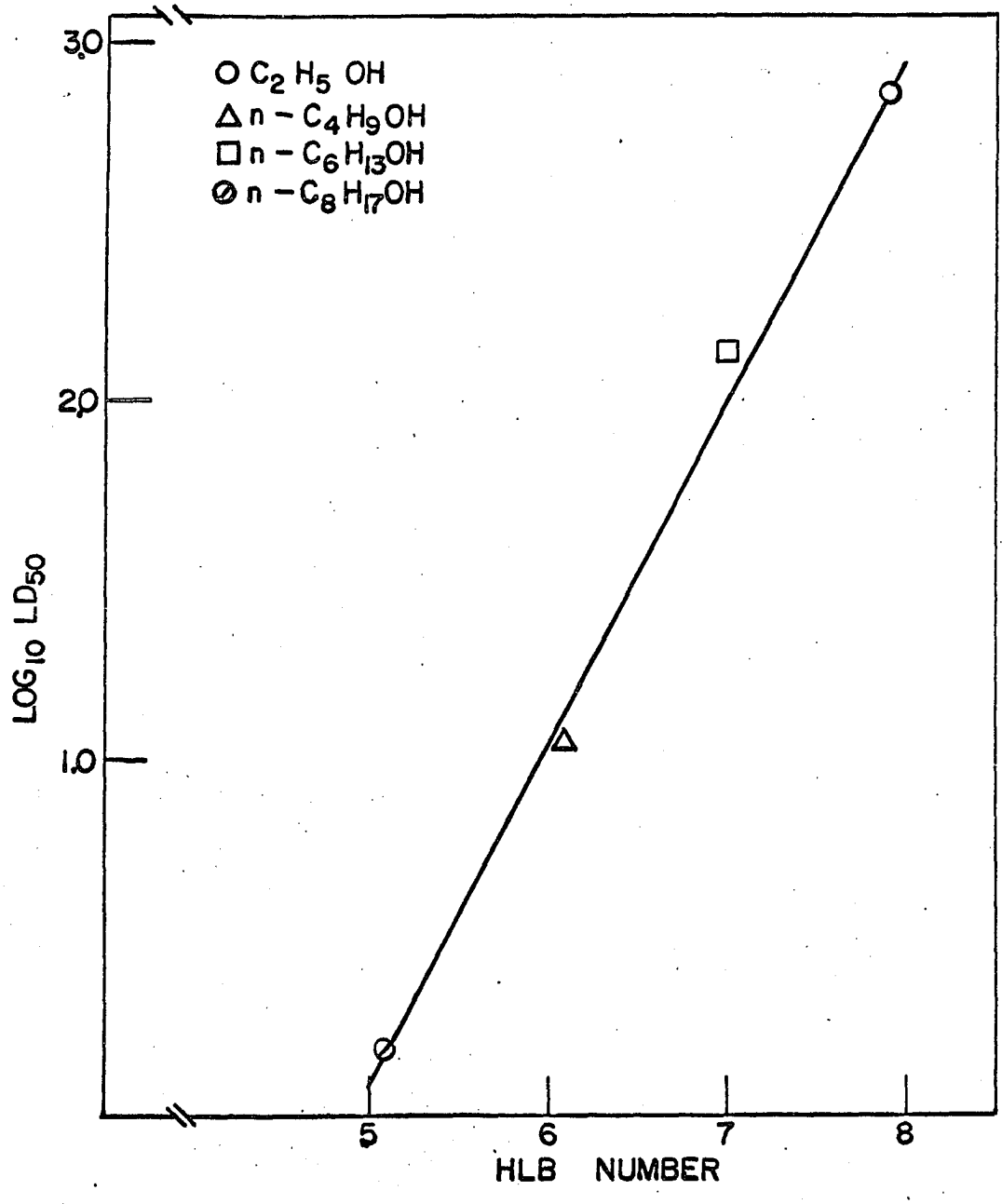

Fig. 2. Relationship of the phytotoxicity of n-alkyl alcohols against Lemna minor to the hydrophile-lipophile balance of the alcohols

It appeared, too, that the phytotoxic effects of these alcohols to Lemna minor were correlated likewise with oil/water distribution coefficients and Hanch's pi constants. Figure 3 illustrates the relationship of the LD_{50}, ie. phytotoxicity, to the distribution coefficient of the alcohols in an olive oil/water system. Similarly, figure 4 shows the relationship of the LD_{50}, ie. phytotoxicity, to the pi constants Hanch (1965) derived for these alcohols. The olive oil/ distribution coefficients used were those determined by Macy (1948). Distribution coefficients were not available for n-hexyl and n-octyl alcohols, and, therefore, values for these alcohols were derived by extrapolation from a curve obtained by plotting the distribution coefficients, available for other alcohols of the series, against the number of carbon atoms in the alcohols.

Phytotoxicity of octoxyethanol surfactants

The phytotoxicity of octoxyethanol surfactants against Lemna minor is shown in the dosage-response curves plotted in figure 5. The slopes of these dosage-response curves and the LD_{50} for each surfactant are given in table 3. The calculation of the relative potencies of the members of the series, as given in table 3, was justified, since probit analysis of the data for this series indicated no significant deviation from parallelism among the dosage-response curves of these surfactants against Lemna minor.

The relationship between the phytotoxicity of octoxyethanol surfactants to Lemna minor and the hydrophile-lipophile balance of the surfactants was shown in figure 6 by plotting the LD_{50}'s of the

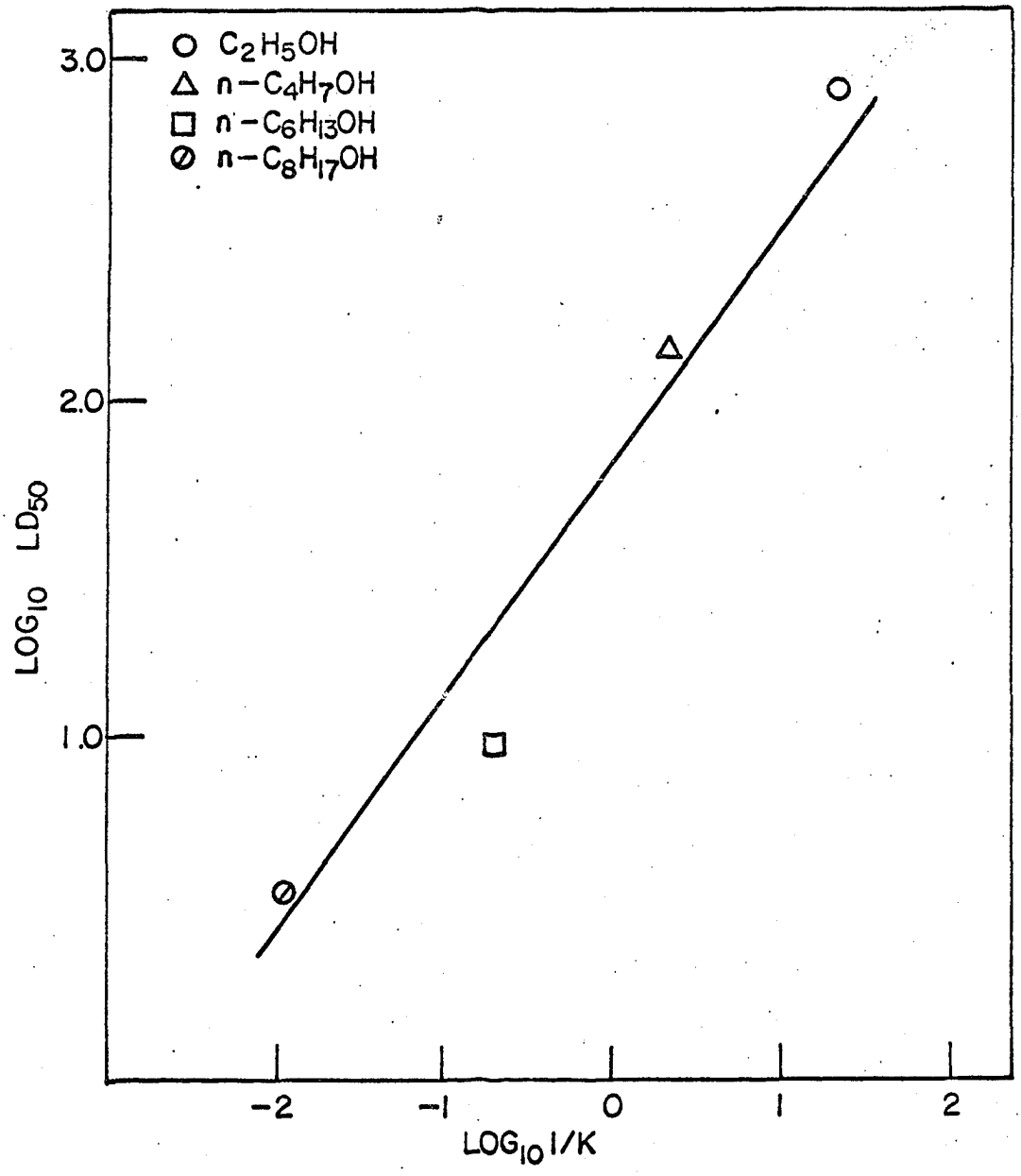

Fig. 3. Relationship of the phytotoxicity of n-alkyl alcohols against Lemna minor to the olive oil/water distribution coefficient of the alcohols

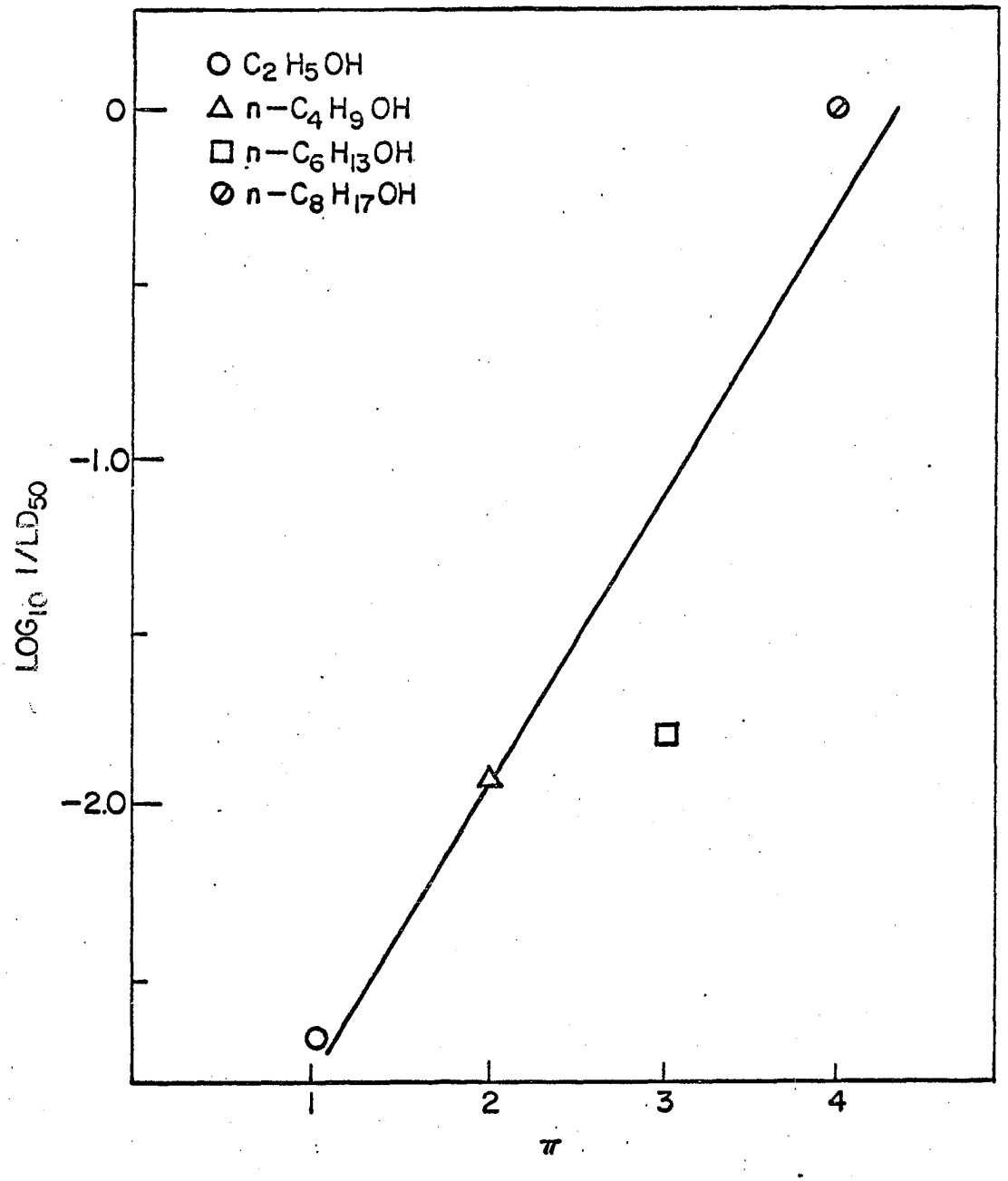

Fig. 4. Relationship of the phytotoxicity of n-alkyl alcohols against Lemna minor to the pi constants of the alcohols

