THE EFFECT OF ADJUVANTS UPON PESTICIDE UPTAKE AND AND PENETRATION OF FOLIAGE

A dissertation submitted to the

COUNCIL FOR NATIONAL ACADEMIC AWARDS

in partial fulfilment of the requirements for the

DEGREE OF DOCTOR OR PHILOSOPHY

BY

ANTHONY WILLIAM McCANN

June 1982

IN COLLABORATION WITH SHELL RESEARCH LIMITED

SITTINGBOURNE RESEARCH CENTRE, SITTINGBOURNE

AND

DEPARTMENT OF CHEMISTRY AND BIOCHEMISTRY

LIVERPOOL POLYTECHNIC

LIVERPOOL JOHN MOORES UNIVERSITY

The effect of adjuvants upon pesticide uptake and penetration of foliage
A.W. McCann
PhD Thesis, Liverpool John Moores University, 1982

Items to be redacted:

Between pages 60-61 Fig. 3

Between pages 61-62 Fig. 4

Between pages 62-63 Fig. 5

DECLARATION

The work described in this dissertation was carried out by the author at Liverpool Polytechnic, Dept., of Chem. and Biochem., and at Shell Research Limited, Sittingbourne Biosciences Laboratories, between October, 1977 and September, 1980, and represents the original work of the author, except where indicated by conventional references. It has not been submitted in whole, or in part for a degree or other qualification at another university.

Signature Anthony W Hillman

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my supervisors, Dr. R.J. Hamilton and Dr. P.A. Sewall at Liverpool Polytechnic, and Dr. G.T. Merrall, Shell Research Ltd., for their guidance and encouragement.

Thanks are also due to the technical staff of Liverpool Polytechnic, Chemistry Laboratories, to Mr. J.A. Brammer and Mr. P.A. Abery of Shell Research Ltd., and to Linda for typing a difficult manuscript.

I acknowledge financial support from the Science Research Council and from Shell Research Limited.

Finally, I would like to thank my wife for her patience and understanding at all times.

DEDICATION

This work is dedicated to the memory of my father,

William James McCann

ABSTRACT

The foliar uptake by wheat of the wild oat herbicide flamprop-methyl has been studied following application of ul droplets of an aqueous solution. The results show that over the time required for the drop to dry the compound does not partition into the surface waxes and crystallises as an external deposit. This deposit is stable to volatilization and photochemical and biochemical degradation, and is depleted by foliar penetration. Adsorbtion by the leaf surface wax is initially rapid and thereafter occurs at a relatively constant rate until the almost complete exhaustion of the surface deposit. Penetration of the epicuticular wax layer generally proceeds at a slower rate than does adsorbtion of the surface deposit and the herbicide is accumulated in the wax layer. The compound is lost from the epicuticular waxes only slowly to the leaf tissues. Movement within the leaf tissues away from the area of uptake is predominantly acropetal to the leaf tip and it is presumed that the compound moves with the transpiration stream.

A similar fate befalls the compound when aqueous solutions of flamprop-methyl are applied as ul droplets to barley or wild oat: no evidence of selectivity between wheat, barley and wild oat as a consequence of preferential uptake was found.

Foliar uptake of flamprop-methyl is shown to depend on the amount of compound applied to unit area of leaf surface and to increase as the amount applied is increased or as the area to which application is made is increased. These trends are discussed in terms of compartmental model of foliar uptake. Foliar uptake of flamprop-methyl is also shown to be slightly influenced by environmental factors; in an

environment in which maximum daily temperatures exceeded 35°C penetration was especially rapid and tended to completion within 24 hours.

The surface properties of aqueous solutions of selected polyoxyethylene non-ionic surfactants have been studied prior to investigating the effect of these compounds on the foliar uptake by cereals of flamprop-methyl. Two types of non-ionic surfactant were included in this study, the alkylphenol ethoxylates as exemplified by the Triton X products (ex Rohn and Haas) and alcohol ethoxylates as exemplified by the Brij products (ex Atlas Chemical Industries).

Products from both sources were analysed using instrumental and separational methods; the results of these analyses supported the manufacturers description of the compounds.

Surface and interfacial tensions of aqueous solutions of the surfactants were determined at various concentrations. Maximum reduction of surface tension in the alkylphenol ethoxylate solutions was found with Triton X-35 and Triton X-45. Maximum reduction of surface tension in the alcohol ethoxylate solutions was found with the tetraoxyethylene dodecyl ether (Brij 30) and was reduced with increasing oxyethylene content with any one hydrophobe. At constant oxyethylene content it was shown that surface activity was dependent on the nature of the hydrophobe. Critical micelle concentrations were determined from plots of surface tension vs concentration for alkylphenol and alcohol ethoxylates.

The spreading and wetting properties of aqueous solutions of alkylphenol ethoxylates were investigated by the Draves test and in terms of calculated spreading coefficients.

The foliar uptake by wheat of ethoxylated non-ionic surface active agents has been studied following topical application of aqueous solutions. The permeability of the cereal leaf to these compounds has been demonstrated: the rate of uptake was shown to depend on the mean oxyethylene content of the surfactant, decreasing as the oxyethylene content was increased within the Triton X series of compounds. In qualitative terms the trend in uptake was paralleled by changes in both partition coefficient and (estimated) diffusion coefficient. The distribution of oligomers within any one surfactant was apparently retained during transcuticular movement. Movement of the surfactant across the epicuticular waxes resulted in an accumulation of the penetrant in the tissues underlying the site of application. Movement of the surfactant in treated leaves was acropetal.

The foliar uptake of flamprop-methyl by wheat has been studied following topical application of the herbicide formulated in aqueous surfactant solutions. Non-ionic ethoxylated surfactants have been found to markedly enhance transcuticular movement of the herbicide but do not promote transport of the compound within treated leaves. The extent to which uptake was enhanced was largely influenced by the concentration of the formulation with respect to the surfactant, was much less dependant on either hydrophobe or hydrophile structure and was apparently independant, within experimental limits, of the herbicide concentration. Optimum surfactant concentrations were between 100 and 1000 ppm. Correlation between penetration enhancement and the surface properties of the formulations was non-existent. The effect of the surfactant on penetration is discussed in terms of a surfactant/lipid interaction which facilitated diffusion of the herbicide. At high

surfactant concentrations a variety of responses were identified ranging from varying degrees of enhancement through to an inhibition of transcuticular movement: these responses were shown to correlate with the resistance to uptake afforded by a persistent surfactant residue on the leaf surface.

CONTENTS

Chapter 2

1. Introduction

    1.1 Introduction

    1.2 Non-ionic surfactants

    1.3 Polyoxyethylene alkylphenols

    1.4 Polyoxyethylene alcohols

2. Experimental

    2.1 Structural analysis

    2.2 Physical properties

3. Results and Discussion

    3.1 Triton X compounds

    3.2 Surface properties

    3.3 Spreading and wetting

    3.4 Brij compounds

Chapter 3

1. Introduction

2. Experimental

    2.1 Uptake studies with tetraoxyethylene octyl ether

    2.2 Uptake studies with Triton X 100

    2.3 Uptake studies with the Triton X series of surfactants

3. Results

    3.1 Chromatography

    3.2 Foliar uptake of Triton X 100

    3.3 Effect of surfactant concentration

    3.4 Oligomer distribution

    3.5 Foliar uptake of Triton X compounds

CHAPTER ONE

Penetration of flamprop-methyl into wheat

I N D E X

3.5 Foliar uptake by wheat

3.6 Translocation out of treated leaves

3.7 Effect of applied dose

3.8 Influence of environmental factors

3.9 Foliar uptake by barley and oat

SUMMARY

The foliar uptake by wheat of the wild oat herbicide flamprop-methyl has been studied following application of μl droplets of an aqueous solution. The results show that over the time required for the drop to dry the compound does not partition into the surface waxes and crystallises as an external deposit. This deposit is stable to volatilization and photochemical and biochemical degradation, and is depleted by foliar penetration. Adsorbtion by the leaf surface wax is initially rapid and thereafter occurs at a relatively constant rate until the almost complete exhaustion of the surface deposit. Penetration of the epicuticular wax layer generally proceeds at a slower rate than does adsorbtion of the surface deposit and the herbicide is accumulated in the wax layer. The compound is lost from the epicuticular waxes only slowly to the leaf tissues. Movement within the leaf tissues away from the area of uptake is predominantly acropetal to the leaf tip and it is presumed that the compound moves with the transpiration stream.

A similar fate befalls the compound when aqueous solutions of flamprop-methyl are applied as ul droplets to barley or wild oat: no evidence of selectivity between wheat, barley and wild oat as a consequence of preferential uptake was found.

Foliar uptake of flamprop-methyl is shown to depend on the amount of compound applied to unit area of leaf surface and to increase as the amount applied is increased or as the area to which application is made is increased. These trends are discussed in terms of a compartmental mode of foliar uptake. Foliar uptake of flamprop-methyl is also

shown to be slightly influenced by environmental factors; in an environment in which maximum daily temperatures exceeded 35°C penetration was especially rapid and tended to completion within 24 hours.

1.1 Introduction

In agriculture plants are sprayed with chemicals to supply nutrients, to control growth, to protect the wanted or to kill the unwanted. The behaviour of these substances in the environment, and hence their efficacy, depends largely on their physical properties, method of application and formulation. To use biologically active chemicals to best advantage it is essential to understand these factors and their interaction with environmental processes in relation to the characteristics of target and non-target organisms.

It is only rarely, if at all, that herbicides can be applied directly to the site of action. Generally such substances must enter the plant and migrate to the site of action at subcellular level. The efficiency with which the chemical is received at the site of action will depend on the efficiency with which it is delivered to an intermediate target and on various processes of transfer within the immediate environment of the chemical and target organism.

The primary pathway for herbicide entry into plants will vary with the specific weed control problem in question. When herbicides are applied directly to the aerial parts of a mixed crop and weed population (post-emergence spray application) the leaves serve as the primary intercepting and absorbing organs. The expression of activity of such sprays will depend on transfer between the external deposit on the leaves and the internal tissues so that the leaf surface is the first potential barrier encountered by the applied herbicide.

It is now well known that the aerial parts of plants are covered by an outer layer of nearly continuous structure which is less permeable to substances than much of the internal structure. This layer,

termed the cuticle or cuticular membrane is a heterogeneous structure with macro and micro morphological features and is complex in terms of its morphology, structure and chemistry. The nature of the cuticle has been extensively reviewed and will not be discussed herein; the reader is directed towards the literature referenced in later sections of this chapter and especially section 1.2 for comprehensive details. Generally, the structure and chemistry of the cuticle play a dominant role in determining the permeability characteristics of this layer and it constitutes a rate limiting factor in the penetration process. However, regardless of the complexities of the process, foliar application has proved extremely effective for a wide range of commercial products as a means of transferring pesticides from an external phase to the site of biological activity within the plant substances.

During recent years it has become apparent that the efficiency of spray applications of post-emergence herbicides may be considerably influenced by the manner in which they are formulated for use. The formulation of spray is a complex problem; a large number of materials are available as spray supplements, of which surfactants form a most important group. It is known that the uptake of many herbicides can be increased when they are applied with certain additives and surfactants are one class of additives active in this way. Several reviews of surfactant effects on pesticide performance have appeared and are referenced in Chapter 4. Most agricultural sprays incorporate a surfactant for a specific purpose: for the stabilisation of emulsions and suspensions, for producing increased wetting or spreading and as stickers for improving the tenacity of deposits. Although the addition of a surfactant may have been designed with one particular

purpose in mind, it may give rise to further effects, ie. on uptake of the active ingredient. Since alternative surfactant products may be available to achieve the original intent, a knowledge of the effect of surfactants on the uptake of the toxicant should permit more intelligent formulation with regard to biological efficiency. Additionally, selectivity of herbicides in crop protection must rely largely on biochemical differences but may also be contributed to by differences in foliar penetration. To take advantage of potential surfactant induced improvements in efficiency and/or selectivity it is important to understand the mechanism(s) by which such additives can modify cuticular permeability.

A considerable amount of work has been published on the use of surfactants in sprays, mostly in ad hoc field trials. Whilst such trials have produced valuable information, the large number of variables which are inherent in all field trials render the results of dubious value in any fundamental study of the effects involved. The present study was undertaken with a view to elucidating the effect of surfactants on the uptake of a specific herbicide by a limited range of plant species. Flamprop-methyl (Figure 1a) was chosen as a suitable compound for study. Most attention was devoted to the uptake by wheat of this compound: this combination offers the advantage that wheat is not susceptible to flamprop-methyl so that uptake can be studied in isolation from biological effects and over an extended time frame. In these studies topical application was used in preference to spray application to reduce the number of variables involved. For a similar reason simple aqueous surfactant solutions of the herbicide were preferred to conventional formulation alternatives. I have discussed

the results of these studies in Chapter 4 in the context of trends established using different surfactant/herbicide formulations.