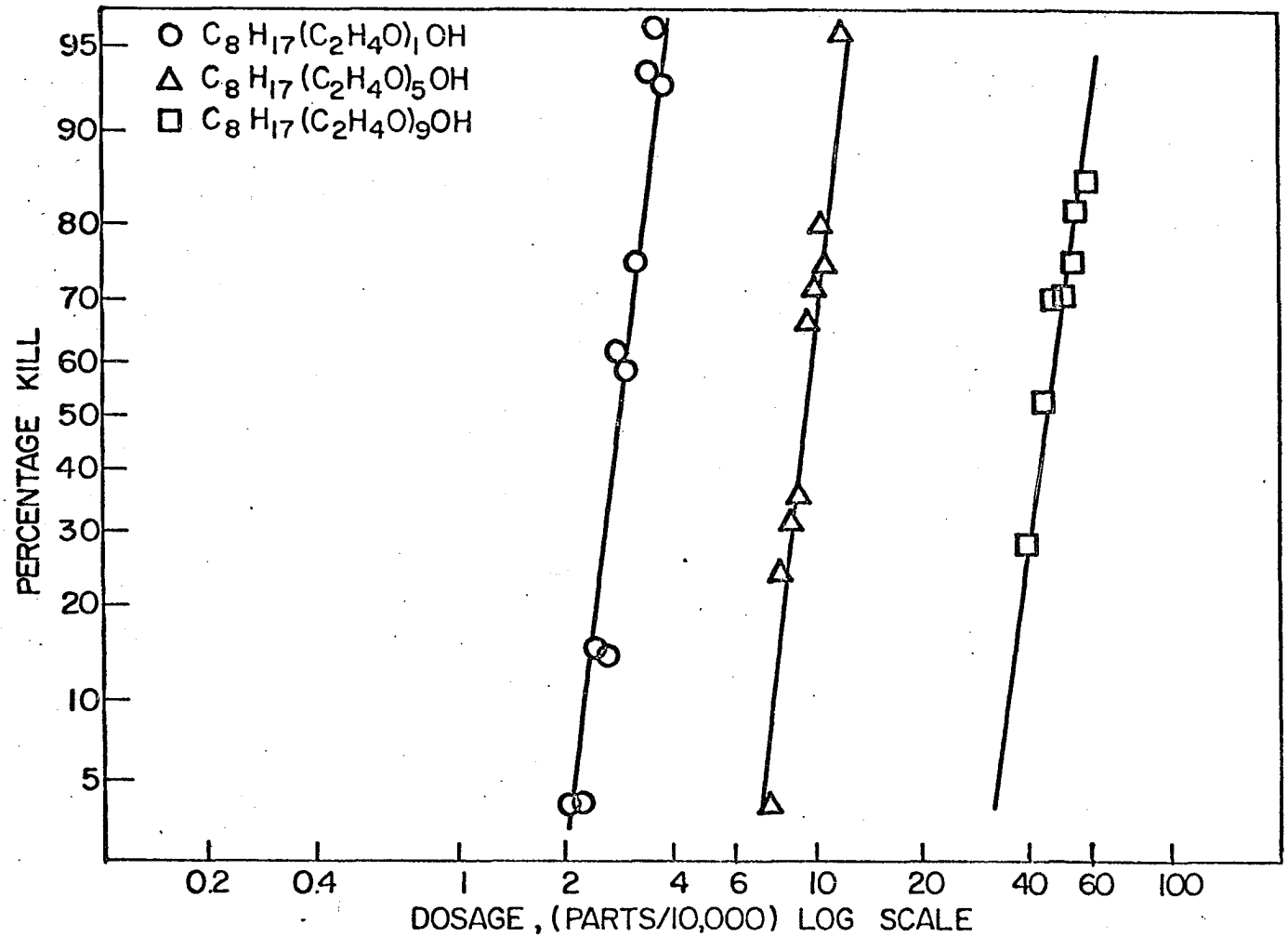

Fig. 5. Dosage-response curves for three octoxyethanol surfactants against Lemna minor

Table 3. Phytotoxicity of homologous series of octoxyethanol surfactants to Lemna minor

Surfactant	HLB No. [a]	LD_{50} [b]	Slope [c]	Relative Potency [d]
Octoxyethanol 1 mole EO	5.1	2.82	1.46	1.00
Octoxyethanol 5 moles EO	12.5	9.28	1.49	3.29
Octoxyethanol 9 moles EO	15.0	45.24	1.20	16.04

[a]Hydrophile-lipophile balance numbers derived by the method of Griffin (1954).

[b]"Median Lethal Dose" or the concentration (parts/10,000) of surfactant required to kill fifty per cent of the plants.

[c]Slope of the dosage-response curve of the surfactant against Lemna minor.

[d]Degree to which the LD_{50} of the most toxic member of the series has to be multiplied in order to obtain the equitoxic concentration for another particular member of the series.

surfactants against their respective HLB numbers. Although it appeared that a relationship existed between phytotoxicity and hydrophile-lipophile balance, it was further apparent that the relationship was non-linear. The phytotoxicity of the members of this homologous series of surfactants against Lemna minor increased, apparently, as an exponential function of the hydrophile-lipophile balance of the surfactants as the series was ascended.

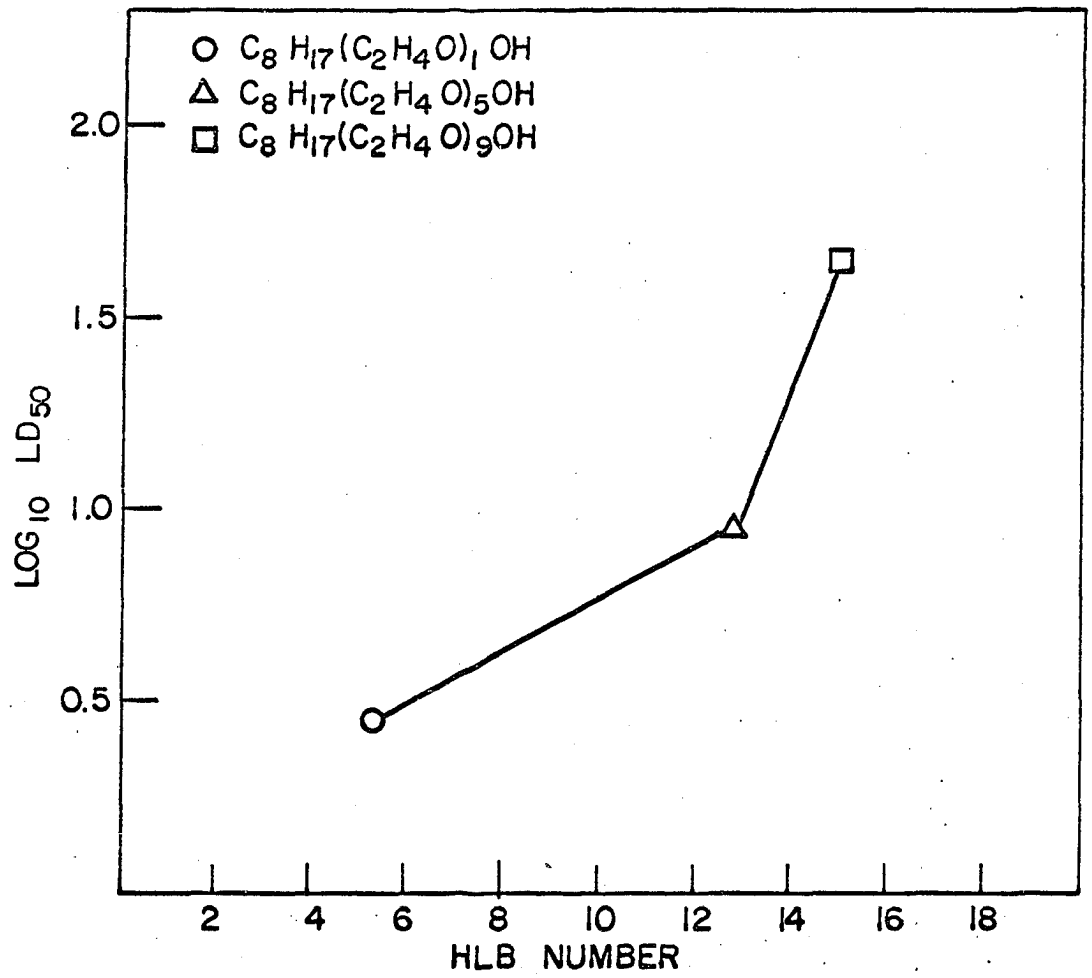

Fig. 6. Relationship of the phytotoxicity of octoxyethanol surfactants against Lemna minor to the hydrophile-lipophile balance of the surfactants

Phytotoxicity of octylphenoxyethanol surfactants

The dosage-response curves obtained for octylphenoxyethanol surfactants against Lemna minor differed from those observed with n-alkyl alcohols and octoxyethanols. While these latter materials produced linear response curves against Lemna minor, the dosage-response curves obtained for the octylphenoxyethanols deviated from linearity at high concentrations. Thus only the response data for surfactant concentrations of ten parts per ten thousand or less were utilized for the purposes of probit analysis. The dosage-response curves for these surfactants against Lemna minor are shown in figure 7, and the initial slopes of these dosage-response curves and the LD_{50} for each surfactant of the series are listed in table 4. Since a fifty per cent kill was not obtained even at high concentrations of the twelve-thirteen mole ethylene oxide member of the octylphenoxyethanol series, the LD_{50} listed for this surfactant in table 4 is an estimate based on the initial slope of the dosage-response curve. Relative potency values are listed also in table 4, since the initial slopes of the dosage-response curves for these surfactants did not differ significantly from parallelism.

The relationship of the phytotoxicity of the octylphenoxyethanol surfactants to their hydrophile-lipophile balance was similar to that shown previously for the octoxyethanol surfactants. In figure 8, the LD_{50}'s of the octylphenoxyethanols were plotted against their respective HLB numbers. From this figure it seemed that the phytotoxicity of the octylphenoxyethanol surfactants was an exponential function of their hydrophile-lipophile balance. Likewise, the LD_{50}'s of octylphenoxyethanol surfactants appeared to be related to the distribution coefficients

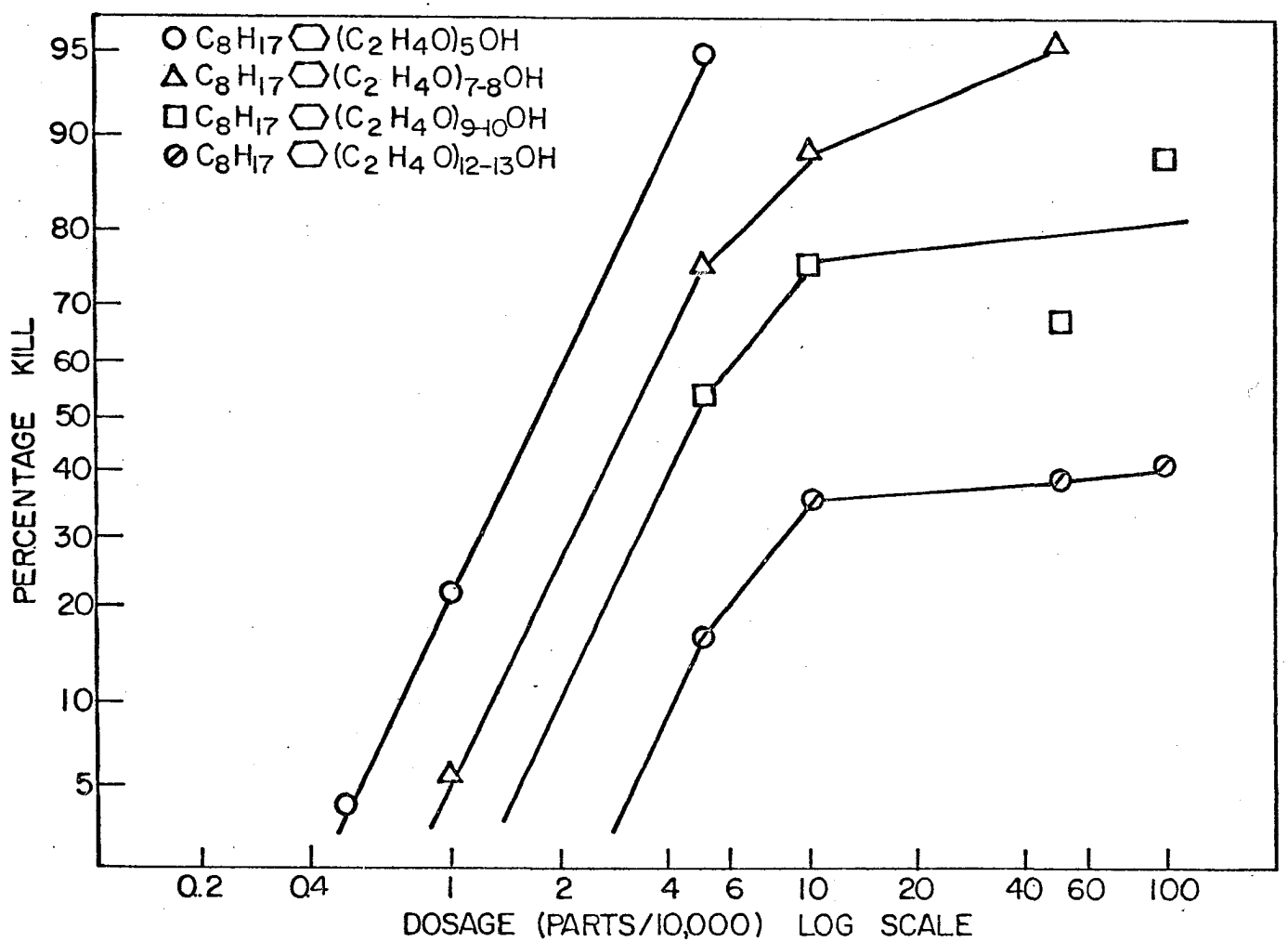

Fig. 7. Dosage-response curve of four octylphenoxyethanol surfactants against Lemna minor

Table 4. Phytotoxicity of homologous series of octylphenoxyethanol surfactants to Lemna minor

Surfactant	HLB No.[a]	LD_{50}[b]	Slope[c]	Relative[d] Potency
Octylphenoxyethanol 5 moles EO	10.2	1.75	3.20	1.00
Octylphenoxyethanol 7-8 moles EO	12.3	3.41	3.03	1.94
Octylphenoxyethanol 9-10 moles EO	13.4	4.75	3.20	2.71
Octylphenoxyethanol 12-13 moles EO	14.5	9.80	2.38	5.84

[a]Hydrophile-lipophile balance numbers derived by the method of Griffin (1954).

[b]"Median Lethal Dose" or the concentration (parts/10,000) required to kill fifty per cent of the plants.

[c]Slope of the dosage-response curve of the surfactant against Lemna minor.

[d]Degree to which the LD_{50} of the most toxic member of the series has to be multiplied in order to obtain the equitoxic concentration of another, particular member of the series.

of the surfactants is an isooctane/water system. This relationship appeared to be linear, as illustrated in figure 9; however, some departure from linearity could be expected if more members of the series were included. The distribution coefficients used were those determined by Greenwald et al. (1961).

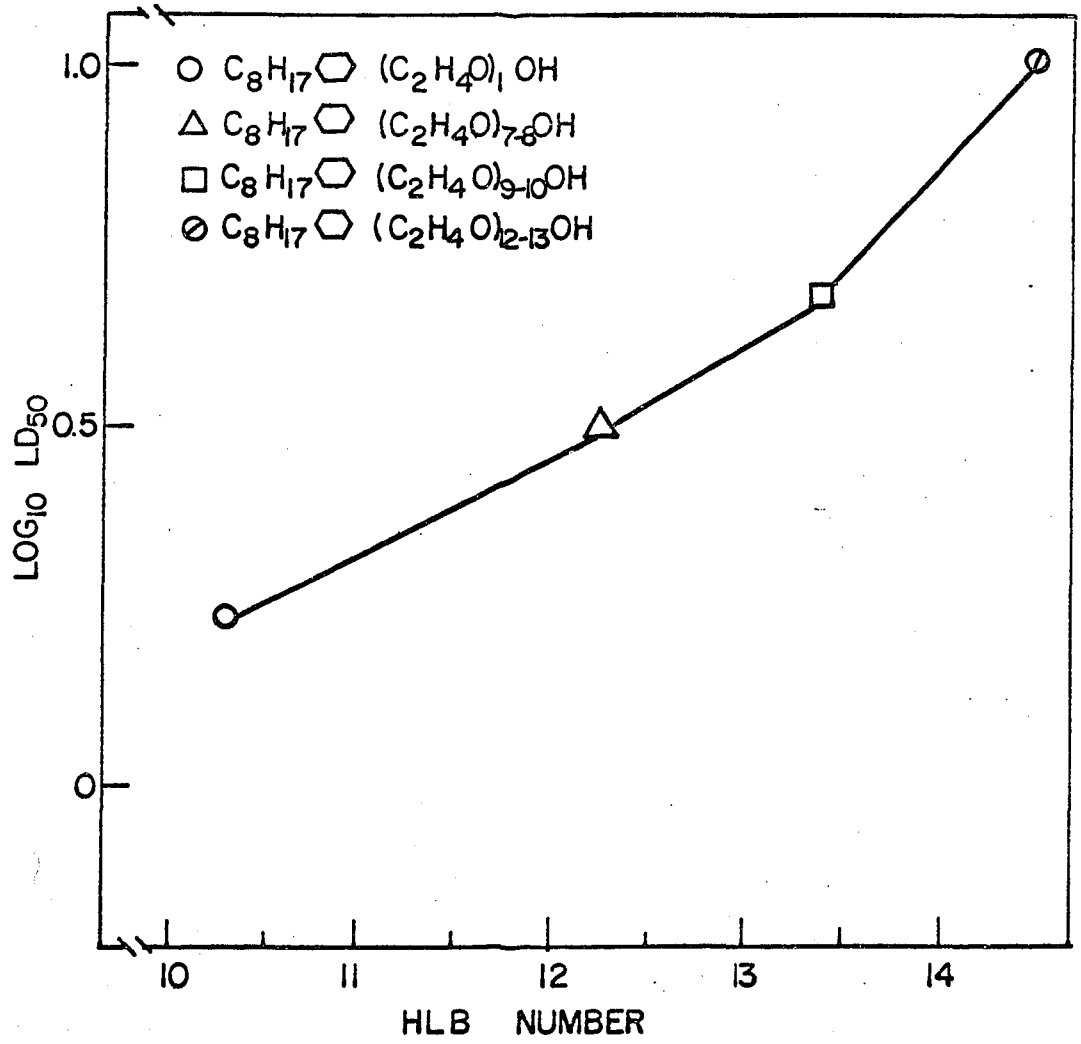

Fig. 8. Relationship of the phytotoxicity of octylphenoxyethanol surfactants against Lemna minor to the hydrophile-lipophile balance of the surfactants

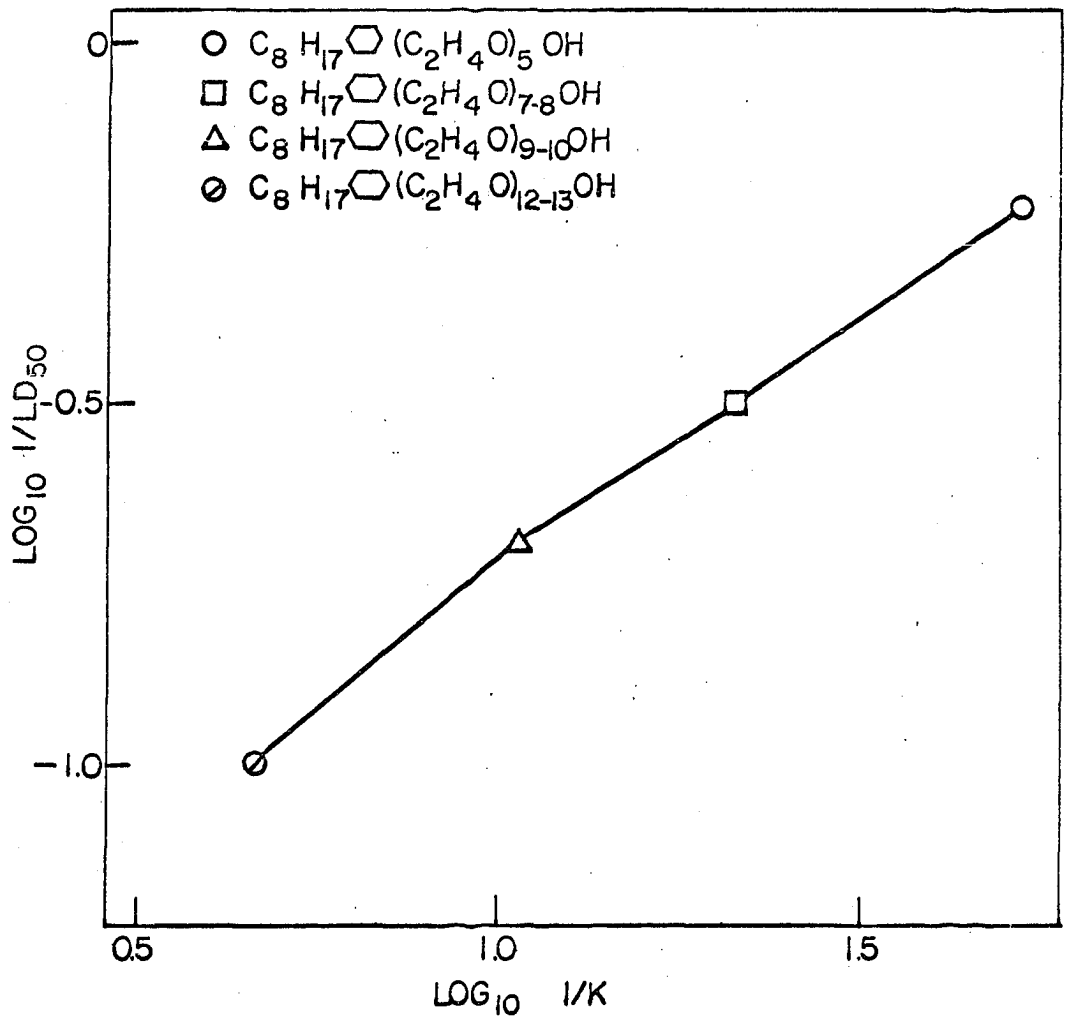

Fig. 9. Relationship of the phytotoxicity of octylphenoxyethanol surfactants against Lemna minor to the isooctane/water distribution coefficients of the surfactants

Phytotoxicity of Surfactant-Herbicide Combinations

In these studies, the combined phytocidal effects of surfactants and herbicides were evaluated. The influence of added surfactants on the phytotoxicity of herbicidal solutions against Lemna minor was assessed; both with surfactants added directly to the herbicide solutions and with the surfactants utilized as a pretreatment prior to herbicide treatments. The effects of a time lapse, or recovery period, after a preliminary surfactant pretreatment on the subsequent phytotoxicity of herbicide treatments to Lemna minor, were studied. Interactions were observed between surfactant phytotoxicity and herbicide toxicity to determine whether simply additive effects were involved or whether, in fact, synergistic effects were present. The effects of herbicide treatments on the phytotoxicity of surfactants to Lemna minor were evaluated.

2, 4-D - Octoxyethanols Mixtures

The effects of added octoxyethanol surfactants on the phytotoxicity of 2,4-D solutions to Lemna minor were evaluated. Figures 10 and 11 show the dosage-response curves obtained for a series of 2,4-D solutions with various octoxyethanol surfactants added. Probit analysis of the data represented by these curves showed that the lines for the 2,4-D - octoxyethanols combinations were essentially parallel. Therefore, the dosage-response curves drawn for these mixtures in figures 10 and 11 were fitted by a common slope. Probit analysis of the data showed that the dosage-response curve for 2,4-D alone was not parallel to the other curves; hence this curve had a different slope.

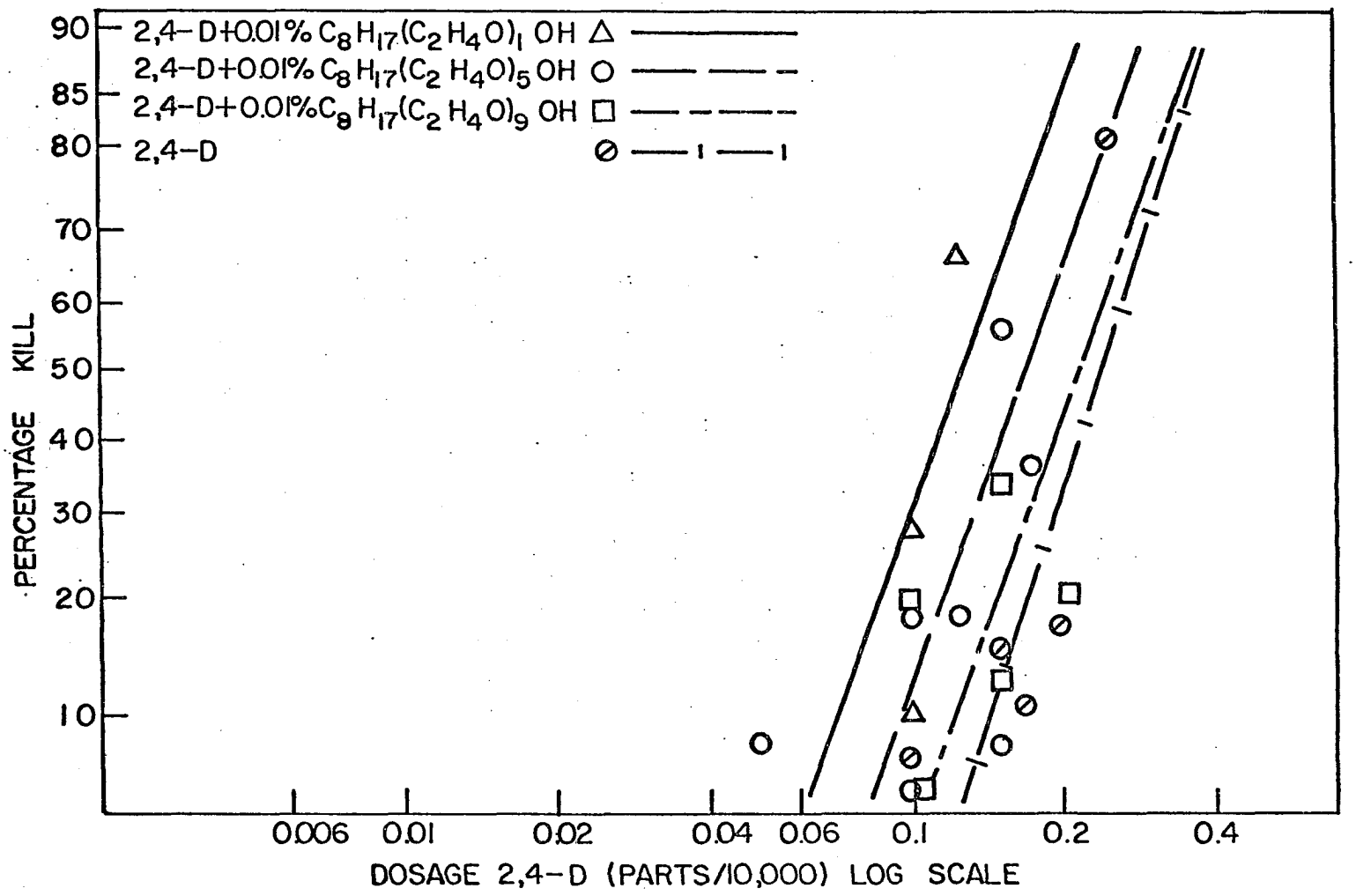

Fig. 10. Dosage-response curves for 2,4-D solutions against Lemna minor with 0.01% octoxyethanol surfactants added.

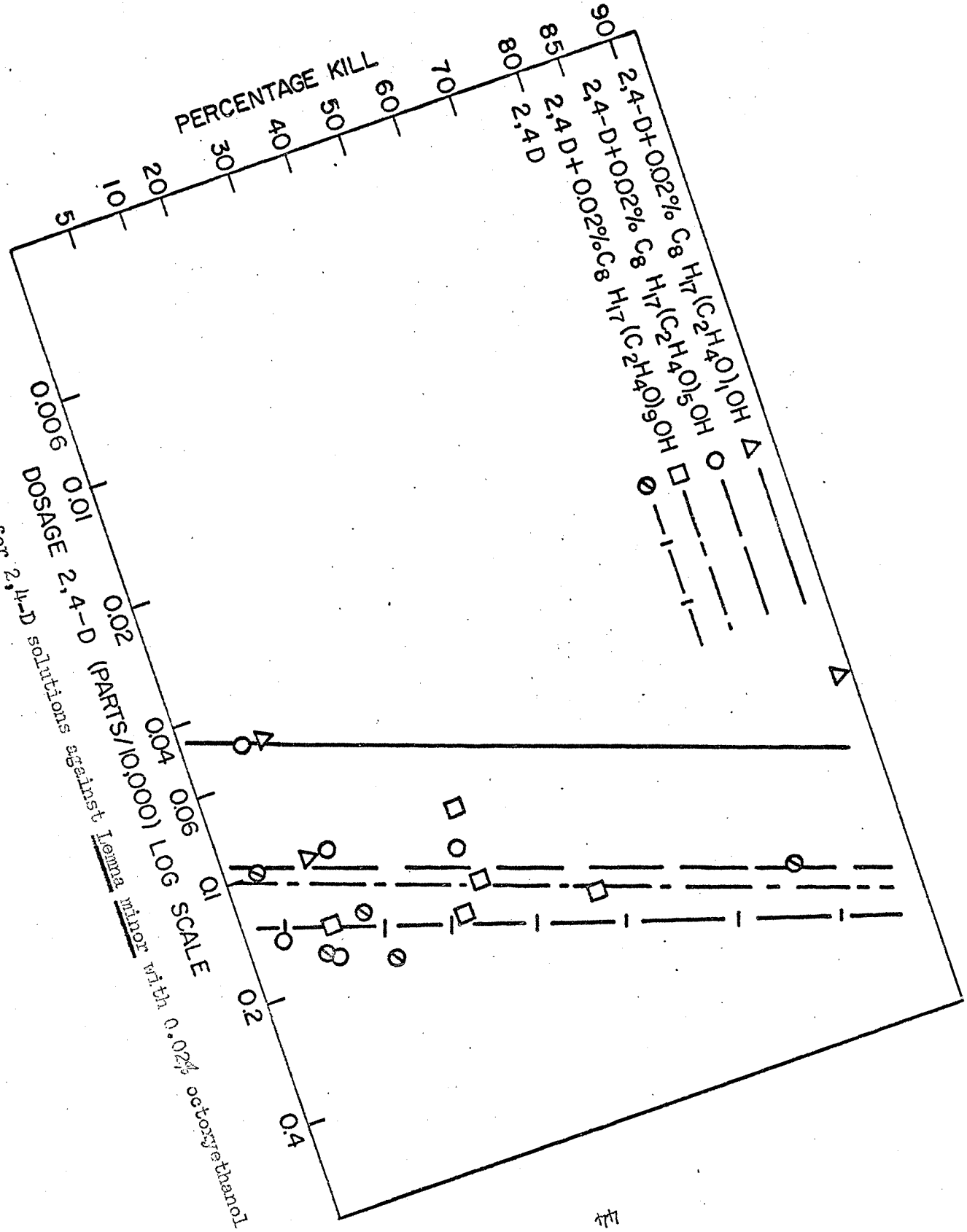

Fig. 11. Dosage-response curves for 2,4-D solutions against Lemna minor with surfactants added