At the outset of this work it was apparent that one could not hope to fully understand the implications of the results of studies involving surfactants without a detailed knowledge of the uptake of the pesticide following application in aqueous solution without additives, or of some knowledge of the fate of the surfactant following foliar application. Thus in this Chapter I will discuss the foliar uptake by wheat of flamprop-methyl; this discussion is prefaced by a consideration of modern theories about foliar uptake and foliar transport, and by some consideration of the practical aspects of studying uptake and transport processes. Although emphasis throughout is on penetration, transport cannot be disregarded in considering interactions with whole plants. I will also introduce much experimental detail in this chapter which is also pertinent to Chapter 4. In Chapters 2 and 3 I have discussed the physico-chemical properties of the aqueous surfactant solutions of flamprop-methyl used in later studies and the foliar uptake by wheat of aqueous solutions of these surfactants.

1.2 Foliar penetration

Foliar adsorbtion has been defined[1,2] as the sequence of events starting with penetration of the leaf surface and progressing via partition and subsequent transport processes to movement within or export from the leaf. Foliar penetration (uptake) represents a stage in the adsorbtion process by which the externally applied compound moves through the outermost plant surface, viz., the cuticular membrane. Penetration in whole leaves, whether detached or on intact plants, and in leaf discs can be influenced by subsequent events in the adsorbtion process, ie., by the rate at which the penetrant is removed from the site of penetration by partition or transport processes. The study of foliar penetration with such material can be complicated by the nexus of factors controlling adsorbtion. Studies on isolated cuticles, have been used to isolate the event of penetration from other processes, but it must be realised that the behaviour of the isolated cuticle may differ from that of the cuticle in vivo.

The past 35 years have seen the publication of large quantities of data[1-26] describing the foliar uptake of a wide variety of organic compounds which have differed markedly in structure and polarity. These studies have encompassed a wide variety of plant types and data have been obtained with whole leaves, leaf discs, isolated cuticles and isolated cells. Steric, polar, electrical, mechanical (eg size to pore shape), and physico-chemical properties of the penetrant molecule have been recognised as of importance to the uptake of exogenous compounds.[5,17,24-26] It has been found, with few exceptions, that relatively non-polar compounds having semi-lipophilic characteristics can most effectively penetrate the leaf surface. In general, for

members of a closely related group of compounds, the tendency for foliar penetration increases as the compounds become less polar and as the lipid solubility is increased.[1] Thus

- The penetration of many organic acids into isolated cuticles has been found[27-29] to increase at a pH below the pKa,
- Non-polar forms of Picloram and 2,4,5 - T have been shown[30] to penetrate the cuticle of Vicia faba more readily than the corresponding polar forms,
- For the phenoxyacetic acids penetration into Phaseolus vulgaris has been shown[31] to increase as the lipid solubility was increased by progressive chlorination,
- Studies[32-36] using model membrane systems have demonstrated the importance of lipid solubility in penetration; in general penetration through these systems is improved by increasing lipid solubility.

Foliar uptake by Phaseolus vulgaris of benzoic acids is decreased with progressive chlorination, in contrast to the result found with the phenoxyacetic compounds.[31] Bukovac[37] found the same contrast to hold for penetration of phenoxyacetic and benzoic acids through isolated tomato fruit cuticles. The contrasting behaviour of the two series of acids illustrates the complex interplay of the factors interplay of the factors influencing uptake. Whereas improved lipid solubility can be inferred on the phenoxyacetic acids with little effect on the pKa value, chlorination of the benzoic acids markedly depresses the pKa value and increases the polarity of the molecules.

A. The Lipid Pathway

There is ample evidence that lipid soluble compounds can penetrate leaf cuticles of many species. Crafts[10] noted that undissociated dinitrophenols and chlorophenoxyacetic acids, the esters of dalapon,

substitued urea compounds and triazines have all proved able to enter leaves through astomatous surfaces. Although exemplifying the permeability of the cuticle to such molecules this listing is by no means comprehensive. A direct pathway for the uptake of such lipid soluble, non-polar compounds has been commonly assumed, (the non-polar, apolar or lipid pathway), involving adsorption at the epicuticular wax surface, movement through the bulk of the cuticular membrane, and desorption into the underlying apoplast.

Diffusion

Movement across the cuticle is considered[22] a physical process occurring primarily by diffusion as opposed to a biological process involving active accumulation. The absence of living, cellular tissue within the cuticle argues against a physiological mechanism,[2,14,38] and a diffusion mechanism has been indicated by kinetic studies of penetration into isolated cuticles.[39-43]

Lieb and Stein[44] have considered diffusional processes in biological membranes to be to some extent analogous to the corresponding processes in hydrophobic synthetic polymers. The molecular basis of diffusion in these polymers has been interpreted[45] in terms of the transient formation of 'holes' resulting from the random thermal motion of the polymer chains, into which the diffusant molecule can enter. The rate of diffusion is determined by the rate of appearance of holes of a requisite size, which in turn is dictated by the formation frequency of holes and the probability distribution of hole sizes in the polymer. The frequency of hole formation is a property of the solid phase and independant of the diffusant; the probability distribution of hole sizes will determine the size selectivity of the diffusion process.[46]

It has been shown[47] from general statistical mechanical considerations that the probability distribution will have a form such that there will be many more small holes than large holes, the energy requirement dictating the predominance of the former. From a consideration of the relative nature of the bonding and molecular packing involved, the frequency of hole formation might be expected to be greater for a polymeric solid than for a crystalline solid. In terms of hole size one would also expect a more favourable situation to exist in a polymeric solid, with the intermolecular spaces in the matrix generally having larger dimensions than spaces in a crystal lattice. Since the gross crystallinity of the wax deposits suggests a semi-crystalline molecular configuration, movement through the epicuticular wax layer would be expected to offer greater diffusional resistance than movement through the underlying cutin matrix.

The quantitative treatment of diffusion which establishes the necessary relationships between concentration, distance and time is based on the fundamental principle (known as Fick's law) that the rate of diffusion in a given direction at any point is proportional to the concentration gradient in that direction. The constant of proportionality is the diffusion coefficient, D, so that Fick's law is expressed mathematically:

$$\frac{F}{A} = -D \frac{\partial C}{\partial x}$$

where F is the amount of substance diffusing per unit time across area A normal to the x direction and $\partial C/\partial x$ is the concentration gradient in that direction. The negative sign is required because diffusion takes place in the direction of decreasing concentration along a negative gradient.

Epicuticular waxes and cuticular transpiration

The wax layer has been shown[13,48] to play a significant role in minimising cuticular transpiration and the permeability of cuticle to water in an in vitro system has[49] been directly correlated with the amount of impregnated wax. Exposure of the surface wax of Vitis vinifera to light petroleum vapour for 30 seconds markedly increased subsequent cuticular transpiration, and this effect was attributed[50] to the disorganisation of the waxes, although the cutin layer may also have been modified. Disruption of the epicuticular waxes by light brushing has been shown[51] to enhance wetting and water loss, and to increase the uptake of 3-chlorophenoxypropionic acid,[52] and ureas,[53] Norris and Bukovac[54] found that water penetration through fractionated components of leaf wax showed marked differences between the various fractions, and it would appear that the permeability of the cuticle to water can be affected by the quantity, chemical composition and physical configuration of the wax. A number of compounds are known to inhibit wax production when applied via the roots, for example TCA and EPTC; inhibited wax production has been shown[13] to result in increased cuticular transpiration, increased spray retention with decreased contact contact angle, and enhanced uptake of foliar applied herbicides.

Epicuticular waxes and foliar uptake

Permeability studies using intact leaves or isolated cuticles from a wide range of species have shown[55] that the epicuticular wax layer constitutes the major barrier to the movement of applied compounds. It has been found[13] that the foliar uptake of both polar and non-polar herbicides is markedly influenced by both the structure and quantity of the surface waxes. Although experiments using radioactive labelled compounds have not, in general, correlated the rate of penetration with

the overall thickness of the cuticle, changes in the thickness and composition of the wax layer induced spontaneously by mutation or following treatment with inhibitors of wax synthesis correlate well with changes in the penetration rate.[55] The penetration of 2,4-D has been inversely related to the amount of epicuticular wax in six weed species[56] and susceptibility to aminotriazole has been explained on the basis of differential cuticular structure/thickness.[57] Recently[58] the permeability of the cuticle from Prunus persica has been shown to decrease progressively with leaf age, a trend closely paralleled by changes in the physical and chemical characteristics of the leaf surface. Bukovac and Norris,[42,43] found that successive removal of the wax from Pyrus communis cuticles increased the penetration of IAA, and that when wax was plated onto the dewaxed cuticles penetration was reduced proportional to the quantity applied. A similar effect had previously[59] been noted for N-isopropyl chloroacetamide. Interestingly, Bukovac and Norris found that the removal of the embedded waxes from the cuticles of de-epicuticular waxed cuticles from Pyrus communis resulted in only slight additional increases in the uptake of IAA.

Partition into leaf tissues

While lipid solubility appears to favour cuticular penetration the solute must partition into the aqueous, apoplast system if uptake is to be maintained. A compound can not be permanently retained within the cuticle simply because it has a high oil - water partition coefficient, although with such a compound partition out of the cuticle (desorption) can be inhibited by very low concentrations in the apoplast.[60-62]

Movement of the solute partitioned into the apoplast can occur by diffusion, mass flow, and/or by metabolic processes. The rapid

uptake of atrazine by Zea mays has been attributed[63] to the high rate of atrazine metabolism.

The interaction of herbicides with lipids has been investigated for highly lipid soluble compounds by St. John and Hilton.[64] The biological activity of foliar applications of lipid soluble pyridazione compounds and the highly lipid soluble herbicide trifluralin were found to be regulated by the addition of isoprenoid compounds and other lipids to the leaf surface. In particular, the isoprenoid tocopherol acetate was found to prevent the expression of biological activity of the pyridazione compounds, which are inhibitors of chloroplast pigment formation, even when these compounds were used at three to four times the dosage required to produce a response in untreated plants. Studies using radioactive labelled pyridazione showed that the alteration in the phytotoxicity was largely due to partitioning of the label into the applied lipid phase external to the plant, so depleting the quantity available to inhibit pigment formation. When these studies were extended to the highly water soluble 3-amino-s-triazole it was found that the applied lipids did not regulate the biological activity. The relative phytotoxicity of trifluralin was correlated with the endogenous lipid content of the dry seeds of 11 species such that species with a high lipid content were found to be more tolerant to this compound than species with a low lipid content. These results suggest that the endogenous plant lipid can also solubilise highly lipid soluble compounds and so regulate the availability of such compounds to the site of biological activity. The epicuticular and embedded waxes may similarly influence the uptake of foliar applications of lipid soluble herbicides, either as a consequence of the solubility of the solute in

the wax phase, the high partition into the lipoid phase making partition into underlying phases very slow, or, more directly, as a result of chemical binding. The immobilization of herbicides as a result of binding within the epicuticular wax has been suggested[29] to occur in Vicia faba with MCPB and both cationic and anionic herbicides may be bound.[65] This mechanism is probably of less importance with non-ionic compounds.

The formulation of organic acids as esters has been used to improve lipid solubility and foliar uptake. When the ester is resistant to hydrolysis it has been found that it is particularly important that the compound be of optimum polar-apolar balance so that both penetration of the cuticle and desorption into the apoplast can be achieved at viable rates.[13,38,66,67] Aliphatic esters penetrate the cuticle but may not move into the apoplast at a useful rate.[68,69] Heavy esters formed by alcohols that have both water and lipid solubility have however proved effective as translocated herbicides, as exemplified by the butoxyethanol ester of 2,4-D.[69] There is evidence to suggest that conversion of the ester to the free acid is often a necessary prerequisite for the expression of biological activity. Norris and Freed[70] suggest that such ester formulations are less suitable for efficient translocation since only the amount partitioning into the aqueous phase can be available for hydrolysis and subsequent transport. Use can however be made of this property in providing selectivity between crop and weed.

B. The Polar Pathway

In many of the reviews of foliar penetration published prior to 1965 it was assumed that the barrier to the uptake of polar, hydrophilic

compounds presented by the cuticular membrane was insurmountable, and uptake of these compounds was considered to occur only by mechanisms which could circumvent, in part or in whole, the lipid layers.[17] The uptake of aqueous solutions of inorganic ions and polar organic compounds was postulated to occur by entry through imperfections in the lipid layer allowing access to the aqueous phase.[3,7,11,71,72] Imperfections in the cuticle which might function as entry sites included areas of mechanical damage, cracks, fissures, natural abrasions, insect punctures, areas damaged by penetrating pathogens and the stomatal aperture. These explanations cannot however satisfactorily explain the collected data on the uptake of polar compounds. The frequency of fissures, breaks and punctures in what appears to be a continuous wax layer is insufficient to account for the observed uptake of many polar compounds.[17] In particular, when experiments have been performed using isolated cuticles, the membrane has been subjected to detailed investigation prior to usage; uptake is not dependent on physical imperfections.[13]

Weaver and DeRose[73] and Adam[74] suggested that aqueous solutions devoid of surface active agents could not infiltrate the stomatal pore by reason of the forces of surface tension. More recently Schonherr and Bukovac[75] have reported that there is a general concensus that pure water does not enter into the intercellular space through open stomata unless an external pressure is applied. Polar compounds can penetrate leaf surfaces on which the stomata are closed[2] and penetration of astomatous surfaces occurs.[41,76-79] That the cuticle is permeable to polar molecules is also demonstrated by the phenomena of cuticular transpiration and excretion, and by the penetration of compounds such as maleic hydrazide[10] and ionized formulations of

2,4-D,[80] and 1-methylpyridiniumchloride.[81] Although not impervious to polar compounds the rate at which penetration of the cuticle proceeds has been commonly found to be lower than that found with non-polar compounds.

The uptake of polar compounds has been postulated to occur via a aqueous pathway, distinct from the non-polar route, although morphological description of the aqueous pathway has rarely been attempted. Pectic or cellulosic extensions of the apoplast exist in the cuticular membranes of some species. Such structures could serve in the uptake of polar compounds by extending the water continuum of the apoplast to the leaf surface. There is however, as yet, little evidence for the existence of these structures as a common feature of leaf structure.

Orgell[82] and Overbeek[3] postulated penetration of a polar solute to occur via a hydrophilic pathway around lipid platelets. Swelling of the cutin matrix with an ample water content could theoretically enhance cuticular permeability by displacing the wax components; the separation of lipid components could also occur as a consequence of high turgidity in the epidermal cell wall. The mechanism can be seen to depend on the affinity of the cutin for water and on the elasticity of the cuticular components.

Crafts[10,83] suggested that pores in the cuticle might function as routes for the foliar uptake of polar solutes. High humidity and the attendant high water content of the leaves were postulated to enable the water continuum of the leaf to approach the outer surface of the cuticle. Under these conditions pores, supposed to span the lipid membrane, might be filled with a water continuum and allow contact between a spray drop or residue and the apoplast, thereby providing a

continuous path for diffusion in a hydrophilic medium. Although subsequent investigations have failed to reveal the existence of any discrete structures that could be recognised as cuticular pores or microchannels in the membrane[13] save for the fibrillar intrusions discussed previously, a mosaic structure of this kind on a molecular scale might be envisaged to exist as a consequence of the hydration of exposed polar functional groups in the cutin matrix. At the present time there does not appear to be sufficient evidence to support any one morphological description of the polar or aqueous pathway.

The concept of an aqueous route appears to be based on the solubility characteristics of the solute and on the observation that, with relatively few exceptions, foliar uptake of these compounds is generally facilitated by a high humidity. Whilst accepting the latter, the relationship between the external humidity and the hydration of the cuticle has been questioned by Hull,[13] the extent of hydration being considered more a function of available soil moisture than of atmospheric humidity under most conditions. Experimentation reviewed by Hull indicates that moisture stress may inhibit the absorption of foliar applications of solutes by adversely affecting transport processes. Although there appears to be no question that a favourable water abalance is important for optimum translocation the relationship with penetration is less clear. In studies with 2,4,5-T and picloram[84] and 2,4-D[85,86] the effect of water stress on the absorption process was found to be confined to inhibition of translocation, penetration being unaffected. It would seem reasonable to presume that the hydration of the cuticle would depend on the water stress of the plant rather than on atmospheric humidity; from this the results obtained with plants

subjected to varying degrees of stress would not support the concept of uptake proceeding either by a swelling of the cuticle or by aqueous pores. The increase in permeability with increasing humidity by a plant physiological mechanism, as distinct from a physical effect on the external deposit of the solute, would not seem to have been established as a universal trend.

Foliar absorption, excretion and transpiration may be localised in special sites and not occur in all areas of leaf with equal intensity.[17] Studies[87] on the binding sites of radioactive ions and urea in isolated cuticles have been interpreted as evidence that penetration does not proceed through the total plane surface. Amongst the areas of preferential permeability identified for various compounds have been included the veins and anticlinal epidermal cell walls. Overbeek[3] has suggested that preferential permeability of the epidermis overlying veins can be explained by the modified form of the cells arising from the extension of the bundle sheath to the epidermis. This feature is common to dicotyledonous species and can be observed in leaf transcections of wheat and Pyrus species;[88] Overbeek suggests that these cells may be capable of absorbing aqueous solutions from the leaf surface.

The embedded wax layer in the cuticle of Pyrus communis is greatly reduced over the anticlinal cell walls and absent from the cuticle overlying major veins. The correlation between the preferentially permeable areas of the cuticle and the discontinuities in the embedded wax layer can be interpreted in terms of these discontinuities constituting pathways for the movement of applied compounds. The greater penetration of the cuticle associated with the abaxial leaf surface may

be due to a decrease in the embedded, orientated waxes relative to the adaxial surface.[13,89]

Attempts have been made to explain areas of preferential permeability existing on the leaf surface in relation to underlying structures termed ectodesmata or ectocythodes.[17,90-93] Much has been written on the role of these structures in mediating foliar uptake, but it would seem[94] that they appear only as a consequence of cuticular penetration and are an artifact which results from a cuticular property rather than vice-versa. Similar structures to the ectocythodes have been demonstrated in agar resulting from the penetration of isolated cuticles. The visualisation of these structures necessitates the penetration of the cuticle by polar solutes, and the work of Franke and Schonherr and Bukovac clearly demonstrate the existence of discrete areas of the leaf surface permeable to polar molecules. The location of these areas corresponds to the areas previously thought rich in ectocythodes, viz., stomatal guard cells, the basal cells of trichomes, veins, and the anticlinal walls of epidermal cells. The size, abundance and characteristics of these permeable sites remain uncertain, as do the underlying morphological characteristics.

The routes postulated for the uptake of polar and non-polar compounds differ in that for the latter a direct pathway involving intracuticular penetration is commonly assumed whilst for polar solutes most theories are based on intercuticular penetration following a hydrophilic phathway around the lipid layer. If the two routes exist it is unlikely that they would constitute exclusive pathways for any compounds save those with extremes of hydrophobic or hydrophilic character. The concept of a lipid and aqueous route would more probably represent preferred paths, so that for any one system the two routes would contibute to the overall uptake of a solute.

1.3 Methods of Studying Foliar Penetration

The measurement of the foliar uptake of chemicals has been approached experimentally in many different ways. Methods differ in the techniques used to apply the chemical to the leaf, in the way uptake was determined, and in the nature of the biological material.

(i) Application to the leaf of exogenous chemicals

The normal commercial practice used to apply foliar herbicides is by conventional spraying or dusting. This method does not readily lend itself to the investigation of foliar penetration, for a number of reasons. The method introduces many variables, for example the statistical scatter of the dosage of the applied chemical, and evaporation of carrier and active ingredient on spraying. The solution to be applied to the foliage as a spray must include formulating additives to stabilise the treatment, and this introduces further variables. Spraying is the method of application most relevant to normal practice, but since the solutions used in the laboratory investigations often differ markedly from the commercial formulations there is little advantage to be gained from using a spray to apply such solutions to the foliage.

The methods used to apply solutions to the leaf in studies of foliar uptake can be divided into those delivering a finite volume of the solution to the leaf and those which maintain a constant composition of solution at the leaf surface. The application of measured volumes of solution to selected areas of the leaf can be achieved with microsyringes, and removes many of the variables introduced by spraying. The method is particularly suitable when radioactive labelled compounds are to be applied; economic and safety considerations preclude conventional spray application with such materials. The main disadvantage of this method

of application is the size of the drop applied to the leaf. The majority of studies report drops in the range 0.1 to 10.0 µl. Volumes from 0.1 to 1.0 µl can be difficult to apply because of the problem of the liquid being retained at the tip of the applicator. In contrast, the drops produced in hydraulic nozzle sprays have a diameter of about 500 µm,[106] a 10 µl drop has about 160 times the volume of such a spray drop. Application by microsyringe thus results in large drops in comparison to the commercial situation. The importance of the difference in drop size is questionable; the differences between commercial formulations and research solutions in terms of the solvent system, the concentration of the active ingredient and the presence of formulation additives may be considered to introduce far greater differences between commercial and laboratory applications than any introduced by drop size.

Sargent[1,2] has suggested that the results of experiments which use the placement of a finite volume of solution on the leaf are difficult to interpret since changes occur throughout the experiment in both the external concentration of the penetrant and in the nature of the cuticle. These difficulties may be obviated by the use of a donor solution of constant composition.

If the difference in drop size between spraying and application by microsyringe is considered important then the use of a donor solution of constant composition, involving volumes many thousand times greater than those found with spraying, represents a more drastic departure from the commercial situation. The use of large donor volumes is intended to eliminate effects of change of donor composition and, as a technique, has been much used in permeability studies on membranes. When whole leaves

have been used the contact between the solution and the leaf has been achieved by a number of techniques, including total immersion of the leaf in the donor solution[107] and partial immersion with the apical half making contact with the donor solution and the petiole end dipping in water.[108] These methods can result in drastic alterations in the structure of the leaf in contact with the donor solution, especially if this solution contains organic solvents or surface active compounds which can solubilize plant lipids and thereby affect the permeability of the leaf cuticle. In penetration studies this must be considered a serious limitation to the technique.

Tubes, positioned on the leaf surface with the ring of contact sealed with a petroleum jelly, have been used to make contact between the donor solution and the leaf (Sargent and Blackman, 1962-1972). A known volume of solution was introduced into the tube and the area of contact and the concentration of the donor solution was maintained constant. This technique was generally found to be more useful with broad leaved plants than with monocotyledonous plants.

(ii) Determination of the uptake of the applied compound

The extent of foliar uptake of the applied compound can be estimated by determining the surface residue of the compound on the leaf surface and/or by measuring the mass of compound in the plant tissues. The use of a large volume of donor solution often requires the measurement of the mass of compound in the plant to determine with any accuracy the extent of uptake. With spray - type deposits uptake can be estimated from the mass of chemical remaining on the leaf surface.

The measurement of the surface residue can be attempted by a washing off technique. The use of a solvent to recover such material from the leaf surface is problematical, in that a distinction must be made between

washing off and washing out. Washing out represents the extraction of penetrated chemical from within the leaf and, if significant, will reduce the apparent uptake achieved. If uptake is an irreversible process and a clear physical distinction exists between the applied, superficial, chemical and that penetrated, then washing out may not represent a significant contribution to the measured value of the mass of chemical on the leaf surface. The use of a organic liquid to recover a chemical from the leaf surface will result in some washing out because of the dissolution of the epicuticular wax layer. The extent to which washing out may occur when water is used to wash the leaf surface does not appear to have been considered in the literature.

If foliar uptake is to be estimated from the surface residue it is essential to consider other mechanisms for the loss of the applied compound from the leaf. The depletion of the surface residue may occur as a result of photochemical or biochemical degradation and by volatilization as well as by leaf penetration. The contribution of these mechanisms to the depletion of the chemical on the leaf surface must be evaluated if this method is to be used. Biochemical degradation is perhaps the least probable mechanism for the depletion of a exogenous compound outside the leaf tissues. Biochemical degradation is however of importance within the plant tissues. The importance of photochemical degradation and volatilization will vary with the chemical being studied. Photochemical degradation has been shown to be particularly important in the depletion of the surface residue of NAA.[109]

One advantage to determining the extent of uptake from surface residue is that the applied compound can be isolated for analysis without the complicating presence of significant amounts of co-extracted material.

Chemical estimation of the amount of exogenous compound present in the plant can be time consuming and lack sensitivity because of the variety of endogenous compounds present in the plant tissues and the small quantities of applied compound involved. The use of radiactive labelled compounds can reduce the purification of extracts that would otherwise be required and improve detection limits. The use of radioactive labelled compounds is also advantageous in the determination of unextractable, irreversibly bound compound which can be measured following oxidation to $^{14}CO_2$.

(iii) The leaf

Penetration has been studied using detached cuticle, leaf discs, detached leaves and intact plants in previous studies of foliar uptake. The leaf cuticle may be isolated from the underlying tissues using ammonium oxalate - oxalic acid,[17] zinc chloride - concentrated hydrochloric acid[110] and the enzymatic technique developed by Orgell and modified by Yamada.[111] The isolated cuticle can be used to study cuticular penetration separated from the subsequent steps involved in the uptake and translocation of chemicals in the intact leaf. Sargent[1,2] has suggested that experimentation with intact leaves can only measure the combined effect of penetration of the leaf and transport in the cellular tissues. This criticism might be extended to include detached leaves and leaf discs. The use of leaf discs can eliminate translocation, but would still combine penetration with transport effects in the apoplast and symplast layers included in the leaf disc.