The observed results showed that the phytocidal action of 2,4-D-surfactant combinations was greater than would be expected if the phytotoxicities of the surfactants and the 2,4-D were simply additive. This conclusion was based on the premise that the dosage-response curve for a mixture of two compounds can be predicted from the individual dosage-response curves for the two constituent compounds of the mixture taken separately. Finney (1952), for instance, pointed out that the expected mortality of a mixture of two toxicants, having independent modes of action, is related to the mortalities produced by the individual constituents of the mixture by the equation:

$$P = P_1 + P_2(1-P_1)$$

where P is the total proportion of the test plants killed and P_1 and P_2 are the proportion killed by the first and second toxicants respectively. In this experiment, when P_1 is the proportion of test plants killed by 2,4-D alone and P_2 is the proportion killed by surfactants alone, the above equation may be reduced to the expression,

$$P = P_1,$$

since the percentage kill for all of the surfactants alone was zero at the levels of surfactants used. One would, therefore, expect the dosage-response curves for the 2,4-D - surfactants mixtures to be identical with that for the 2,4-D alone. The lack of parallelism between the slopes of the dosage-response curves for the mixtures and 2,4-D alone, together with the observed differences in the phytotoxicity of the 2,4-D alone and with surfactants added, as presented in table 5, is evidence that the dosage-response curves were, in fact, not identical. Although Finney (1952) pointed out that no exact statistical test is

Table 5. Phytotoxicity of 2,4-D solutions against Lemna minor as influenced by added octoxyethanol surfactants

2,4-D - Surfactant Combination	HLB No.[a] of Surfactant	LD_{50}[b] 2,4-D Solution	% Reduction in in LD_{50} due to added surfactants
2,4-D + 0.01% octoxyethanol EO_1	5.1	0.126	35
2,4-D + 0.01% octoxyethanol EO_5	12.5	0.169	28
2,4-D + 0.01% octoxyethanol EO_9	15.0	0.214	8
2,4-D + 0.02% octoxyethanol EO_1	5.1	0.086	63
2,4-D + 0.02% octoxyethanol EO_5	12.5	0.175	25
2,4-D + 0.02% octoxyethanol EO_9	15.0	0.190	18
2,4-D alone	---	0.233	0

[a]Hydrophile-lipophile balance numbers derived by the method of Griffin (1955).

[b]"Median Lethal Dose" or the concentration (parts/10,000) required to kill fifty per cent of the plants.

available for this type of "independent joint action", these results suggested that the phytotoxicities of these surfactants and of 2,4-D are more than additive, or in other words, that the surfactants had a synergistic or enhancing effect upon the action of 2,4-D.

The phytotoxicities of 2,4-D solutions, to Lemna minor, with various octoxyethanol surfactants added are presented in table 5. It is evident that the LD_{50} of a 2,4-D solution against Lemna minor was reduced by the addition of octoxyethanol surfactants to the solution. It is

further apparent from this data that the increased phytotoxicity of 2,4-D solutions which resulted from the addition of octoxyethanol surfactants was related to the hydrophile-lipophile balance of the surfactants added. This relationship, shown graphically in figure 12, suggested that, within a homologous series of surfactants, the surfactants with low HLB numbers were more effective in enhancing the phytotoxicity of 2,4-D solutions.

As a further test for synergistic effects of octoxyethanol surfactants on the phytocidal action of 2,4-D to Lemna minor, the phytotoxicity was determined for mixtures of these two chemicals combined in the proportions of 1:1, 7:2 and 10:1. The dosage-response curve for each of these mixtures and for octoxyethanol and 2,4-D alone are plotted in figure 13. Shown, also, are the dosage-response curves predicted for each of these mixtures, by application of the previously mentioned equation of Finney (1952). Since the levels of octoxyethanol present in the 1:1 and 7:2 mixtures were non-toxic when the surfactants were tested alone, it was concluded that the phytotoxicity of these mixtures was determined solely by the amount of 2,4-D present. The predicted dosage-curves for 1:1 and 7:2 mixtures based on the phytotoxicity of the 2,4-D present in the mixtures, were parallel to the dosage-response curve for 2,4-D alone. Octoxyethanol, when tested separately at the level present in the 10:1 mixture, produced phytocidal effects on Lemna minor; therefore, the dosage-response curve predicted for this mixture was determined by the combined mortalities due to both the octoxyethanol and 2,4-D. The slope of this 10:1 mixture is therefore slightly steeper than the dosage-response curve shown for the 2,4-D alone. Comparison of the

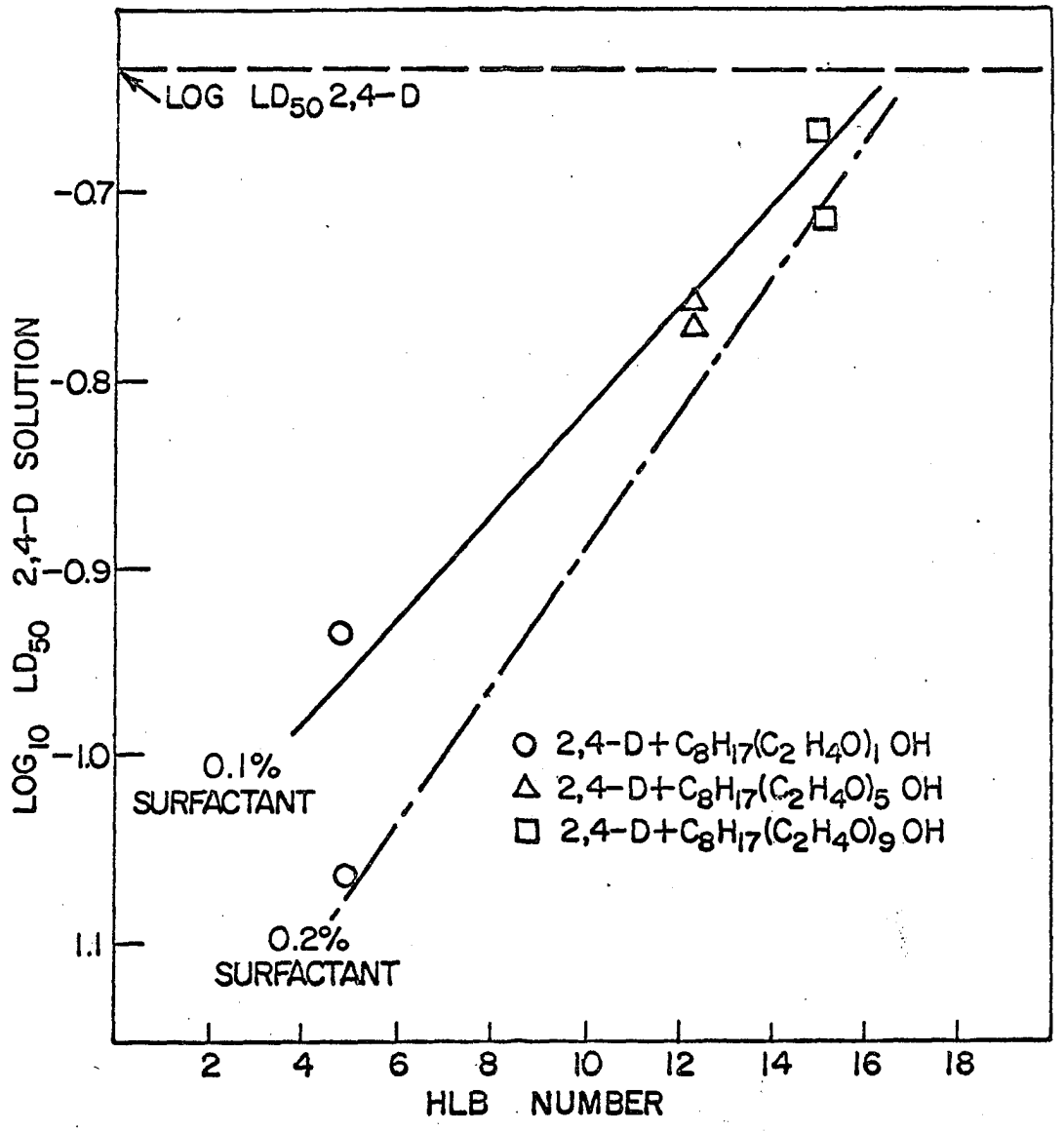

Fig. 12. The relationship of the phytoxicity (LD_{50}) against Lemna minor of 2,4-D solutions with added octoxyethanol surfactants to the hydrophile-lipophile balance of the surfactants

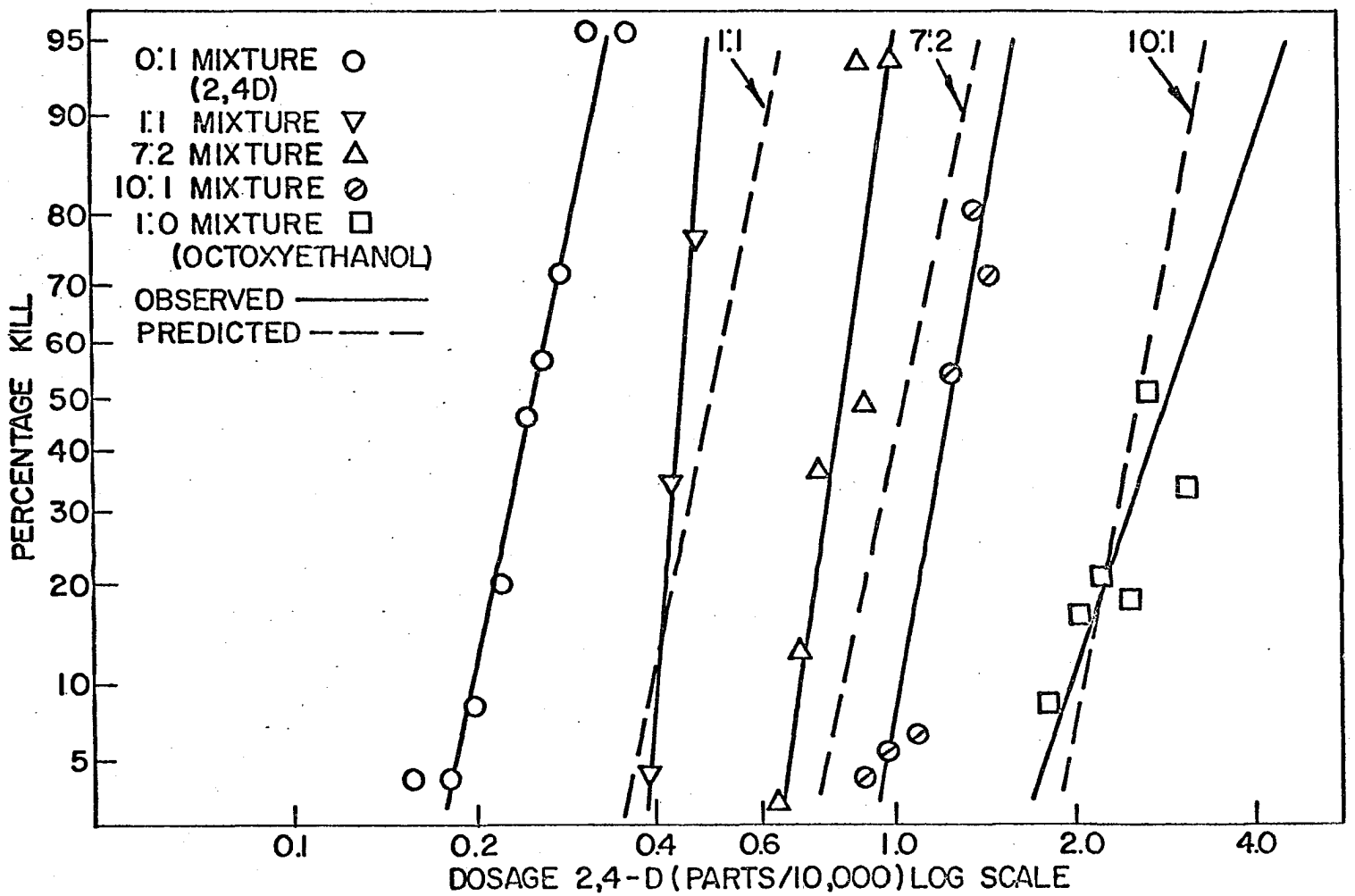

Fig. 13. Dosage-response curves for 2,4-D - octoxyethanol mixtures against Lemna minor

observed dosage-response curves, for each of these mixtures, with the predicted dosage-response curves showed that the phytotoxicity of each mixture was greater than expected on the basis of independent joint action. This was reflected, also, in the comparison of the observed LD_{50} with the predicted LD_{50} as tabulated in table 6, for each mixture. Again, as Finney (1952) pointed out, there is no exact statistical test available for this type of joint action; however, the wide departure of the observed dosage-response curves from the predicted dosage-response curves for these mixtures substantiates the previous results which showed that octoxyethanol surfactants synergized or enhanced the phytocidal action of 2,4-D against Lemna minor.

In another experiment, the effects of forty-eight hour pretreatments of the test plants with solutions of three different octoxyethanol surfactants on the phytotoxicity of subsequent 2,4-D treatments to Lemna minor were studied. Figure 14 shows the dosage-response curves plotted for 2,4-D against Lemna minor pretreated with one part per ten thousand solutions of one, five and nine mole ethylene oxide adducts of octoxyethanol. Similarly, figure 15 shows the dosage-response curves obtained for 2,4-D solutions against Lemna minor pretreated with two parts per ten thousand of these surfactants. Each figure shows the dosage-response curves obtained in these respective experiments for a 2,4-D solution against Lemna minor without a surfactant pretreatment. In these experiments there was a slight mortality among test plants pretreated with the surfactants but not subjected to a subsequent treatment with 2,4-D. Thus, the mortalities observed after subsequent

Table 6. Phytotoxicity of 2,4-D - octoxyethanol surfactant mixtures against Lemna minor

Octoxyethanol-2,4-D Mixture	Predicted LD_{50}[a]	Observed LD_{50}[a] and 95% fiducial limits	Observed LD_{50}[a] as a per cent of predicted LD_{50}[a]
1:1	0.49	0.43(0.42-0.45)	- 16
7:2	1.02	0.81(0.76-0.86)	- 21
10:1	2.51	1.24(1.13-1.37)	-102

[a]"Median Lethal Dose" or the concentration (parts/10,000) required to kill fifty percent of the plants.

2,4-D treatments were corrected for the effects of the surfactant pretreatments. Assuming no synergistic effects, the dosage-response curves obtained with the 2,4-D solutions after surfactant pretreatments would be expected to be identical to those obtained for 2,4-D treatments without surfactant pretreatments. Examination of figures 14 and 15 showed, however, distinct evidence for synergism. The slopes of the dosage-response curves for 2,4-D with a surfactant pretreatment were less than the slopes of the curves for 2,4-D without a surfactant pretreatment, with all surfactants used, except the nine mole ethylene oxide adduct of octoxyethanol. The LD_{50}'s tabulated in table 7, and corresponding to these dosage-response curves showed that these results differed from those obtained when the same surfactants were added directly to the 2,4-D solution. Pretreatment with octoxyethanols containing five or nine moles of ethylene oxide actually decreased the toxicity of 2,4-D against Lemna minor, although the reduction in the LD_{50} for 2,4-D was statistically significant only with the nine mole adduct.