The detached cuticle has been criticised as a substrate for permeability studies; Hull[13] identified the main objections to the technique as;

1. Chemical alteration of the membrane induced by isolation techniques.

2. Physical imperfections caused by subjecting the membrane to stress.

The enzymatic isolation of the cuticle of the pear leaf has been studied by Norris and Bukovac[89] using normal and polarised light and electron microscopy. This study provided evidence of an almost complete lack of alteration in cuticular structure during isolation. Enzymatic isolation was found to separate cuticle and underlying tissue along the pectin layer. Pectic materials embedded in the cutin were retained in the detached cuticle, no change was found in the position and degree of orientation of embedded waxes, and the epicuticular wax was retained without structural alteration. The detached cuticle was not completely free of cellulosic debris, so that some contribution from the apoplast to the measured permeability of the cuticle would remain. Gross physical imperfections in the detached cuticle, for example cracks, can be discerned with non - penetrating marker dyes and inspection under high magnification and imperfect membranes discarded.[13] These studies would appear to have fully answered the criticisms listed above. A more serious criticism of the detached cuticle as a representation of the attached leaf cuticle concerns the possible difference in the permeabilities of the membrane in vivo and when isolated. If the permeability of the cuticular membrane depends more on its relationship to subsequent layers than to its composition, for example as a consequence of the juxtaposition of preferential routes between cuticle and apoplast, then the isolated cuticle might be more permeable than when in vivo. It has however been postulated[112] that the isolated cuticle may be less permeable than the attached cuticle. The pectinaceous layer underlying the attached cuticle is water retaining. Variations in the water

content of this layer could modify the interactions of the layers above. When the pectin layer is saturated it will swell and have a greater volume than when under some degree of water stress, and the embedded cutin waxes in this situation may be separated into discrete platelets. In the absence of a pectin layer, as in the detached cuticle, the embedded waxes may form a near continuous layer which may result in decreased cuticular permeability. Leaf discs maintained in a turgid state by working in a saturated atmosphere,[41] or by lying on filter paper in contact with a water reservoir[67] would retain a water saturated pectin layer and open wax structure. The use of a saturated atmosphere restricts the situations in normal practice to which measurements made with such a substrate would be relevant.

Detached leaves can be maintained in the turgid state by immersing the petiole in water. Although detached leaves offer no advantage over intact plants with regard to separating cuticular penetration from subsequent steps, they can be more convenient to work with. Active transport processes in the detached leaf have been reported[113] to have been maintained for 24 hours subsequent to detachment so that it would seem to be possible to study uptake under consitions of normal metabolism for a limited time with these substrates. The use of whole plants allows penetration to be studied with the retention of normal metabolism over the life of the plant. As already mentioned, the major difficulty with such studies is that the interpretation of the results may be problematical, as a consequence of the complication of interactions in the pectin and underlying tissues.

1.4 Herbicide transport

The transport of herbicides has been discussed in several reviews.[10,68,95-98,103-106] Hull[13] identified three probable mechanisms by which lateral and logitudinal movement of substances can occur within the lamina of the foliage leaf:

(i) intercellulary via the apoplast within the cell wall or along the cell wall interface. The cell wall is a porous structure and is freely permeable[99] to dissolved substances so that even large molecules such as sucrose pass through with ease. Physical diffusion in the pores of the wall is commonly assumed[23] to be the mechanism for apoplastic movement to the xylem tissues.

(ii) intercellulary via the symplastic continuum, and

(iii) within the conductive tissues of the vascular bundles, either by symplastic movement in the phloem or apoplastic movement in the xylem.

An organized transport system capable of rapid conduction of solute from the cuticle to the vascular bundle has been concluded not to exist[99,100] and movement between the site of entry and the main conducting tissues appears to be relatively slow in comparison to movement in the conducting systems. The exact velocities at which different compounds penetrate from the leaf surface to the vascular system do not appear to have been studied to the same extent as velocity of movement within the conductive tissues. Day[101] determined that 2,4-D moved from the leaf surface of bean plants to the phloem at a rate of about 0.003 cm per hour; in comparison movement in the phloem occurred at a rate of 10 - 100 cm per hour. Also using bean, Little and Blackman[102] determined velocities for the movement of phenoxy

compounds (2,4-D, 2,4,5-T) between leaf surface and underlying vascular bundles only slightly lower than that found by Day.

A. Apoplastic transport

Transport in the apoplast is observed with the ureas, phenylureas and triazines; monuron has been cited[77] as a classical example of a herbicide that is transported only in the apoplast. For such compounds penetration into the leaf can be slow as a consequence of the lack of export from the treated foliage; symplastic mobility is negligible and there is little or no basipetal movement. Within the apoplast transport is in the direction of the transpiration stream, the driving force for long distance movement in this system, and labelled compounds moving in this way produce a wedge-shaped pattern on an auto-radiograph of a treated leaf as the compound moves distally from the point of application and in a direction away from the source of the transpiration stream.[95] Movement within the apoplast is strongly directional, following the movement of water to the leaf tip (monocots) or margins (dicots) with solutes accumulating where water is lost by evaporation.[23]

B. Symplastic transport

The export of herbicides from leaves is thought to occur almost exclusively via the phloem[68,103] so that symplastic mobility is a prerequisite for translocation out of the treated leaf. For a foliar applied compound to be exported from the leaf requires (i) penetration of the plasmalemma, (ii) movement to the vascular bundle, and (iii) loading into the phloem. Movement from the area of cuticular penetration to the vascular bundle may occur in part or in whole in the apoplast;[104] however, for compounds that show no movement in the transpiration stream

it would seem reasonable[95] to presume that the solute is rapidly adsorped into the symplast.

Franke[17] has suggested that movement into the protoplast is a three stage process involving (i) diffusion into free space (ii) adsorption to the surface of the plasmalemma by physico-chemical binding, and (iii) movement into the protoplast by a process requiring metabolic energy. Free space constitutes the intermicellar spaces and spaces between the microfibrils in the cell wall. Whilst the cell wall is freely permeable the plasmalemma appears to constitute a barrier to movement into the protoplast.

Penetration of undissociated molecules into lipid layers from an aqueous solution occurs[105] in three steps:

(i) adsorption into the membrane, which involves breaking the hydrogen bonds formed by the hydrated solute molecule, overcoming van der Walls forces, and the formation by thermal agitation of a hole in the membrane lattice.

(ii) diffusion through the membrane, and

(iii) desorption from the membrane, which involves a reversal of (i).

Early studies[114] on the permeability of natural membranes to non-electrolytes suggested that penetration was a function of molecular size and partition coefficient, expected if membranes were a simple bilayer. Many of the physical properties of natural membranes are possessed by artificial lipid bilayers, but Price[23] has noted that the bilayer has a higher electrical resistance (10^6 - 10^9 ohm.cm^{-2}) and surface tension (0.2 - 0.6 mN.m^{-1}) than the membrane (10^2 - 10^5 ohm.cm^{-2}, and 0.1 - 3 mN.m^{-1} respectively), and that the membrane exhibits far more selectivity with regards to the types of compound

which can penetrate it. The lower electrical resistance and surface tension of membranes suggest that they are not completely bilayer in structure and that the presence of proteins provides alternate routes by which solutes can penetrate the membrane.

Another difference between membranes and bilayers is that many membranes are able to accumulate solutes to an equilibrium concentration different from that expected from the chemical potential.[23] Robertson and Kirkwood[68] have suggested that non-electrolytes penetrate natural membranes by facilitated diffusion while active uptake of electrolytes occurs.

Facilitated diffusion can occur[115] by three mechanisms; (i) the diffusing molecule may combine with a carrier which transports it across the membrane, (ii) diffusion may take place through a channel or pore which has different physico-chemical properties to the bulk phase, or (iii) the solute molecules may interact to form a dimer with superior permeability characteristics. Facilitated diffusion is concentration dependent (the process can be saturated) and can be influenced by the presence of competing solutes, or inhibitors (competitive and non-competitive inhibition). Active uptake is similar to facilitated uptake in saturation effects, competition and specificity, but whilst the latter occurs under the driving force of thermal agitation, active uptake is linked to metabolism;[23,68] Examples of both types of diffusion have been reported; facilitated diffusion has been reported for urea[87] and active uptake was suggested[116] as the mechanism by which maleic hydrazide penetrates the symplast. The selectivity inherent in either of these processes may explain the lack of phloem mobility exhibited by compounds such as monuron.

After penetrating the plasmalemma a solute may move by 'short distance' transport in the symplast to the minor veins, there to be loaded prior to long distance transport in the phloem. Little is known about the cell to cell transport system that carries the herbicide from the epidermis to the phloem.[68] Plasmodesmata can be visualised as preferential intercellular transport channels;[24,38] assimilates, sugars and possibly exogenous compounds move between cells via these protoplasmic connections.[24,98,103] It has been suggested[83] that cell to cell diffusion may be activated by protoplasmic streaming.

Short distance transport apparently leads to an active accumulation of the solute in specialised parenchyma cells associated with the sieve tubes of the minor veins.[117] This accumulation forms an important part of phloem loading[96] the process by which the major translocated substances are selectively and actively delivered to the sieve tubes in the source region before translocation.[118] It has been suggested[119] that movement from these cells to the sieve tubes occurs via the plasmodesmata in the lateral and end walls. There is evidence that plasmodesmata are especially abundant at specific, high transport sites such as between the mesophyll and phloem[120] or between the mesophyll and bundle sheath cells,[121] but only the specialised parenchyma cells - the companion cells - connect directly with the sieve tubes.[122] The general absence of plasmodesmata between parenchyma and sieve elements points to a specific role of the companion cells in sieve tube functioning.

In discussing the transport of organic substances in plants, Kursanov[123] emphasises the intricate mechanism involved in the transfer of sugars and amino acids from the mesophyll to the conductive

tissues of the minor veins. Sucrose is apparently produced in the chloroplasts and moves into the protoplast.[124] Movement in the mesophyll is primarily in the form of hexose phosphates[13] and may take place via the symplast or in the free space via the cell walls.[125,126] On entering the bundle sheath parenchyma, in which high phosphatase activity has been observed,[13,38] the phosphate is split off and the hexoses condensed into sucrose, which is subsequently discharged into the sieve tube elements. The fine vascular bundles can[127] contain three to four times more total sugar than the adjacent mesophyll cells and transport into the bundle must consequently involve considerable metabolic activity. Phosphatase activity has been found in the companion cells of several plant species.[13]

Several variations of the mechanism involved in the transfer of carbohydrate assimilates from the leaf mesophyll to the sieve elements have been suggested.[13] It is generally agreed that the phloem loading component of translocation must be an active step[98] and that transport into and out of the phloem requires a supply of metabolic energy;[117,123,128] studies with ATP, a metabolic stimulant, and DNP and KCN, metabolic inhibitors, indicate an energy requiring process for phloem loading.[118,129] It is widely believed that the specialised mechanism for loading sucrose into the phloem against a concentration gradient may also except, selectively, some exogenous solutes. The findings of Field and Peel[130] that several exogenous compounds can be loaded into willow bark phloem against a concentration gradient supports this view.

It has been suggested[131] that discrimination between substances determining their movement or non-movement in the phloem occurs at the loading stage. As an example of this, Crisp[132] has suggested that as

a prerequisite for active uptake into the phloem tissues certain compounds must have the carboxyl group; other functional groups e.g., alcohols, amines, ketones, acetoxy groups, will not substitute in the active uptake process for the acidic moiety, (the weak acid hypothesis). Crisp and Look[133] explored this hypothesis for the phloem mobility in soybean of 4-chlorophenoxyacetic acid and derivatives thereof: the acid and derivatives that converted into the acid all showed active uptake and phloem loading, whilst other derivatives showed only passive movement and were not phloem mobile.

The symplastic movement of foliage applied translocated herbicides occurs in the phloem along with photosynthates. Movement is thought to accompany that of assimulates along a source to sink pattern, the herbicide moving en masse with the flow stream.[96] The rate of translocation of assimilates within this system has been reported as 85 cm per hour in soybean[134] and between 40 and 100 cm per hour in sugarbeet and pumpkins.[3] It would seem that not all solutes move at the same rate; sucrose has been reported[134] to be translocated faster than fructose or glucose and studies with ^{32}P and tritiated water have shown respective flow rates of 60 cm per hour and 180 cm per hour at the same time. Overbeek[3] has analogized this to movement in a mobile phase over a stationary phase leading to chromatographic separation in which the solvent front moves ahead of the solutes.