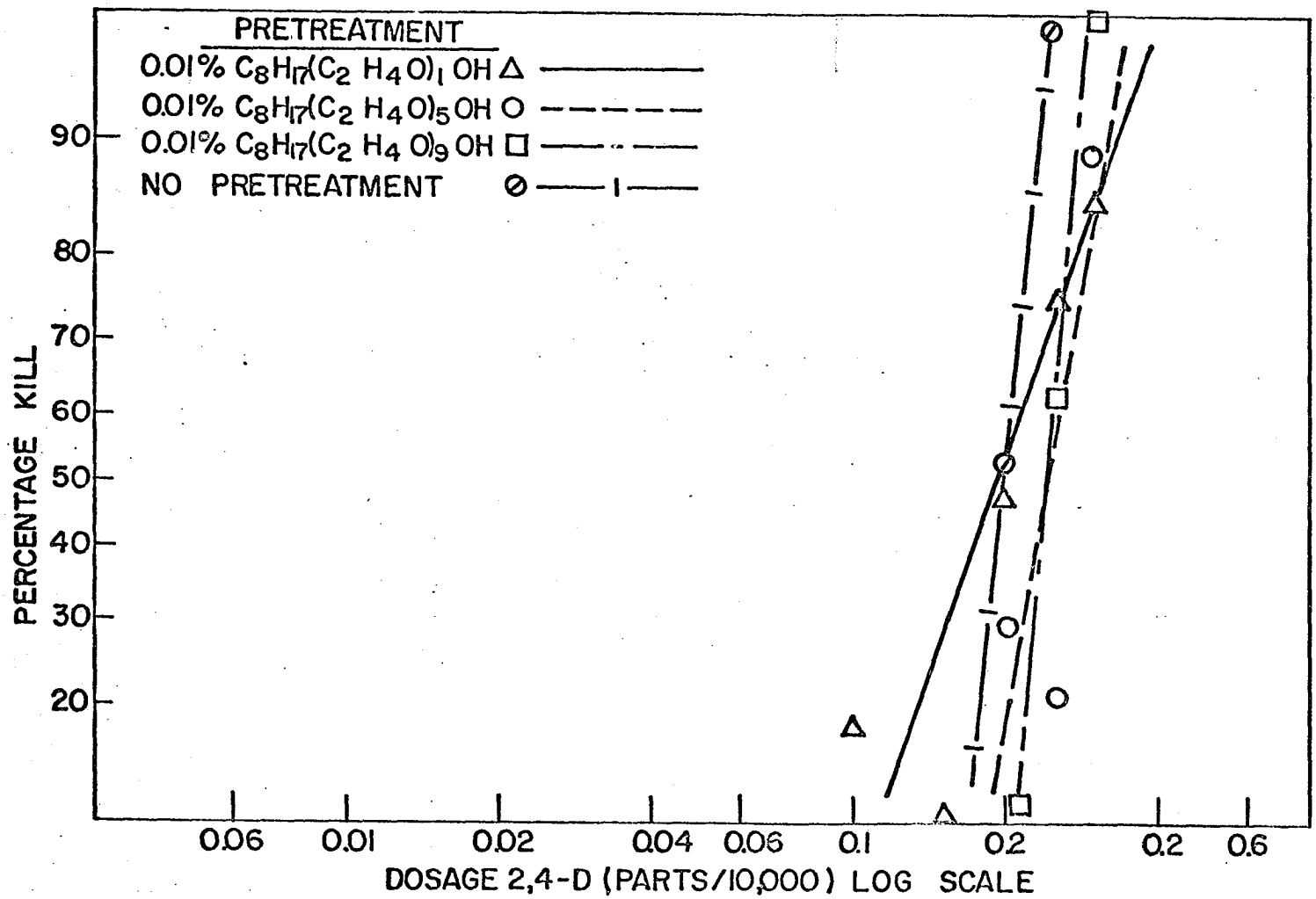

Fig. 14. Dosage-response curves for 2,4-D against Lemna minor pretreated with 0.01% octoxyethanol surfactants

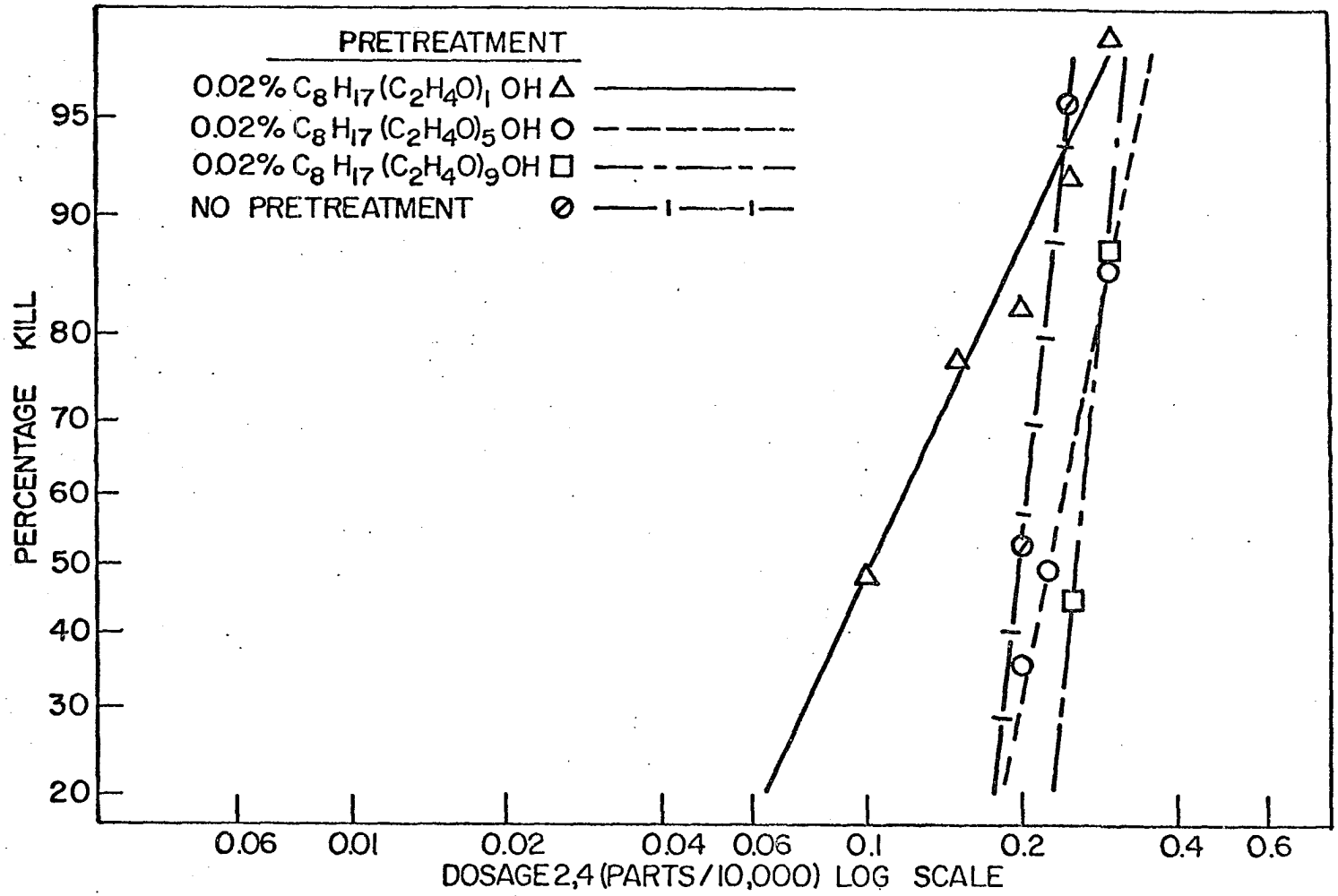

Fig. 15. Dosage-response curves for 2,4-D against Lemna minor pretreated with 0.02% octoxyethanol surfactants

Table 7. Phytotoxicity of 2,4-D solutions against Lemna minor as influenced by pretreatment of the test plants with octoxyethanol surfactants

Pretreatment	HLB No.[b] of surfactant	LD_{50}[c] 2,4-D solution and 95% fiducial limits	% Reduction in LD_{50} due to pretreatment
0.01% Octoxyethanol EO_1	5.1	0.196(0.122-0.277)	1
0.01% Octoxyethanol EO_5	12.5	Non-significant res.	--
0.01% Octoxyethanol EO_9	15.0	0.241(0.234-0.247)[a]	-22
0.02% Octoxyethanol EO_1	5.1	0.104(0.083-0.120)[a]	48
0.02% Octoxyethanol EO_5	12.5	0.234(0.198-0.261)	-18
0.02% Octoxyethanol EO_9	15.0	0.256(0.249-0.264)[a]	-29
No pretreatment (control)	--	0.198(0.176-0.215)	0

[a]Indicates significant difference from control at the 5% level of statistical probability.

[b]Hydrophile-lipophile balance numbers derived by the method of Griffin (1955).

[c]"Median Lethal Dose" or the concentration (parts/10,000) required to kill fifty per cent of the plants.

In contrast, octoxyethanol caused an increase in the LD_{50} of 2,4-D when used as a pretreatment. This increase was statistically significant when the test plants were pretreated with two parts per ten thousand of this surfactant but was not statistically significant when the test plants were pretreated with one part per ten thousand of this surfactant. These results revealed that surfactant pretreatment both synergized and antagonized the phytocidal action of 2,4-D depending upon the hydrophile-lipophile balance of the surfactant used. Pretreatment of Lemna minor

with octoxyethanols with low HLB numbers enhanced the phytotoxicity of subsequent 2,4-D treatments, while pretreatment with octoxyethanols possessing high HLB numbers reduced the phytotoxicity of 2,4-D to Lemna minor.

An additional experiment was conducted with this surfactant series to evaluate the effect of a recovery period, after surfactant pretreatment, upon the phytotoxicity of a subsequent 2,4-D treatment to Lemna minor. Figure 16 shows the dosage-response curves for 2,4-D against Lemna minor pretreated with a two parts per ten thousand solution of octoxyethanol followed by recovery periods of zero, twenty-four, forty-eight, and seventy-two hours. The LD_{50}'s extrapolated from these curves are listed in table 8. Statistically significant regressions were obtained for the data representing zero and forty-eight hour recovery periods. The slopes of these two curves differed significantly, showing that a recovery period reduced the slope of the dosage-response curve for the phytotoxicity of 2,4-D treatments preceeded by surfactant pretreatments. Statistically significant regressions were not obtained for the data representing twenty-four and seventy-two hour recovery periods. However, probit analysis of the data showed, by means of a "Chi square" test, that the dosage-response curves for the twenty-four, forty-eight and seventy-two hour recovery periods did not differ significantly from parallelism. It was thus shown that a recovery period following surfactant pretreatment altered the slope of the dosage-response curve for 2,4-D against Lemna minor; however, it was inferred that there was little additional change in the slope of the dosage-response curve resulting from an increase in the length of the

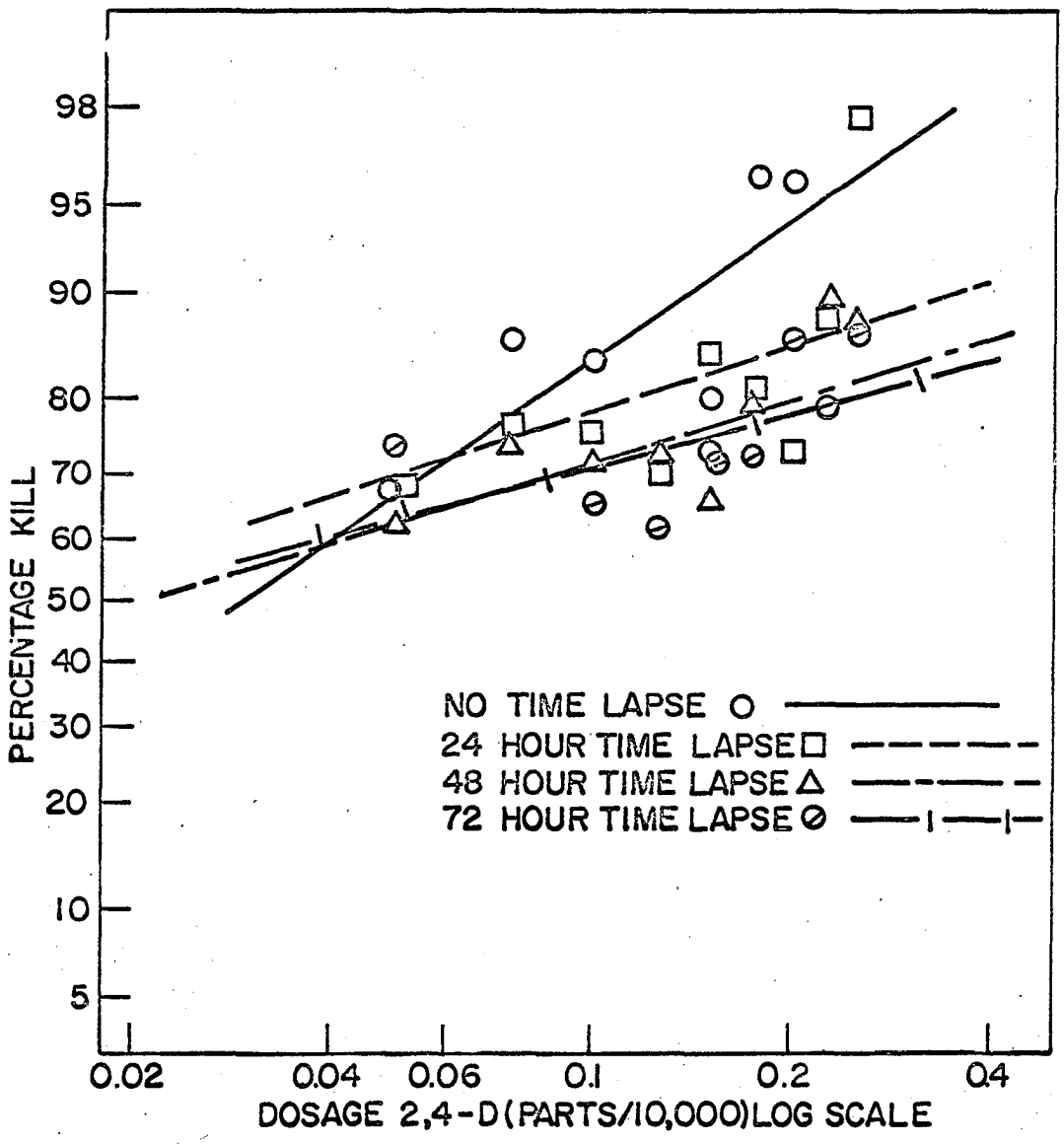

Fig. 16. Dosage-response curves for 2,4-D solutions against Lemna minor pretreated with octoxyethanol with various time lapses between pretreatment and 2,4-D treatment

Table 8. Phytotoxicity of 2,4-D solutions against Lemna minor after pretreatment with octoxyethanol with various time lapses between pretreatment and 2,4-D treatment

Time lapse in hours	LD_{50}[b] 95% fiducial limits	Slope of the dosage-response curve
0	$0.030(8.8\times10^{-5}-3.7\times10^{-2})$	1.93
24	0.009[a]	0.79[a]
48	$0.022(1.1\times10^{-3}-4.5\times10^{-2})$[c]	0.86
72	0.020[a]	0.79[a]

[a]Approximate values.