Translocation in the phloem sieve tubes is to regions of high metabolic activity (active growth) with the site of accumulation (the sink) depending on (i) the maturity and (ii) the position on the plant of the treated leaf. The import/export characteristics of an individual leaf will vary according to age[24]; initially it will act as a sink which

gradually diminishes in intensity until maturity, when it becomes a nett exporter of assimilates. Presumably carbohydrates are not exported from developing leaves because of the energy requirements for growth exceeding supply.[98] This suggests that the export of herbicides may be inhibited from young leaves.

Translocation of assimilates and herbicides may be strongly influenced by the relative position of source and sink, which will continue to vary with plant development. The general pattern of translocation in the plant is from the upper mature leaves to the expanding leaves, from the lower mature leaves to the roots, and from median leaves in both directions.[95;98,103] Young fruits and flowers are extremely active sinks[10] and at the development of these the direction of transport from the various sources may change to that of the dominant sink.[24] In moving from source to sink, assimilates and phloem mobile herbicides typically by-pass mature exporting leaves[10] and accumulate in sinks to very high levels.

The dependence of symplastic mobility on metabolic activity has been demonstrated by the autoradiographic studies of Yamaguchi[135] on several plant species. Following spot application of ^{14}C-2,4-D, a compound which typifies the wholly symplastic transport pattern, Yamaguchi found that by reducing metabolic activity (by DNP) a concomitant reduction in phloem mobility was obtained. Apoplastic mobility was enhanced. Similar results were also obtained by starvation and anoxia. 2,4-D is strongly bound in living cells and extensive movement would seem to be dependent on an active source and sink.[136]

C. Xylene - Phloem interchange

Intermediate between the urea and triazine herbicides, none of which

are phloem mobile, and 2,4-D, which seldom shows apoplastic movement distal to the point of application, are dalapon, dicamba, MH, naptalam, amitrole, barban, 2,2,3-TBA, and N-m-tolylphthalmic acid, all of which show both apoplastic movement within the leaf and symplastic movement out of the leaf.[10,13,98,137-141] Of these, amitrole is unstable in plants[138] and it is unclear as to whether degradation occurs before or after movement, or if it moves in complexes. This is not a consideration with either dalapon[140] or dicamba.[137] Many of these compounds differ from 2,4-D in that they are not accumulated so avidly by living parenchyma and move more freely across the mesophyll.

2,3,6-TBA, MH, and dalapon, (and possibly other herbicides) are translocated to the roots as a consequence of phloem mobility; subsequently the compounds move acropetally in the transpiration stream, re-entering the symplast within the foliage and, repeating the cycle, circulating within the plant. Sagar[142] found that although dalapon was translocated in the phloem little was present in the roots when applied to upper leaves or when the transpiration rate was high since it tended to leak into the xylem. Currier and Dybing[99] suggest that xylem - phloem and phloem - xylem interchange occurs readily, whilst Hay[98] concludes that compounds may move between phloem and xylem in the stem: however the fact that different compounds applied in the same way to similar plants can move in different directions suggests that exchange between the conducting systems in the leaf is not free.

Factors influencing the efficiency of herbicide transport

It is uncertain whether translocation is more dependent on the amount of herbicide penetrating the cuticular membrane and available for movement, or, alternatively, that the concentration gradient across the

cuticle depends on the efficiency of translocation from the tissues adjacent to the site of uptake. The amount of MCPA (or MCPB) actually translocated from treated leaves has been shown[29] to be inversely related to the level present in the leaf tissues. With these compounds, which uncouple oxidative phosphorylation, interference with ATP synthesis in the treated leaf can inhibit energy requiring processes associated with movement.[29] Pretreatment with MCPA or MCPB can affect the translocation of other exogenous compounds in Vicia faba.[61] Kirkwood[24] and Price[23] have cited many additional examples of inhibition, and, more rarely, stimulation of herbicide translocation by pretreatment with the same or other herbicides, as a result of direct or indirect effects on energy requiring processes associated with translocation and possibly related to phloem loading. Crafts[10] has suggested that, by accelerating ontogenetic changes in the sieve tubes so that premature obliteration sets in, 2,4-D can restrict movement of phloem mobile, translocated herbicides.

Distribution can be markedly influenced by contact injury. Sharma[143] investigating the movement of asulam in wild oats and flax, found in wild oats initial movement to the roots, migration from phloem to xylem, and movement in the transpiration stream. In contrast, the compound was seen to be retained in the treated leaves of flax. This was correlated with contact injury at the site of application resulting in restricted movement out of the treated leaf.

It is known[144] that many herbicides affect cell membrane permeability, including extensive disruption of cell and organelle membranes, which may in turn influence the efficiency of herbicide distribution. Permeability changes may be caused by a direct effect on

the membrane, as with surfactants[145] and 2,4-D[146] or by indirect effect; the membrane can be damaged by a toxic by-product, e.g., peroxide production by bipyridal herbicides[147] or from a modification of membrane synthesis such as the inhibition of lipid synthesis by dinoseb.[64] Price[23] considers membrane damage and symplast disorganisation a common result of herbicidal activity.

Herbicide distribution can be markedly influenced by fixation at metabolically non-active sites; vacuolar adsorption and protein and lipid binding in leaf tissues may occur.[24] The binding of 2,4;D and other phenoxy compounds has been considered by Ashton[148], O'Brien,[149] and in review articles by Crafts[10] and Robertson and Kirkwood.[103] In general it appears that movement within the plant is not a necessary consequence of foliar penetration and that immobilisation can occur either within the treated leaf or in a region of the plant through which the transporting stream passes. Additionally, the rate of translocation of a herbicide is not necessarily related to the rate of flow of water along which it is normally transported. O'Brien[149] demonstrated that although the rate of foliar penetration of 2,4-D by Avena can be twice that for Phaseolus, the herbicide is retained in the treated leaf of the former but translocated freely in the latter, even though the treated leaves of both species are at the same time readily exporting assimilates.

1.5 Methods of studying translocation

The study of the translocation of applied exogenous compounds necessitates finding the compound (and its degradation products) in the plant. Research on the absorption and translocation of exogeneous compounds is dependent on the ability to measure minute quantities of such materials in different tissues. A number of techniques have been reported for identifying the distribution of exogenous compounds in plants.

Biological assay is one technique that has been used to study translocation. Any compound having biological activity will evoke a growth response from a test organism, and, if the conditions of experimentation are exactly reproduced, a response of the same magnitude may be subsequently elicited with the same concentrations of the active compound. With the hormone type herbicides biological response has been used to provide information on translocation.[98,150] Early studies[151] on the phenoxyacetic herbicides used the appearance of epinasty as a qualitative indication of movement, and the angle of bending of bean epicotyls as a quantitative measurement of translocation. Such curvature measurements provide a bioassay by which the herbicide content in solutions obtained by extracting plant tissues from treated plants can be quantified. The technique can prove unreliable if endogenous compounds are extracted from the plant tissue which inhibit or enhance the growth process being used as the bioassay.[152-154] Suitable precautions, for example parallel studies with control solutions, can reduce this hazard. The main limitations of this technique are that it is only suitable for compounds for which there are good bioassays, the plant response to the chemical increasing in an order related to the dose,

and that the variation of plant response as a consequence of environmental variables and inherent genetic variability set a low limit to the precision of biological assay techniques.[150]

The main techniques used previously to study translocation involved the use of radioactive labelled compounds. With such materials two approaches to the problem of locating the applied activity have been extensively used, radio-autography and extraction and counting of the extracts and liquid scintillation counting.

(i) Radio-autography

The practical basis of radio-autography is to expose prepared plants to an X-ray film, the resulting darkened areas on the developed film indicating the distribution of the radioactivity in the plant. The technique allows the general distribution of activity in the plant to be identified, but is subject to a number of limiatations. The plant material to be exposed to the film must be dried because of the long exposure times required and the possibility of chemical interference with the sensitive film. Redistribution of water soluble substances during sample preparation gave rise to artifacts in the early work with radio-autography.[155,156] Plants can be cut into sections before drying, but this can lead to an accumulation of the activity present at the cut surface.[98] Many of the problems associated with sample preparation have been overcome by the freeze drying method of Yamaguchi and Craft.[136] In their method the plant is frozen with dry ice and dried under vacuum prior to exposure, so arresting any movement of and in water.

Identification of the activity visualised on the film is one limitation of radio-autography. Although the darkened area formed

locates the activity in the plant, it does not necessarily mean that the applied compound is present. Degradation of the applied compound may occur in the plant and the label can be in degradation products or assimilated into new products, for example conjugates formed with plant sugars. Additional tests to confirm the identity of radioactive compounds found by radio-autography are necessary if the darkened areas on the film are to be equated with the herbicide.[1,148] Amitrole is unstable in plants and autographs do not produce a true representation of the transport of this compound.[98] Dalapon, in contrast, appears to be reasonably stable and its distribution can be deduced from radio-autography.[98] The problems associated with identification may be solved by using a compound containing labels in different positions in the molecule,[157] or by doing parallel studies on degradation. If it can be shown that no degradation occurs within the time frame of the tests, the image formed by radio-autography gives a qualitative appreciation of the distribution of the applied compound. The lack of an image on the film is a good indication of the absence of translocated material, providing the tissue being investigated is not too dense. The major limiation to radio-autography is that the technique does not give a reliable quantitative measurement of the compound in the plant. It is not possible to accurately correlate the image intensity with the quantity of compound present because the intensity depends on the density of the tissues through which the radiation must pass. Radiation from vascular tissues may be completely absorbed by overlying tissues because of this.

(ii) Extraction technique

A quantitative measure of translocation can be obtained by counting the radio-activity in extracts of treated plants. The technique involves extraction of the plant material with some suitable solvent, purification of the extract, and separation by fractionation or chromatography when degradation is a factor to consider, prior to counting. Extraction of plant tissue always gives rise to endogenous compounds going into solution as co-extractants. When it is intended to quantify the final solution by liquid scintillation counting the presence of coloured co-extractants is particularly undesirable. Quenching of scintillation by colour reduces the efficiency of counting, and the sensitivity of the analysis. Endogenous material that does not absorb visible radiation will generally not interfere with the method and can be ignored. Colour in the extraction solutions can be destroyed by bleaching with chlorine, and does not represent a limitation to the method.

As with radio-autography, extraction and counting of the activity from treated plants may not differentiate between the applied compound and metabolites or degradation products. Some selectivity may be achieved by suitable choice of solvent for the extraction. One advantage of this technique over radio-autography is that the method allows differentiation to be achieved without recourse to parallel studies. The extraction solution can be fractionated by a number of techniques and the fractions counted, or can be divided, part being used to determine the total activity and part to determine the number and identity of the species contributing to the total activity.

Fractionation of the extract may be achieved by Thin Layer Chromatography, when the activity on the plate can be located by scanning with a suitable detector and quantified by removing the areas of silica and counting. HPLC and GLC may also be used for fractionation and quantification. With the sensitivity of liquid scintillation counting, the technique of extraction and counting allows a comprehensive, quantitative, picture of the distribution of the applied compound to be obtained.

In the absence of a radioactive label the investigation of the translocation of the exogenous compound is complicated by co-extracted endogenous compounds, which may necessitate elaborate purification of the extraction solutions. If a characteristic chemical reaction of the herbicide to produce a coloured complex or compound can be found then this may provide a method of analysis.[150] An advantage of such a colorimetric method is that it can be very specific to the exogenous compound, but the introduction of a chemical reaction in the analysis may introduce errors from losses or incomplete reaction. Instrumental methods, in contrast to colorimetric techniques, can be direct and sensitive, but are more susceptible to the problems of endogenous impurities. Chromatography on two or more stationary phases allows positive identification of extracted material.

1.6 Compartmental modelling of foliar uptake

It was suggested earlier that the foliar uptake of a solute molecule occurring via a direct pathway can be discussed in terms of sorption into the epicuticular wax layer, diffusion across this layer and desorption into the underlying cutin and apoplast. This permeation of the outermost leaf can be considered in terms of simple physico-chemical principles which can be incorporated into compartmental models. Bridges and Farrington[150] and Price[23] have considered uptake and transport in terms of models: partition, diffusion and movement in flowing liquid steams have been identified as the main movement processes, opposed by binding and accumulation in backwaters. Solubility, partition coefficient, charge status and binding characteristics can be recognised as important physical properties of the solute.

Figure 1 shows a three compartment model which may represent movement of applied compounds through a lipid phase. The basic premise of the model is that movement across the lipid phase occurs through the bulk of this phase by diffusion. Movement between compartments is assumed to occur by partition. At zero time an instantaneous, finite concentration of solue (C_1) is developed in compartment 1. At later times some concentration (C_3) is developed in compartment 3. Within compartment 3 movement may be by diffusion or movement in flowing liquid streams. If compartment 3 is considered to represent a sink then solute will be accumulated therein; conversely if this compartment is swept out by flowing liquid streams then C_3 will tend to zero.

Partition between compartments 1 and 2 will be governed by the equilibrium distribution coefficient ($K_{1,2}$). Partition rate constants will be very high relative to diffusion rate constants[158] and it is

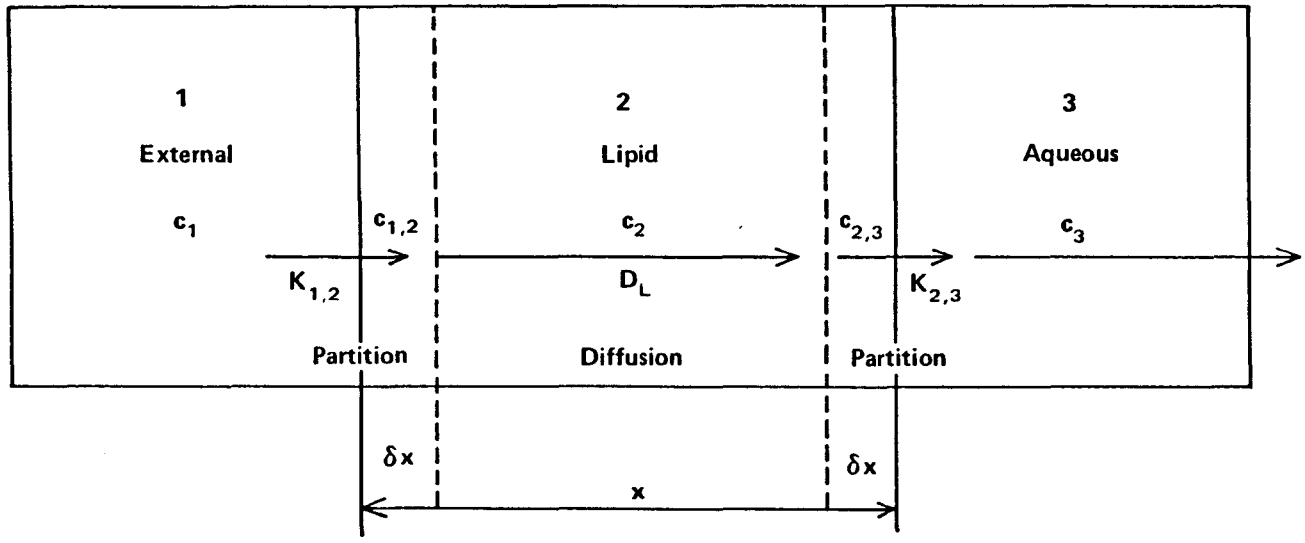


Fig 1 Compartmental model of foliar uptake

The cuticle is represented as a homogeneous lipid phase(2) separating some (unspecified) external phase (1) and an internal, aqueous phase (3). Movement through the model is by partition across interfaces and diffusion across the lipid phase. Solute may be retained in compartment 3 or may be lost by diffusion or movement in flowing liquid streams.

assumed that equilibrium distribution across interfaces is attained instantaneously. As a consequence, and from above, the interfacial concentration $C_{1,2}$ must be attained at zero time.

The interfacial concentrations $C_{1,2}$ and $C_{2,3}$ establish the concentration gradient across the lipid phase. Figure 2 shows the effect of partition at membrane interfaces on the concentration gradient across the membrane when the partition coefficient favours sorption by the membrane. The concentration gradient is the concentration distribution across the membrane and is related to the flux across the membrane by Ficks laws of diffusion introduced earlier. When diffusion occurs in such a way that the distribution of concentrations within the system remains constant a steady state is said to exist and Ficks law may be expressed as

$$\frac{F}{A} = D \frac{C_1 - C_2}{X}$$

where C_1, C_2 and X retain their meanings from Figure 1. D is assumed to be constant. Conditions approximating to the steady state are to be expected when solute diffuses across a membrane between two large reservoirs whose concentrations remain effectively constant. These conditions may be met when the volume of compartment 1 is much larger than the interfacial volume, ($C_{1,2}$ constant), and loss from the inner interface is relatively rapid, ($C_{2,3}$ tends to zero): under these circumstances the flux may be given by

$$\frac{F}{A} = D_L \frac{C_{1,2} - C_{2,3}}{X}$$

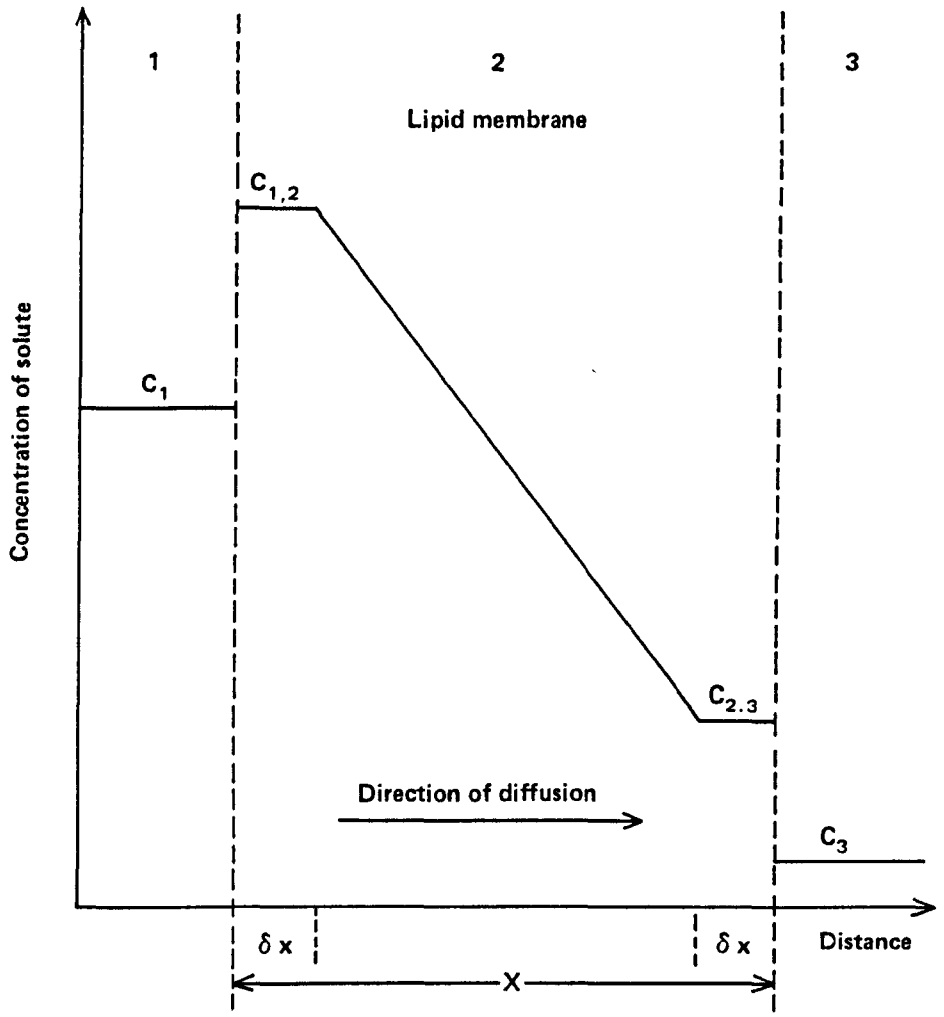


Fig 2 Concentration distribution (gradient) across membrane showing effect of partition at membrane interface

This model is qualitative at this stage because most of the basic data are not available. In qualitative terms the model may be of use in gaining some understanding of foliar uptake. Thus, it is apparent that movement across compartment 2 would be expected to be highly dependent on the partition characteristics of the solute. Given that $K_{1,2}$ will be the ratio of C_1 to $C_{1,2}$, then for a given value of C_1 the interfacial concentration might be directly dependent on the partition coefficient. Assuming other factors are constant and $C_{2,3}$ unchanged, any increase in $C_{1,2}$ will then be manifested in the rate of uptake of the solute by the membrane. A direct correlation might be predicted between the partition characteristics of the solute and the rate of uptake if the partition were varied without affecting diffusion across the membrane.

It is of course unlikely that $K_{1,2}$ might be made more favourable without adversely affecting $K_{2,3}$. This need not be a constraint if solute is lost from compartment 3 such that C_3 tends to zero. An additional constraint to uptake is provided by the saturation capacity of the interfacial zone (δx) for the solute, which will be of importance when partition occurs at a much higher rate than subsequent diffusion transfer. If $C_{1,2}$ is limiting, any changes in C_1 and/or $K_{1,2}$ may above certain values have no affect on movement through compartment 2. In such a situation increasing C_1 at constant volume might result in decreased percentage uptake. Increasing the cross-sectional area of the interface (by dilution) might facilitate uptake in these circumstances since the same flux could be established over an increased area.

Diffusion across unit area of the lipid phase occurs at a rate determined by the diffusion coefficient and by the concentration gradient. The diffusion coefficient will be dependent on the nature of the lipid phase and on the nature of the solute. When diffusion is considered in terms of movement via holes in the solid as described earlier then the molecular volume of the solute would be expected to be of importance: at some upper limit of molecular volume size exclusion should occur.

The concentration gradient will reflect the extent to which solute can be removed from the inner interface and can be greatly influenced by events in compartment 3. Mechanisms serving to deplete C_3 will favour continued uptake by maintaining the concentration gradient. Such processes will be of particular importance for lipophilic solutes for which low values of C_3 might neutralize the effective concentration gradient across the interface. It is also possible that solute may be rapidly lost from compartment 3 and accumulated at some location remote from the site of uptake until saturation at this location inhibits further loss from compartment 3 and, in turn, inhibits continued uptake.

In this context it is of interest that Hull[13] has suggested that foliar uptake may be positively related to water solubility of the solute given that in general a high lipophilic tendency favoured such adsorption. The foliar uptake by cotton of alkylamino-s-triazines has been shown[11] to increase as the water solubilities of the compounds increased between 5 and 750 ppm. Movements of these compounds was apoplastic within the transpiration stream. Such a pattern of transport results in an accumulation of solute within the treated leaf and this could inhibit

desorption out of the epicuticular waxes. The variation in foliar uptake with these relatively lipophilic compounds may be attributed to partition into the leaf tissues constituting a rate determining stage in the absorption process.

1.7 Aminopropionate Herbicides

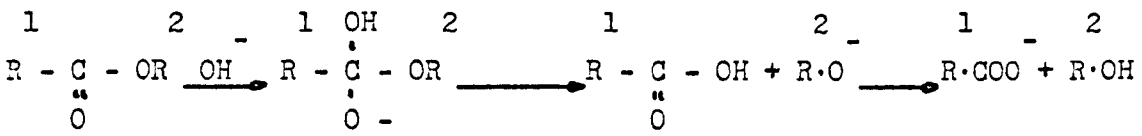
The aminopropionate herbicides are selective herbicides for the control of wild oat in wheat and barley[159-161] and are applied post-emergence in the spring as foliar applications. The general structure of these compounds is shown in Figure 3.

Flamprop-methyl(methyl(∓)-2-(N-(3-chloro-4-fluorophenyl)benzamido)-propionate), flamprop-isopropyl(isopropyl(∓)-2-(N-(3-chloro-4-fluorophenyl)benzamido)-propionate) and benzoylprop-ethyl (ethyl(∓)-2-(N-benzoyl-3,4-dichloroanilino)-propionate) can be seen to have closely related structures: Flamprop-methyl(3a) and flamprop-isopropyl(3b) are derived from the parent carboxylic acid flamprop((∓)-2-(N-(3-chloro-4-fluorophenyl) benzamido)propionic acid:3c). Benzoylprop((∓)-2-(N-(3,4-dichorophenyl)benzamido)propionic acid:3e) differs from flamprop only in the nature of one of the halogen atoms. These compounds are typically lipophilic with very low aqueous solubilities and dissolve readily in o-xylene.

The basis for the biological selectivity shown by the esters has been investigated by Jeffcoat and Norton Harries.[159-161] The ester, which is not phytotoxic, is hydrolysed to the biologically active acid which can be subsequently detoxified by conjugation with plant sugars. The expression of biological activity depends on the ease of de-

esterification of the ester to the parent carboxylic acid within the treated plant.

Grayson and Stokes[162] have investigated the hydrolysis of the esters in both acidic and basic media (Figure 4). The hydrolysis of the ester was found to occur by a bimolecular acyl bond fission (B_{AC}"2) mechanism, viz.,



The rate of hydrolysis was greater in basic than in acidic media, the rate of hydrolysis at ambient temperatures and at pH values of 7 and below being so slow as to be impossible to determine. The products of hydrolysis were similar with acid and base hydrolysis. Hydrolysis using mild reaction conditions yielded the constituent acids and alcohols (Figure 4). Further degradation was found to occur under more severe reaction conditions with debenzoylation, cleavage of the amide bond, occurring to give the N-phenyl substituted amino acid and benzoic acid (Figure 4: (iv) and (v) respectively). The amino acid was found to be unstable in very acidic media, and the corresponding aniline (VI) was isolated.