[b]"Median Lethal Dose" or the concentration (parts/10,000) required to kill fifty per cent of the plants.

[c]Indicates significant difference from control at the 5% level of statistical probability.

recovery period beyond twenty-four hours.

2,4-D - octylphenoxyethanols mixtures

The effects of octylphenoxyethanol surfactants on the phytotoxicity of 2,4-D solutions against Lemna minor were evaluated in the first of the experiments in this series. Figure 17 shows the dosage-response curves obtained for 2,4-D solutions against Lemna minor, with octylphenoxyethanols containing five, nine to ten and twelve to thirteen moles of ethylene oxide added to the solutions. Probit analysis of the data represented by these curves showed that the regression lines were not parallel. Hence, the dosage-response curves shown in figure 17 were obtained by separate analyses of the data for each treatment after

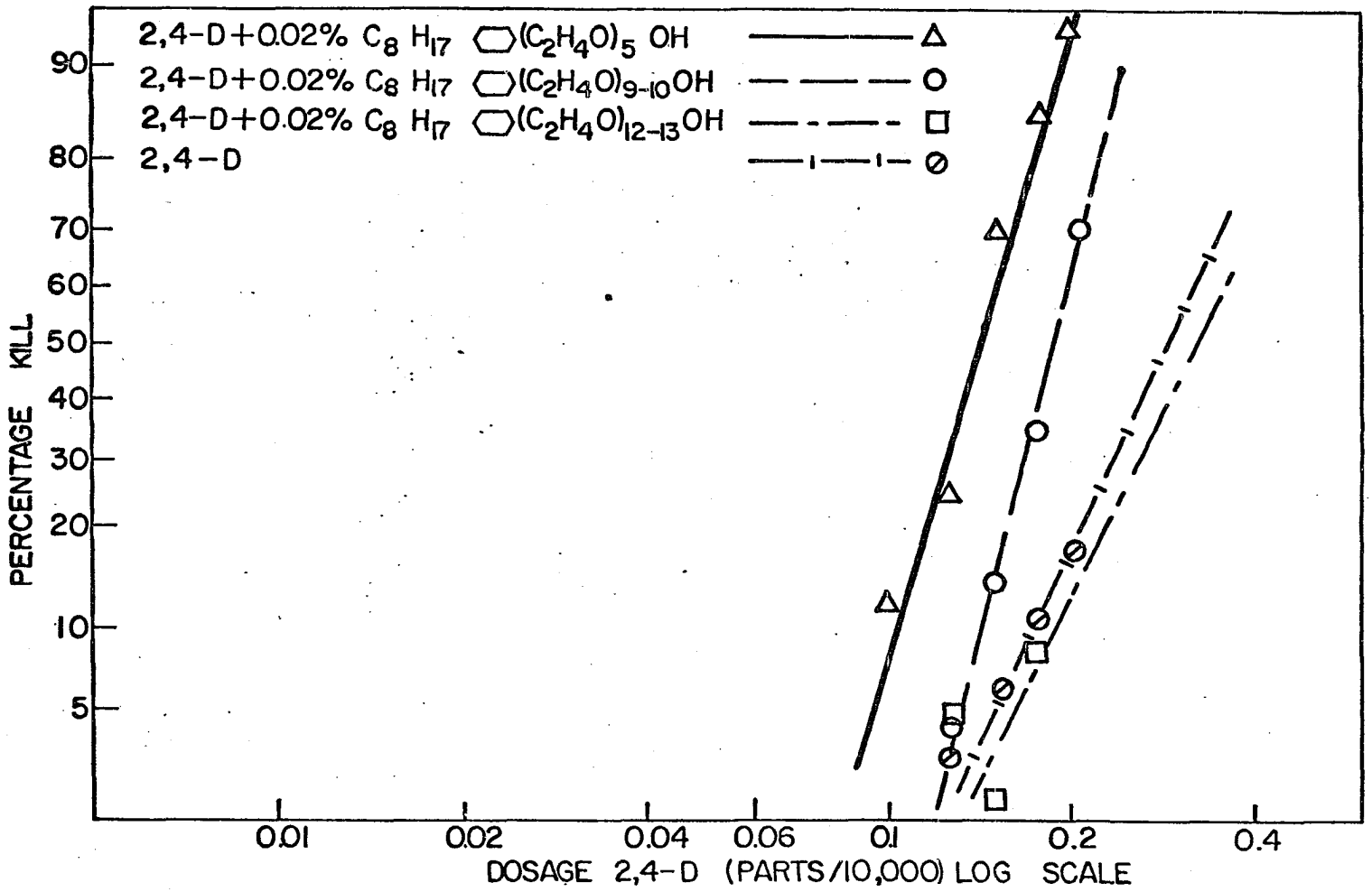

Fig. 17. Dosage-response curves for 2,4-D solutions against Lemna minor with 0.02% octylphenoxyethanol surfactants added

correction of the data for the independent phytocidal action of the surfactants.

Table 9 presents the LD_{50}'s of the 2,4-D solutions with each of the three octylphenoxyethanol surfactants added. The addition of octylphenoxyethanol surfactants enhanced the phytotoxicity of the 2,4-D solutions, as shown by the reduced values of the LD_{50}. The lower the HLB number of the added surfactant, the greater was the observed enhancement of the phytotoxicity of the 2,4-D solutions. This relationship is shown graphically in figure 18.

In a second experiment, the phytotoxicity of 2,4-D against Lemna minor was determined after a forty-eight hour pretreatment of the test plants in solutions of three octylphenoxyethanol surfactants. Figure 19 shows the dosage-response curves obtained for 2,4-D solutions against Lemna minor after the test plants were pretreated with octylphenoxyethanols containing five, nine to ten, and twelve to thirteen moles of ethylene oxide. The observed mortalities for 2,4-D treatments following these pretreatments were corrected, in the probit analysis of the data, for the mortalities observed among test plants subjected to pretreatment with these surfactants but not subsequently treated with 2,4-D. Consequently, it was expected that the observed dosage-response curves obtained for 2,4-D treatments following surfactant pretreatments would coincide with the dosage-response curves observed for 2,4-D treatments without surfactant pretreatments. However, the probit-response versus log-dose regression, computed for the data obtained in this experiment with plants treated with 2,4-D without surfactant pretreatment, was not statistically significant. Hence, the dosage-response curve in

Table 9. Phytotoxicity of 2,4-D solutions against Lemna minor as influenced by added octylphenoxyethanol surfactants

2,4-D - Surfactant mixture	HLB No.[b] of surfactant	LD_{50}[c] 2,4-D solution and 95% fiducial limits	% Reduction in LD_{50} due to added surfactant
2,4-D + 0.02% Octylphenoxyethanol EO_5	10.3	0.137(0.127-0.145)[d]	56
2,4-D + 0.02% Octylphenoxyethanol $EO_{9.7}$	13.4	0.184(0.177-195)[d]	40
2,4-D + 0.02% Octylphenoxyethanol $EO_{12.3}$	14.5	0.317[a]	- 5
2,4-D	---	0.308(0.272-0.379)	0.0

[a]Since the probit regression for this solution was not statistically significant the value listed here is an approximation.

[b]Hydrophile-lipophile balance numbers derived by the methods of Griffin (1955).

[c]"Median Lethal Dose" or the concentration (parts/10,000) required to kill fifty per cent of the plants.

[d]Indicates significant difference from control at the 5% level of statistical probability.

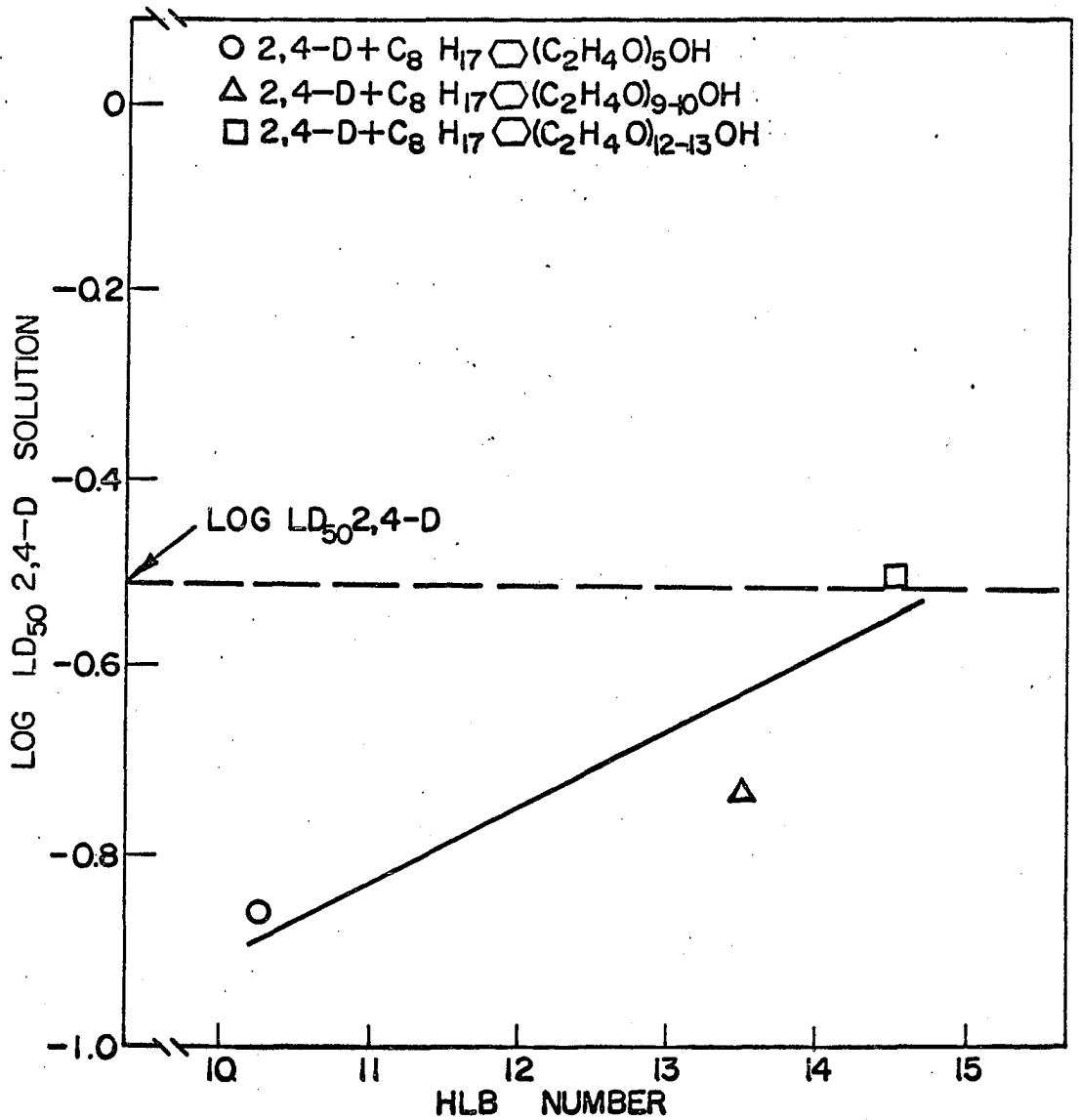

Fig. 18. The relationship of the phytotoxicity (LD_{50}) of 2,4-D solutions with added octoxyethanol surfactants to the Hydrophile-Lipophile Balance (HLB) of the surfactants

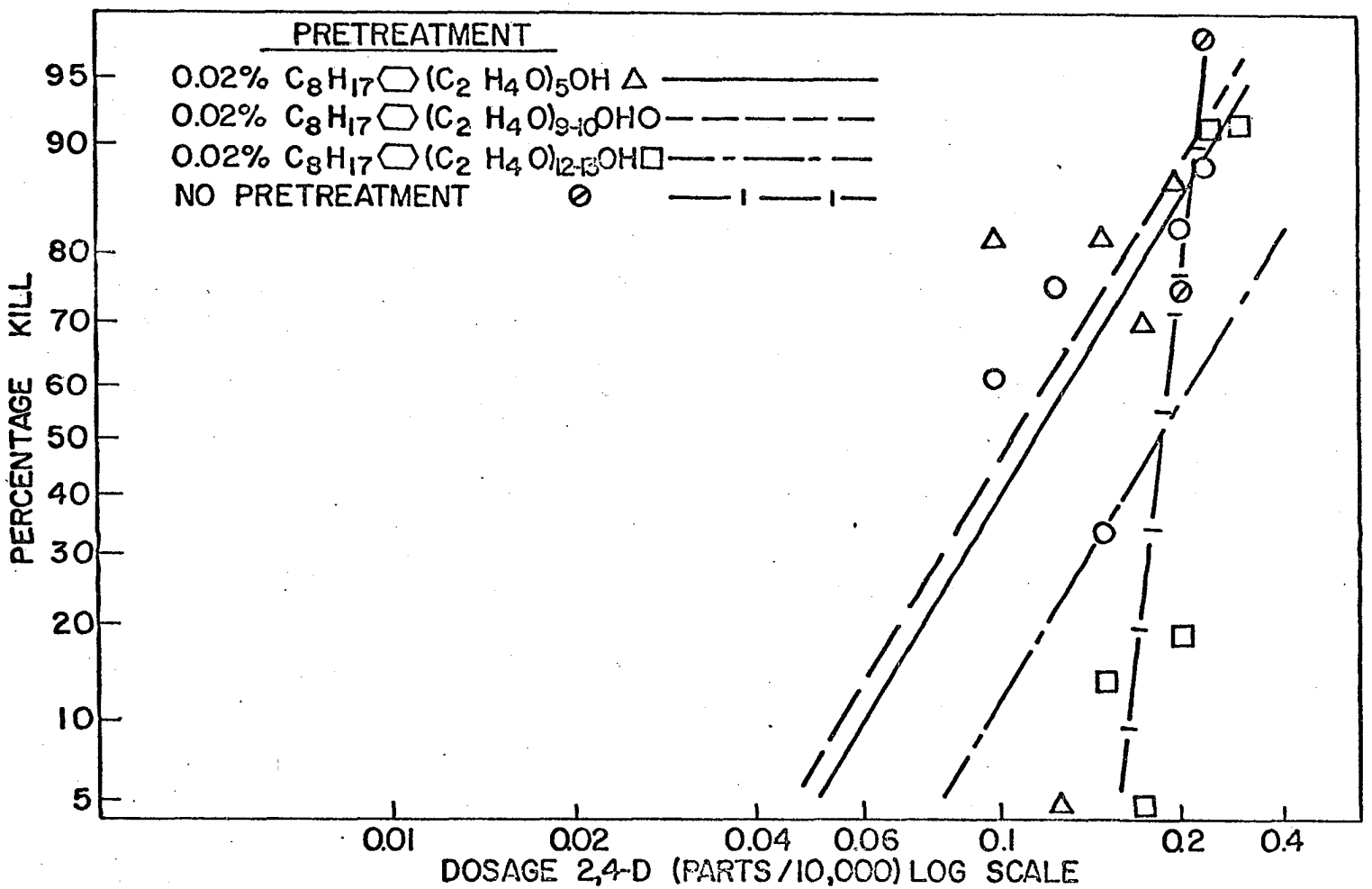

Fig. 19. Dosage-response curves for 2,4-D solutions against Lemna minor pretreated with 0.02% octylphenoxyethanol surfactants

figure 19, representing the phytotoxicity of 2,4-D without surfactant pretreatment is an approximation. However, simultaneous probit analysis of the 2,4-D data with and without pretreatment with octylphenoxyethanol surfactants showed that the dosage-response curve obtained for 2,4-D without pretreatment was not parallel to the dosage-response curves obtained for 2,4-D treatment following surfactant pretreatments. It was concluded from this that surfactant pretreatments altered the dosage-response curve of 2,4-D against Lemna minor, although a quantitative estimate of the change in slope could not be obtained with this data.