The metabolic degradation of the aminopropionate herbicides has been established by Roberts.[163-165] No gross differences were found between the degradation pathways of flamprop-methyl, flamprop-isopropyl, and benzoylprop-ethyl in any one species[163] or between wheat, barley and wild oat for any one ester.[164] In all instances the major metabolite was the parent carboxylic acid, which occurred in free and conjugated forms. It has been shown[166] that part of the conjugation of the acid in cereals is with the monosaccharides glucose and galactose.

The degradation of the ester in the plant was also contributed to by debenzoylation, aryl hydroxylation, and binding of the ester with low molecular weight proteinsor peptides, but these pathways were found to be of only minor importance. The metabolic pathway of flamprop-methyl in wheat is shown in Figure 5.

The biological activity of the carboxylic acids which resulted from the metabolic degradation of the esters was found[159-161] to involve the inhibition of stem elongation, through an effect on cell expansion, in susceptible plants. Cell elongation was inhibited in both the leaves and the stem of wild oat, and the resulting plants were unable to compete successfully with the resistant cereal crop. The selective retardation of cell elongation in Avena spp. was shown to be dependent on the relative rates of de-esterification and subsequent conjugation with plant sugars (Figure 6). The relative rates of these two reactions determines the concentration of the free acid in the plant. The basis for the selectivity of these herbicides is largely dependent on K_1, (Figure 6) the rate at which the parent acid is produced in the plant.

Jeffcoat and Harries[159] suggested that the acid was generated by enzyme catalysed hydrolysis within susceptible plants, and that the rate would depend on the level of esterase (ester hydrolase) in the plant. It is known[167] that wheat and corn contain complex systems of esterases and that carboxylic ester hydrolases vary in different species, in different strains of the same species, and in different parts of the same plant.[168] Hill[169] isolated a hydrolysing esterase fraction from wild oat which converted benzoylprop-ethyl into the corresponding acid. From his work it was not possible to differentiate

Fig 6 The basis of selectivity of aminopropionates

$$\text{ESTER} \xrightarrow{K_1} \text{ACID} \xrightarrow{K_2} \text{CONJUGATE}$$

$$\frac{d\,[\text{ESTER}]}{dt} = -K_1\,[\text{ESTER}]$$

$$\frac{d\,[\text{ACID}]}{dt} = K_1\,[\text{ESTER}] - K_2\,[\text{ACID}]$$

$$\frac{d\,[\text{CONJUGATE}]}{dt} = K_2\,[\text{ACID}]$$

If $K_1 = K_2$. then [ACID] = 0
If $K_1 > K_2$, then [ACID] > 0
If $K_1 \gg K_2$, then [ACID] = Phytotoxic concentration

between the fraction containing a specific esterase for the hydrolysis of the aminopropionate or several non-specific hydrolases. The hydrolysis of exogenous compounds requires that plant enzymes be somewhat non-specific.

Esterase activity for benzoylprop-ethyl could not be found in preparations from resistant strains of wheat. Certain fractions from these wheat preparations were found to inhibit esterase activity when added to the hydrolysing preparations from wild oat. Esterase activity was found in the wheat preparations for other compounds; certain fractions showing no activity towards benzoylprop-ethyl readily hydrolysed diclofop-methyl.[170] It would seem from these studies that wheat hydrolases may have either a differential specificity between the ester containing herbicides diclofop-methyl and benzoylprop-ethyl, or, more probably, that the hydrolases possess an inhibitor specific for the aminopropionate herbicides.

De-esterification of the aminopropionates was found[159] to occur most rapidly in oats and least rapidly in wheat. The rate at which the acid was removed by conjugation was found to prevent the accumulation of a phytotoxic concentration of acid in wheat ($K_1 \simeq K_2$, $(ACID) \simeq 0$). Although the rate of detoxification was higher in oat than in wheat it could not prevent the occurrence of phytotoxic levels of the acid in wild oat because of the very rapid de-esterification in this species ($K_1 \gg K_2$).

The degradation of benzoylprop-ethyl in barley was shown[159] to be intermediate between that found in wheat and oats, the relative rates of de-esterification and detoxification resulting in some accumulation of the acid within the plant. The accumulation of a

phytotoxic concentration of acid in barley was prevented[161] if flamprop isopropyl was substituted for benzoylprop-ethyl; de-esterification of the isopropyl ester was less rapid than with the methyl or ethyl esters and selective control of wild oat in barley could be gained.

Biological activity with the aminopropionates must produce retardation of stem elongation for useful herbicidal activity to result; as a consequence of this the rate of translocation of the acid from foliage to stem via phloem must be considered when discussing selectivity and efficacy of these compounds. A further factor involved in this consideration must be the relative inherent phytotoxicities of the acids flamprop and benzoylprop. Studies[159,161] of symplastic mobility have shown that the esters possess little or no phloem mobility, moving acropetally in treated leaves. The acids flamprop and benzoylprop were found to be phloem mobile and to be readily translocated to the stem of treated plants. Flamprop was moved about five times more rapidly than benzoylprop. Flamprop has an inherent activity against oat of approximately twice that of benzoylprop.[160] The greater transport of flamprop results in a relatively high accumulation in the stem apex. This results in both an increased inhibition of cell elongation and a reduction in apical development. The latter is a particularly favourable feature since it leads to reduced seed formation in oat.

2. Experimental

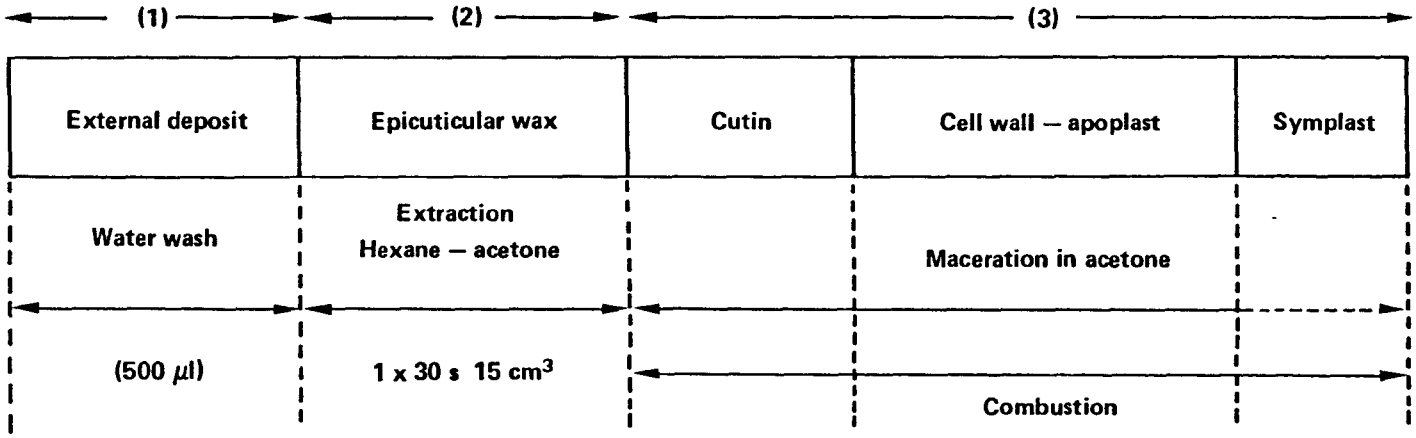
2.1. Materials

Flamprop-methyl labelled with ^{14}C in the halophenyl ring was obtained ex Shell Research Ltd,. Sittingbourne Biosciences Laboratories, as a solution in acetone (0.3 $ug.cm^{-3}$) with a specific activity of 25.7 $uCi.mg^{-1}$. The non-labelled compound was obtained as recrystallised material (m.pt: lit, 84°-86°C; found, 84°-86°C). Aqueous solutions of the herbicide were prepared freshly prior to use in triply distilled water freed from organic impurities (surface tension 71.0-71.8 $mN.m^{-1}$).

The plant species used in these studies were grown from seed under glass in pots of a John Innes Number 2 compost. Wheat (Triticum vulgare) cv sappo, barley (Hordeum vulgare) cv Imber, and wild oat (a mixed population of Avena fatra and A. Ludoviciana) were grown to the third or fourth leaf stage prior to treatment. All plants were maintained by sub-irrigation.

2.2 Environmental conditions

Within the glasshouse temperature and relative humidity were not controlled and were subject to seasonal and diurnal variation. Figure 8 shows the variation in temperature and relative humidity (Thermohygograph; Casella, London) over a period during which the maximum daily temperature varied between 17°C and 24°C. It can be seen that temperature was subject to a variation of 13° between 11°C and 24°C, and that the relative humidity varied between about 45% and 74%. Significantly lower humidities were monitored at higher temperatures and at 30°C the relative humidity typically was about 30%.



(1) compartment 1 Figure 1.

(2) compartment 2 Figure 2.

Fig 7 Selective recovery of material from leaves treated by topical foliar applications

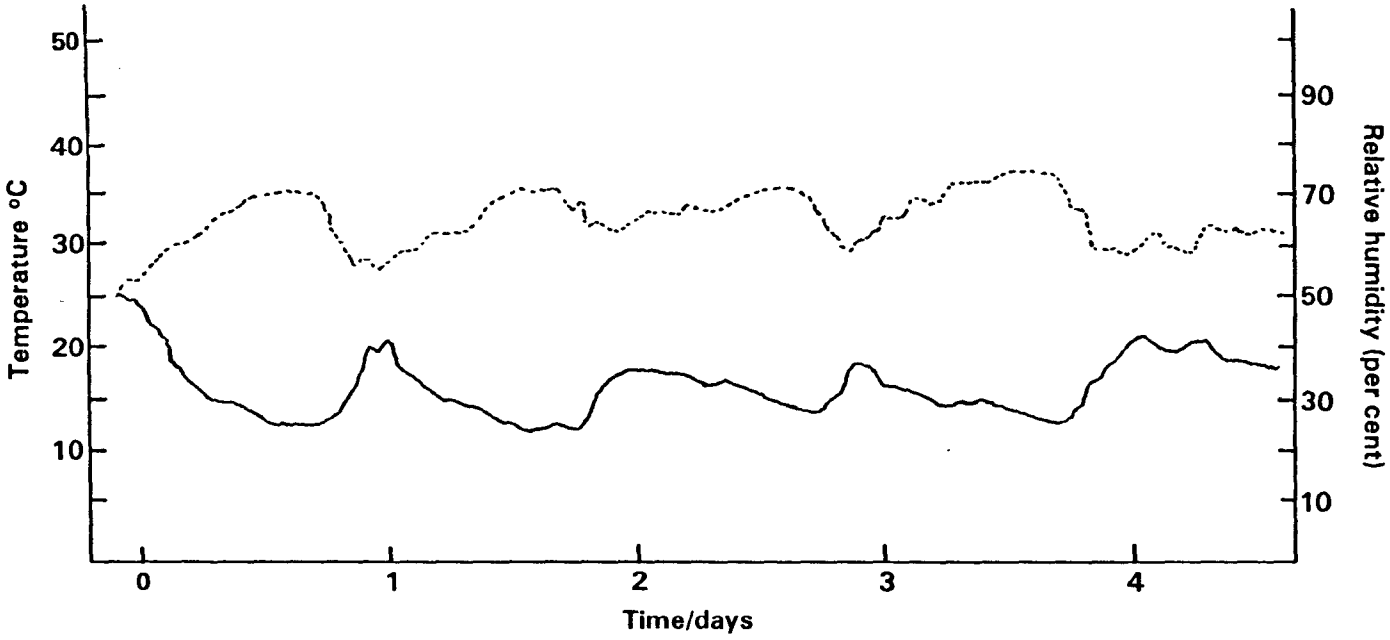


Fig 8 Variation in temperature (solid line) and relative humidity (broken line) in the greenhouse over a four day period in which the maximum daily temperature ranged from 17° to 24°C

Daylight in the glasshouse was augmented by 6 x 40 Watt Atlas fluorescent tubes suspended above the plants, which were used to maintain a 16 hour photoperiod. Light intensity at the plants was measured at 11000 lux..

In some experiments plants were transferred to a controlled environment three days prior to treatment. In these studies plants were grown on at 25°C ± 1°C and at a relative humidity of 40% ± 10% under artificial light provided by 4 x 40 Watt Atlas fluorescent tubes suspended 40 cm above the plants on a 16 hour photoperiod.