Since the probit-response versus log-dose regressions computed for 2,4-D solutions, subjected to pretreatment with octylphenoxyethanol surfactants, were parallel, the dosage-response curves for these treatments were fitted to the data by a common slope as shown in figure 19. The LD_{50} corresponding to each of these dosage-response curves is listed in table 10. Again, it should be pointed out that the LD_{50} listed for 2,4-D without pretreatment is an approximation since its the probit-response versus log-dose regression was not statistically significant.

Statistically significant differences were not observed between the LD_{50} for 2,4-D without pretreatment and the LD_{50} for 2,4-D treatment subjected to pretreatment with each of the octylphenoxyethanol surfactants. These data, however, did suggest that pretreatments with these surfactants enhanced the phytotoxicity of 2,4-D to Lemna minor. Further the data suggested that the enhancement of the phytotoxicity of 2,4-D by pretreatments with octylphenoxyethanol surfactants was related to the

Table 10. Phytoxicity of 2,4-D solutions against Lemna minor as influenced by pretreatment of the test plants with octylphenoxyethanol surfactants

Pretreatment	HLB No.[b] of surfactant	LD_{50}[c] 2,4D - solution	% Reduction in LD_{50} due to surfactant pretreatment
0.02% Octylphenoxyethanol (5 moles ethylene oxide)	10.3	0.115	40
0.02% Octylphenoxyethanol (9-10 moles ethylene oxide)	13.4	0.108	43
0.02% Octylphenoxyethanol (12-13 moles ethylene oxide)	14.5	0.186	2
No pretreatment	---	0.190[a]	0.0

[a]Since the probit regression for this solution was not statistically significant the value listed here is an approximation.

[b]Hydrophile-lipophile balance numbers derived by the methods of Griffin (1955).

[c]"Median Lethal Dose" or the concentration (parts/10,000) required to kill fifty per cent of the plants.

HLB balance of the surfactants.

Paraquat - octoxyethanols mixtures

The phytotoxicities of paraquat-surfactant combinations against Lemna minor were evaluated using the techniques described for 2,4-D - surfactant combinations. The phytocidal action of three nonionic surfactants combined with paraquat were tested separately within the same experiment. Each of these surfactants was combined with paraquat to permit the assessment of paraquat toxicity at several levels of surfactant dosage and, conversely, the assessment of surfactant toxicity at several levels of paraquat.

The effects of three octoxyethanol surfactants, containing, respectively, one, five and nine moles of ethylene oxide, on the phytotoxicity of paraquat against Lemna minor were evaluated. These surfactants at several concentrations were added to the paraquat solutions which varied in concentration from 5×10^{-5} to 15×10^{-5} parts per ten thousand. The phytotoxicities of paraquat solutions were evaluated at these same dosages without added surfactants. The slopes of probit-response versus log-dose regressions and the LD_{50}'s computed for these solutions are shown in tables 11, 12 and 13.

Each surfactant reduced markedly the phytotoxicity of paraquat solutions to Lemna minor. At low levels of surfactant, the phytotoxicity of paraquat at all levels tested, was reduced to zero; hence, it was impossible to compute probit-response versus log-dose regressions and construct dosage-response curves for these data. As the dosage of the surfactant was increased, the toxicity of the surfactant-paraquat

Table 11. Phytotoxicity against Lemna minor of paraquat solutions with various levels of octoxyethanol (EO_1) added

Level of octoxyethanol(EO_1) (parts/10,000)[1]	LD_{50}[c] and 95% fiducial limits	Slope of probit-response regression
0	7.39(5.57-8.92)	4.56
1.6	---[b]	0.00
1.8	---[b]	0.00
2.0	---[b]	0.00
2.2	---[b]	0.00
2.4	N.S.[a]	N.S.[a]
2.6	N.S.[a]	N.S.[a]
2.8	N.S.[a]	N.S.[a]
3.0	N.S.[a]	N.S.[a]
3.2	5.32(7.75-15.9)	2.64

[a]N.S. indicates that the probit-response versus log-dose regression was not statistically significant, therefore a slope or an LD_{50} was not obtainable.

[b]No plants were killed by this surfactant-paraquat combination.

[c]"Median Lethal Dose" or the concentration (parts/10,000) required to kill fifty per cent of the plants.

combinations also increased. These phytotoxic effects may have been due, in part, to the inherent phytotoxicity of the surfactants alone. After corrections were applied to the data for the mortality due to independent surfactant toxicity, no statistically significant probit-response versus log-dose regressions were obtained for most of the paraquat solutions. This is shown also by the slopes of the dosage-response versus log-dose

Table 12. Phytotoxicity against Lemna minor of paraquat solutions with various levels of octoxyethanol (EO_5) added

Level of octoxyethanol(EO_5) (parts/10,000)	LD_{50}[c] and 95% fiducial limits	Slope of probit-response regression
0	7.39(5.57-8.92)	4.56
6.5	---[a]	0
7.0	---[a]	0
7.5	---[a]	0
8.0	72.48(27.19-?)	2.25
8.5	N.S.[b]	N.S.[b]
9.0	N.S.[b]	N.S.[b]
9.5	N.S.[b]	N.S.[b]
10.0	N.S.[b]	N.S.[b]
10.5	N.S.[b]	N.S.[b]

[a]No plants were killed by this surfactant-paraquat combination.

[b]N.S. indicates that the probit-response versus log-dose regression was not statistically significant, therefore a slope or an LD_{50} was not obtainable.

[c]"Median Lethal Dose" or the concentration (parts/10,000) required to kill fifty per cent of the plants.

regressions and the LD_{50}'s presented in tables 11, 12 and 13. However, further examination of tables 14, 15 and 16 suggested that some additional toxicity, other than that attributable to the independent toxic action of the surfactants, was obtained with surfactant-paraquat treatments.

Table 13. Phytotoxicity against Lemna minor of paraquat solutions with various levels of octoxyethanol (EO_9) added

Level of octoxyethanol (EO_9) (parts/10,000)	LD_{50}[c] and 95% fiducial limits	Slope of probit-response regression
0	7.39(5.57-8.92)	4.56
35.0	---[b]	0
37.5	N.S.[a]	N.S.[a]
40.0	N.S.[a]	N.S.[a]
42.5	N.S.[a]	N.S.[a]
45.0	N.S.[a]	N.S.[a]
47.5	N.S.[a]	N.S.[a]
50.0	N.S.[a]	N.S.[a]
52.5	N.S.[a]	N.S.[a]
55.0	N.S.[a]	N.S.[a]

[a]N.S. indicates that the probit-response versus log-dose regression was not statistically significant, therefore a slope or an LD_{50} was not obtainable.

[b]No plants were killed by this surfactant-paraquat combination.

[c]"Median Lethal Dose" or the concentration (parts/10,000) required to kill fifty per cent of the plants.

The data from this experiment did not show whether the toxicity observed was due to the independent toxic action of the paraquat, the independent toxic action of the surfactant, the synergistic action of paraquat on surfactant toxicity or, conversely, the synergistic action of the surfactants on paraquat toxicity. Additional extensive investigations

Table 14. Phytotoxicity of paraquat-octoxyethanol(EO_1) combinations against Lemna minor after adjustment for the expected phytotoxicity of the surfactant alone. (Phytotoxicity expressed as percentage kill)

Paraquat concentration (parts/10,000)	Octoxyethanol(EO_1) dosage (parts/10,000)							
	0	1.6	1.8	2.0	2.4	2.6	2.8	3.0	3.2
5.0×10^{-5}		4.0	0.0	10.0	14.4	6.3	50.5	23.1	47.0
7.5×10^{-5}		0.0	2.0	1.0	1.6	19.5	63.6	71.4	65.4
10.0×10^{-5}		0.0	0.0	0.0	1.6	1.5	1.0	0.0	76.5
12.5×10^{-5}		0.0	0.0	0.0	3.8	62.9	53.9	--	--
15.0×10^{-5}		0.0	0.0	0.0	4.6	0.0	12.1	--	--

Table 15. Phytotoxicity of paraquat-octoxyethanol(EO_5) combinations against Lemna minor after adjustment for the expected phytotoxicity of the surfactant alone (Phytotoxicity expressed as percentage kill)

Paraquat dosage (parts/10,000	Octoxyethanol (EO_1) dosage (parts/10,000)									
	0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0	10.5
5.0×10^{-5}		0	1.2	8.5	13.6	17.6	15.0	26.9	44.0	62.8
7.5×10^{-5}		0	12.8	22.4	38.6	23.3	33.3	1.8	36.8	56.2
10.0×10^{-5}		0	1.4	22.4	29.8	43.4	34.0	14.3	3.3	99.9
12.5×10^{-5}		0	12.0	25.9	10.4	42.3	11.6	20.0	30.2	8.8
15.0×10^{-5}		0	12.5	9.5	27.9	25.8	29.1	28.3	6.8	54.4

Table 16. Phytotoxicity of paraquat-octoxyethanol(EO_9) combinations against Lemna minor after adjustment for the expected phytotoxicity of the surfactant alone. (Phytotoxicity expressed as percentage kill)

Paraquat dosage (parts/10,000)	Octoxyethanol(EO_9) dosage (parts/10,000)									
	0	35.0	37.5	40.0	42.5	45.0	47.5	50.0	52.5	55.0
5.0×10^{-5}		0	0	0	0	5.5	11.8	7.9	3.8	1.8
7.5×10^{-5}		0	3.0	5.0	1.6	1.5	15.2	0	10.8	0.6
10.0×10^{-5}		0	0	5.0	3.6	3.4	4.9	17.6	16.3	6.7
12.5×10^{-5}		0	3.0	8.0	3.6	13.8	4.9	11.6	35.9	28.6
15.0×10^{-5}		0	4.0	0	5.8	5.3	8.5	24.1	26.4	--

will be required to resolve whether the reduction in the phytotoxicity of paraquat solutions by the addition of surfactants was the result of changes in the physiochemical properties of the paraquat solutions or whether, in fact, the surfactants antagonized the phytocidal action of paraquat.

The effects of paraquat on the phytotoxicity, against Lemna minor, of octoxyethanols containing one, five and nine moles of ethylene oxide were evaluated in this same experiment. Tables 17, 18 and 19 present the LD_{50}'s obtained for solutions of these surfactants with paraquat added at several dosages. Relative potencies are presented also in tables 18 and 19 for the five and nine mole adducts of octoxyethanol with various levels of paraquat added to the solutions. Since the probit-response versus log-dose regression for octoxyethanol solutions with paraquat added did not meet the test of parallelism, relative potencies were not computed for these solutions. Dosage-response curves were not shown for these data, since they were quantitatively very similar, closely aligned and presented a confusing picture when plotted graphically.

The results presented in tables 17, 18 and 19 show that the phytotoxicity of these surfactants was reduced by the addition of paraquat to their solutions. Although the reduction of the phytotoxicity of octoxyethanol solutions by paraquat was not statistically significant, the data does suggest that such reduction occurred. With the five and nine mole adducts of octoxyethanol, the reduction of phytotoxicity by addition of paraquat to the solutions was significant with most concentrations.

Table 17. Phytotoxicity against Lemna minor of octoxyethanol solutions with various level of paraquat added

Paraquat dosage (parts/10,000)	LD_{50}[b] and 95% fiducial limits	% Reduction in LD_{50} due to paraquat
0	3.02(2.72-5.59)	0.0
5×10^{-5}	2.77(2.55-3.12)	8.8
7.5×10^{-5}	2.69(2.52-2.89)	10.9
10×10^{-5}	2.92(2.76-3.20)	3.3
12.5×10^{-5}	N.S.[a]	-
15×10^{-5}	2.90(2.74-3.45)	4.0

[a]N.S. indicates that the probit-response versus log-dose regression was not statistically significant.

[b]"Median Lethal Dose" or the concentration (parts/10,000) required to kill fifty per cent of the plants.

Table 18. Phytotoxicity against Lemna minor of solutions of a fire mole ethylene oxide adduct of octoxyethanol with various levels of paraquat added

Paraquat dosage (parts/10,000)	LD_{50}[b] and 95% fiducial limits	Relative[c] Potency	% Reduction in LD_{50} due to paraquat
0	11.57(10.89-13.31)	1.00	0.0
5×10^{-5}	10.78(10.36-11.58)	0.93	6.8
7.5×10^{-5}	10.71(10.36-11.29)	0.94	7.4
10×10^{-5}	10.70(10.28-10.37)	0.90[a]	7.5
12.5×10^{-5}	10.30(10.02-10.73)	0.90[a]	11.0
15.0×10^{-5}	10.55(9.58-15.76)	0.88[a]	8.7

[a]Indicates a value significantly different from that for the control at the 95% level.

[b]"Median Lethal Dose" or the concentration (parts/10,000) required to kill fifty per cent of the test plants.

[c]Degree to which the LD_{50} of the most toxic member of the series has to be multiplied in order to obtain the equitoxic concentration for another particular member of the series.

Table 19. Phytotoxicity against Lemna minor of solutions of a nine mole ethylene oxide adduct of octoxyethanol with various levels of paraquat added

Paraquat dosage (parts/10,000)	LD_{50}[b] and 95% fiducial limits	Relative[c] Potency	% Reduction in LD_{50} due to paraquat
0	49.71(48.40-51.05)	1.00	0
5.0×10^{-5}	46.72(45.79-47.66)[a]	0.94[a]	6.0
7.5×10^{-5}	45.65(43.35-48.30)[a]	0.92[a]	8.2
10.0×10^{-5}	45.60(42.71-49.02)	0.92[a]	8.3
12.5×10^{-5}	46.52(44.33-49.28)	0.93[a]	6.4
15.0×10^{-5}	46.16(45.06-47.30)[a]	0.93[a]	7.1

[a]Indicates a value significantly different from the control at the 95% level of statistical probability.

[b]"Median Lethal Dose" or the concentration (parts/10,000) required to kill fifty per cent of the plants.

[c]Degree to which the LD_{50} of the most toxic member of the series has to be multiplied in order to obtain the equitoxic concentration for another particular member of the series.

DISCUSSION

The patterns of toxicity to Lemna minor, an aquatic plant, observed with both surfactants and surfactant-herbicide combinations were in general agreement with those obtained in other investigations where surfactants and surfactant-herbicide mixtures were applied as foliar sprays to terrestrial plants. These findings demonstrate the same relationship of observed surfactant phytotoxicity to HLB, and lend further support to the concept that cell membranes are a probable major site of surfactant action in the plant.

The investigation was concerned primarily with an evaluation of surfactant toxicity and the enhancement of the action of herbicides on an aquatic plant under conditions where the contact between plant cells and surfactant were relatively constant over the test periods and where considerations of cuticle and the drying effects of the environment were minimized or eliminated.

That surfactants may indeed by phytotoxic entities is well substantiated. The work of investigators such as Furmidge (1959b) and Jansen et al. (1961) suggested that the chemical structure of surfactant molecules is a strong determinant of their phytocidal activity. This suggestion was amplified by the work of Buchanan (1965) which "...showed among surfactants a probable relationship between hydrophilic and hydrophobic properties and toxicity to plant tissues...". None of these investigators, however, have offered a physicochemical hypothesis relating surfactant phytotoxicity to HLB of the surfactants. The present study was concerned, in part, with a further examination of this

relationship between surfactant phytotoxicity and HLB.

In this study strong correlations were observed, within homologous series of surfactants, between phytotoxicity against Lemna minor and HLB. The physicochemical basis for this observed relationship is apparent upon consideration of theories advanced in the field of pharmacology to explain the physiological action of drugs. Apparently both the physical and chemical properties of molecules play a role in the biological activity of such molecules. The physical properties of the molecules are thought to influence their ability to permeate into and accumulate within cells, while the specific biological effects are induced by the chemical reactivity of the molecules. The ability of surfactants to permeate plant cells may therefore be attributed largely to properties of the surfactants which are related to their HLB.

The role of physical phenomena in determining the toxicity of drugs was pointed out by the "Overton-Meyer lipoid theory". This theory was based on the strong correlations which were observed between the lipid solubility of sustances and their biological activity. These correlations, in the essence of this theory, were taken to mean that the toxicity of a substance is related to its ability to penetrate the lipoid barriers of cells. Differing isotoxic concentrations, measured in the external phase, for various chemical substances are, according to this theory, determined by the cell lipoid/external phase distribution coefficient. Certain oils have been suggested to have properties similar to those present in cellular membranes; hence, distribution coefficients determined for chemical substances between water and such oils have been closely correlated with the isotoxic concentrations of

these chemical substances. Toxicity was thus shown to be closely correlated with oil/water distribution coefficients; the larger the distribution coefficient, the greater is the ability of the chemical to penetrate cell membranes and, consequently, the greater its toxicity.

That the observed relationship of the phytotoxicity of surfactants to HLB is explainable in terms of the Overton-Meyer theory was suggested by the findings of Davies (1957) which showed that the HLB of a series of alcohols was linearly related to the olive oil/water distribution coefficients of these alcohols. Hence, it would be expected that the phytotoxicity of these alcohols would be related to their distributions in oil/water systems. The results of the present study showed that the phytotoxicity, to Lemna minor, of the n-alkyl alcohols tested was related linearly to their olive oil/water distribution coefficients. A linear relationship was observed also between the phytotoxicity, to Lemna minor, of octylphenoxyethanol surfactants and their distribution coefficients in an isooctane/water system. Since distribution coefficients were not available for the octoxyethanol surfactants, the relationship of the phytotoxicity of these surfactants to their distribution coefficients was not examined. However, the results obtained with the n-alkyl alcohols and octylphenoxyethanols suggested that the observed relationship between the phytotoxicity of surfactants and their HLB may be explained by the Overton-Meyer theory. The lipoid solubility of a surfactant is a function of its HLB; consequently, if the Overton-Meyer theory is valid, the rate of penetration of the surfactant into plant cells should be a function of its HLB. For surfactants having similar chemical reactivity, phytotoxicity should be a function of the

rate of penetration into the cell and, therefore, should be also a function of HLB.

In addition to HLB numbers and distribution coefficients, Hansch et al. (1965) have derived another physical constant of chemical molecules which may further be used to elucidate the role of phase distribution effects upon the phytocidal action of surfactants. They have related the biological activity of series of chemical compounds to a substituent constant π, which is defined as $\pi = \log(P_X/P_H)$ where P_H is the octanol/water partition coefficient of a parent compound and P_X that of a derivative. Hansch et al. (1965) generalized that the biological activity of chemicals can be related to this constant by the following equation.

$$\log BR = \log 1/C = -k\pi^2 + k'\pi + k''$$

In this equation, BR is the biological response, C is the molar concentration of drug producing an equivalent biological response, and k, k' and k'' are constants. As the basis of their hypothesis, Hansch and the others present the following argument.

> "...assume the partition coefficient (fat:water) of the lowest member of a congeneric series of drugs is zero. In this situation the drug will not be able to move through a lipid barrier and will be restricted to the aqueous phase. Assume the partition coefficient is gradually increased with each successive member to infinity. This last member will be localized in the first lipophilic area with which it comes in contact. Neither of these derivatives at the extremes will be able to find the sites of action which are dispersed throughout a multicompartment cell or organism, and hence will have no biological activity.
>
> Some derivative between the first and the last member will have an ideal partition coefficient, P_o, such that this member can achieve the maximum concentration at the sites of action with a minimum change in free energy. This drug will produce the maximum response."

The phytotoxicity of n-alkyl alcohols to Lemna minor was related to

Hansch's π constant. As Hansch pointed out, the occurence of a linear increase in toxicity upon ascending a homologous series is a special case of the more general, quadratic relationship described previously. When only a few members of the series are examined a linear increase in biological response may occur upon ascending a homologous series. When, however, a sufficient number of the members of the series are examined, a quadratic relationship is observed. This probably explains the observed linearity of the relationship of the phytotoxicity, to Lemna minor, of n-alkyl alcohols to Hansch's π constant, π constants were not available for the octoxyethanol and octylphenoxyethanol surfactants tested in this study, and, consequently, the relationships of the phytotoxicities of these surfactants to their π constants were not assessed. Nevertheless, the observed results with the n-alkyl alcohols suggested that HLB was related to Hansch's π constant.

Analysis of the observed relationship between surfactant phytotoxicity and HLB in terms of Hansch's hypothesis suggests that the exponential relationship observed between phytotoxicity and HLB, within homologous series of nonionic, ethylene oxide-ether surfactants, may be explained on the basis of the relative lipid solubilities of the members of the series. Upon ascending these series lipid solubility decreased and HLB increased. Members of the series with low EO content were quite lipid-soluble and had low HLB numbers. These members partitioned easily into the lipid membranes of cells of the test plants and thus penetrated into the cells of these plants. As the EO content of the hydrophilic portion of these molecules was increased, the lipid solubility decreased and HLB increased. Consequently, as the series was

ascended, members of the series were encountered whose lipid solubility was so slight that there was little partitioning of the surfactants into the cell membranes and the surfactants remained in the aqueous phase surrounding the test plants. In contrast, additions to the hydrophobes, eg. lengthening an alkyl chain, could be expected to increase the lipid-solubility and decrease the HLB of these surfactants, as was observed with the n-alkyl alcohols. Such changes in the hydrophobic portion of surfactant molecules could be expected to increase the partitioning of the surfactants into the lipid membranes of the cells, and thus increase the rate of penetration of these surfactants into cells of test plants. Increases in the lipid solubility beyond a certain point will, however, reduce the ability of the surfactants to partition into cellular membranes since the surfactants will become completely absorbed by the lipids of these membranes. This, then, suggests an optimum HLB number in relation to maximum permeation of plant cells by surfactants.

Although the phytotoxicity of surfactants within homologous series of surfactants can be shown to be related to HLB, a generalization of this relationship extended to include diverse types of surfactants was not feasible. As stated previously, the toxicity of chemical molecules is determined by their chemical reactivities as well as by physical distribution phenomena. Therefore, two surfactants having identical HLB but differing in chemical composition would be expected to differ in their relative toxicities, although the factors which determine the HLB of the surfactants also play a significant role in determining observed phytotoxicity. It can be further shown that, in general, surfactants having similar chemical composition produce parallel dosage-response

curves while those differing in chemical composition produce dosage-response curves which are not parallel. The relative potency of surfactants, produce parallel dosage-response curves, is the same at all levels of kill. The relative potency of surfactants, producing dosage-response curves with differing slopes, is a function of dosage. For example, comparisons of two surfactants producing intersecting dosage-response curves, would be expected to show that at low surfactant concentrations one of the surfactants was more toxic than the other, whereas at higher surfactant concentrations the reverse would be true with the second surfactant then being more toxic than the first. Hence, the relationship of surfactant phytotoxicity to HLB is valid, quantitatively, only within series of surfactants producing parallel dosage-response curves.

These results confirm, in part, the findings of Jansen (1964) who showed that variations in both the hydrophilic and hydrophobic portions of the surfactant molecule were associated with observed differences in the phytotoxicity of surfactant-herbicide mixtures; but seemingly refute his conclusion that "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". The results of the present study show a distinct relationship between the enhancement of the phytotoxicity of 2,4-D against Lemna minor and the relative toxicity of the surfactants tested. This relationship was however restricted to homologous series, since chemical as well as physical properties are associated with surfactant phytotoxicity. Jansen (1964), in referring to a second property of surfactants which Griffin (1955) called "chemical type" has suggested

that this property "...is probably more important to herbicide activity and surfactant toxicity than HLB itself...". Nevertheless the importance of HLB in the enhancement of herbicide activity cannot be discounted in considerations of enhancement by homologous series of surfactants.

Explanation of the enhancement of herbicides by surfactants may be attributable to a disruption of the molecular organization of cell membranes by surfactants and consequent alteration of the permeability of these membranes to herbicides. The more phytotoxic the surfactant, the greater would be the expected cellular disorganization and alteration of cell permeability. Moreover, since surfactant phytotoxicity is considered largely a function of HLB, the changes in membrane permeability could also be expected to be a function of HLB, within the limits of the previously discussed premise regarding the relationship of surfactant toxicity to HLB. This hypothesis is consistent with the findings of Buchanan (1965), whose data suggested a relationship between HLB and the effects of surfactants in modifying cell permeability of beet slice tissue.

Mechanisms other than alteration of membrane permeability may be important, too, in the enhancement of herbicides by inherent phytotoxicity of surfactants. Surfactants may inactivate enzymes responsible for the degradation of certain herbicides within plant cells, thus increasing the effective concentation of herbicide within the cells. Surfactants may disrupt the lipid structure of plant cells, and thus modify the complexing and storage of herbicides in the lipid components of cells with a consequent change in the availability of herbicides to receptor sites.

This study demonstrated further the important role of HLB both in the expression of inherent surfactant phytotoxicity and in the enhancement of herbicide action by surfactants. While the exact mechanism remains unexplained, the results observed with this present bioassay system lend further support to the concept that disruption of cellular organization is a probable common denominator of surfactant action on plants cells. Future productive research must include investigation of surfactant effects at the cellular level.

SUMMARY

The phytotoxicities of selected surfactants and surfactant-herbicide combinations were studied by evaluation of the phytocidal action of these materials against the duckweed, Lemna minor L. Dosage-response relationships were established, by the methods of "Probit Analysis", for the phytotoxicities of n-alkyl alcohols, ethoxylated octyl alcohol, and ethoxylated octyl-phenol. Similar dosage-response relationships were established for combinations of the ethoxylated octyl alcohol and ethoxylated octyl phenol surfactants with 2,4-D and paraquat.

Dosage-response curves derived for homologous series of ethoxylated, octyl alcohol and octyl phenol, nonionic surfactants showed that the "relative potency" of the surfactants within these series was correlated with the "Hydrophile-lipophile Balance" (HLB) of the surfactants. It was shown how this observed relationship was the result of phase distribution phenomena which may be explained by the "Overton-Meyer Lipoid Theory" which hypothesizes that the more lipid-soluble a chemical molecule is, the more readily it will penetrate cell membranes, and, consequently, the more toxic it will be. Reasons are given as to why this phytotoxicity - HLB relationship does not hold for diverse types of surfactants.

Dosage-response curves derived for 2,4-D solutions, with members of homologous series of ethoxylated octyl-alcohol and ethoxylated octyl-phenol surfactants added to the solutions, showed that the phytotoxicity of these solutions was enhanced by these surfactants. Moreover, it was observed that the enhancement of 2,4-D by the members within homologous

series of these surfactants was related to the HLB of the surfactants. Since the inherent phytotoxicity of surfactants was shown to be a function of HLB, it was concluded that the relationship of herbicide enhancement to HLB may be attributable to the ability of these surfactants to disrupt the molecular organization of cell membranes, thereby altering the permeability of these membranes to herbicides.

Pretreatment of Lemna minor with ethoxylated octyl-alcohol and ethoxylated octyl-phenol surfactants showed that such pretreatment increased the phytotoxicity of subsequent 2,4-D treatments. Again, this observed increase in the phytotoxicity of 2,4-D was correlated with HLB, when pretreatments by the members of homologous series of surfactants were considered. A recovery period between surfactant pretreatment and 2,4-D reduced the slope of the dosage-response curve for 2,4-D but did not significantly alter the LD_{50}.

Assessment of the phytotoxicity of paraquat-surfactant combinations showed that the phytotoxicity of paraquat was markedly reduced by low concentrations of ethoxylated octyl-alcohol surfactants. However, as surfactant concentration was increased the phytotoxicity of the paraquat-surfactant combinations increased. Much of the increase in toxicity was due to the inherent phytotoxicity of the surfactants, but some toxicity remained after that attributable to the surfactant alone was accounted for. However, it was not ascertained whether the remaining toxicity was attributable to the individual constituents or to the joint action of the paraquat-surfactant combinations. On the other hand, it was shown that paraquat enhanced the phytotoxicity of these surfactants.

This study suggests that inherent surfactant phytotoxicity is a

major factor governing herbicidal enhancement by surfactants. Since this inherent surfactant phytotoxicity is related to the relative hydrophilic and lipophilic properties of the surfactants, a logical explanation is offered for the observed relationship of herbicide enhancement to surfactant HLB. Alteration of celluar organization and membrane permeability are probable consequences of surfactant phytotoxicity related to herbicide enhancement.

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