2.3 Techniques

The experimental method used to investigate the foliar penetration of flamprop-methyl can be divided into four phases; (i), treatment by topical application of a fixed volume of an aqueous solution of the herbicide, (ii), selective extraction of treated plants, (iii), purification of extracts and (iv), quantitative and qualitative analyses of the various extractions. Treatment and recovery techniques were similar in all experiments.

Purification of extracts and analysis differed according to whether radioactive labelled compound had been used to treat the plants. The amount of herbicide in extracts was quantified by either Liquid Scintillation Counting (LSC) or Gas Liquid Chromatography (GLC), the latter technique being used when non-labelled compound had been used to treat plants. The theory and practice of LSC and GLC have been discussed fully elsewhere.[171,172] GLC was only used to investigate the distribution of the herbicide in plants and was not used to investigate the formation or distribution of metabolites. Flamprop-methyl was identified in GLC studies by the relative retention

time. When LSC was used to quantify the amount of herbicide in plant extractions the solutions were also qualitatively investigated by Thin Layer Chromatography (TLC) to identify species contributing to the total activity.

(i) Application

Plants at the third or emerging fourth leaf stage were selected for uniformity of appearance. A fixed volume (typically 10 µl) of an aqueous solution of flamprop-methyl was applied directly to the upper (adaxial) surface of the horizontally mounted second leaf with a Hamilton microsyringe by expressing the required volume from the syringe and transferring the resultant drop by touching the liquid to the leaf surface. Application was usually to an area of the leaf 10 cm from the leaf tip.

(ii) Recovery

Aerial portions of the treated plants were harvested by severing the stem at soil level and separated into treated leaves, first leaves, upper foliage (acropetal to treated leaf) and stems.

a) Recovery from the area of treatment (Figure 7)

Compound external to the epicuticular wax layer was recovered by a water wash (500 ul) applied as a number of drops from a hypodermic syringe to the area to which the herbicide solution had been applied.

The amount of flamprop-methyl in the epicuticular waxes was determined by immersion (1 x 30 s) of the water washed leaf in a solution (15 cm^3) of redistilled hexane (b.pt 64-67°C) containing acetone (10%(v/v)AR grade).

b) Recovery from plant tissues

The dewaxed leaf was allowed to stand until dry (about five mins)

and was then divided transversely into two cm. strips which were combined for replicated treatments and charged to the bomb of a Sorvall Omni-mixer (Model 17106). Extraction was effected by maceration (2 x 60 s) in acetone (10 cm^3). The mixture was filtered through a Whatman Number 1 paper to separate the extract from residual plant material. The first leaves, upper foliage and stems of treated plants were also, separately, macerated.

c) Recovery from maceration residues

The maceration residue of plants treated with radioactive labelled compound was collected and dried overnight. The dessicated solid was weighed into paper cups and oxidised by combustion (Packard Sample Oxidizer: Model B306). $^{14}CO_2$ was absorbed into Carbo-Sorb (ex Packard : 7 cm^3) and the solution was displaced into a scintillation vial with alkaline scintillation solution (Permfluor V, ex Packard : 13 cm^3) and recovery quantified by LSC. The efficiency of sample combustion and of recovery was determined by oxidizing samples containing known amounts of activity prior to and at intervals throughout the analysis of plant residues.

(iii) Purification of extracts : (a) for LSC

The maceration solutions were in general strongly coloured and required some purification if they were to be counted with moderate to good efficiency. Decolorisation was effected by evaporating extracts to dryness in a scintillation vial with nitrogen and partially redissolving the residue in acetone (500 µl). Chlorine gas was passed over the surface of this mixture for about 10 s. and the vial was shaken and allowed to stand (5 mins) before excess chlorine was displaced with nitrogen gas.

(b) for GLC (Figure 10)

Acetone solutions from plant macerations were purified by chemical partition prior to GLC analyses. The solution was evaporated to 10.0 cm^3 and an aliquot (4.0 cm^3) was pipetted into a 10 cm^3 measuring cylinder and extracted with a solution of acetonitrile/water (3:2)(3 x 3 cm^3). The extracts were combined in a 50 cm^3 measuring cylinder and diluted with 30 cm^3 of brine. This solution was extracted with redistilled petroleum spirit (b.pt 64°-67°C)(3x5 cm^3). The combined extract was filtered through a pad of anhydrous sodium sulphate, additional solvent being used to elute material adsorbed on the drying agent. These washings were combined with the extract, the total evaporated to dryness and the residue redissolved in hexane/acetone (4.0 cm^3).

(iv) Analysis

(a) Liquid Scintillation Counting (LSC)

Radioactivity in the extracts of plants treated with ^{14}C-herbicide was measured with a Packard Tri-Carb Liquid Scintillation Counter (Model 3320), a three channel instrument equipped with an external standardisation facility, co-incidence counting and refrigeration of samples. Disintegrations from carbon-14 were measured in channel one, which was set with a gain of five per cent and with the discriminator almost fully open (50-1000). Channels two and three were set for quench correction using the External Standards Ratio (ESR) method, with a gain of 0.4 per cent and discriminator settings of 80-1000 and 200-1000 respectively. Extracts were evaporated to dryness in a stream of dry nitrogen and the residues dissolved in a toluene solution (15 cm^3) containing 2-(4'-t-butylphenyl)-5-(4''-biphenyl)-1,3,4-oxadiazole, (Butyl BPD, 8 g per 1000 cm^3) and iso-octylphenoxypolyethoxyethanol,

(Triton X 100, 250 cm^3 per 1000 cm^3), and the activity in the solution determined by counting for between 1 and 10 minutes. Carbon-14 decay in each solution was counted three times; in addition each solution was counted for one minute with the external standard in position to determine the degree of quenching. From the measured counts a mean value was calculated which was corrected for background and transformed to disintegrations per minute at 100 per cent counting efficiency.

(b) TLC/LSC

A known volume of extract was evaporated to one tenth of its original volume and 100 ul was applied as a series of 5 µl drops to a pre-coated TLC plate (DC-Fertigplatten SIL G-25 UV 254, a 0.25 mm silica gel plate with fluorescent indicator UV 254). The applied solution commonly covered a band 15 x 0.5 cm, and was eluted over about 15 cm with hexane/acetone (7:3). The developed plate was inspected with a thin layer scanner (Panax RTLS 1) and areas of radioactive silica were removed from the plate and counted by LSC.

(c) Gas Liquid Chromatography (GLC)

GLC analysis was performed using a Pye 104 instrument fitted with a ^{63}Ni Electron Capture Detector (ECD) on 1.5 m x 3 mm OD glass columns packed with 2% OV 225 on Gas Chrom Q (100/120 mesh) and conditioned for 120 hours at 230°C before use. Nitrogen (100%) was used as carrier gas at an inlet pressure of 28 psi and at a flow rate of 60 $cm^3.min^{-1}$. Chromatography was performed isothermally at 218°C. Injection port temperature was 240°C. ECD was operated in pulse mode with a pulse space of 150 us and at a temperature of 320°C.

The amount of flamprop-methyl in a extract was determined by comparing the detector response for the sample against a calibration

curve of detector response as a function of herbicide concentration (Figure 9). Calibration was with a solution of known concentration; flamprop-methyl was dissolved in acetone to give a 30.0 ppm solution which was volumetrically diluted with hexane/acetone (9:1) to 0.015 ppm. A calibration curve was constructed from triplicated determinations of ECD response after injection of one of seven volumes of the standard solution between 2 and 8 ul, (Figure 9). Calibration was effected immediately prior to analyses of plant extracts and was re-evaluated at intervals throughout analysis.

2.4 Partition studies

Cereal plants (wheat, oat, barley) at the emerging fourth leaf stage were treated with a 30 ppm aqueous solution of ^{14}C-flamprop-methyl. At selected times during the period required for the applied drop to evaporate (at zero time and 10, 15, 30, 45, 60, 75, 90, 95, 97.5 and 100 mins after treatment) the residual drops were recovered by touching a piece of glass-fibre paper to the liquid. The leaf surface was subsequently washed with water (500 µl) and the radioactivity recovered in the paper and in the wash water was separately quantified by LSC.

This experiment was repeated using paraffin wax surfaces formed on glass and using wheat plants deprived of water for five days immediately prior to treatment.

2.5 Stability of surface deposits

(i) Volatilization

A 30 ppm aqueous solution of ^{14}C-flamprop-methyl was applied to glass coverslips at a rate of one 10 ul drop per coverslip. At zero time and subsequently at 24 hour intervals over a period of seven

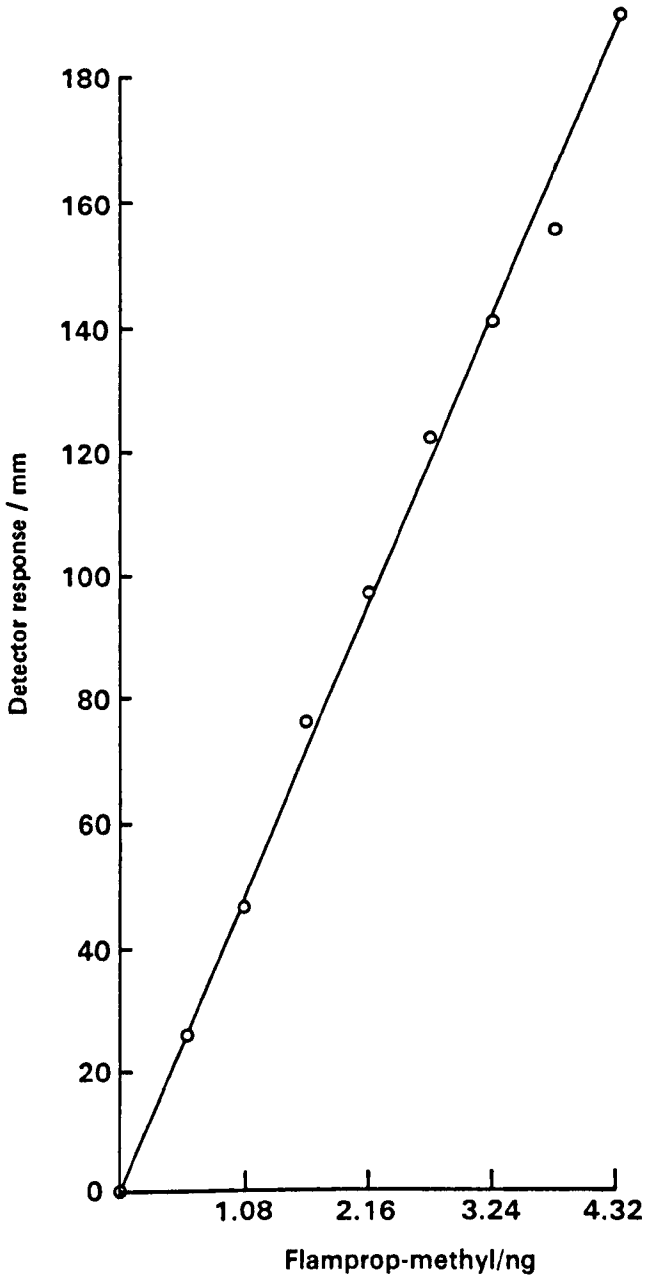


Fig 9 GLC/ECD calibration curve for flamprop-methyl

days the residual activity on the surfaces was determined by immersion of the coverslips in scintillation solution and counting the solutions using LSC. All analyses were based on triplicated determinations of each value.

This experiment was repeated over a time frame of 10 days following topical application to coverslips pretreated by immersion (60 s) in a bath of paraffin wax (BDH: clearing point 65-71°C) maintained at 80°C. The wax layer formed on the glass was allowed to harden overnight prior to treatment.

(ii) Photochemical stability

Glass coverslips treated with ^{14}C-flamprop-methyl were sealed in clear-glass vials and left to stand for 10 days, being exposed to direct sunlight for about 140 hours over this period. Activity was recovered from the opened vials by an acetone wash and was quantified by LSC. Species contributing to the total activity were investigated by TLC/LSC.

2.6 Foliar uptake by wheat

Wheat was grown from seed in the glasshouse to the third leaf stage and transferred to the controlled environment three days prior to treatment. The adaxial surface of the second leaf on selected plants was treated by topical application of an aqueous solution (30 ppm) of flamprop-methyl: 0.3 ug of the herbicide was applied in a single 10 ul drop to each plant. Plants were harvested at zero time and 1, 2, 4, 7, 10 and 14 days after treatment. Additionally treated leaves were harvested 90, 150 and 180 mins after treatment. A minimum of five replicates were taken at any one time and up to ten replicates were taken 10 and 14 days after treatment. Recovery of flamprop-methyl and

quantitative analysis of the extracts were performed according to the techniques described earlier.

2.7 Effect of dose applied on foliar uptake

Wheat plants established in the controlled environment were treated at the emerging fourth leaf stage with aqueous solutions of flamprop-methyl. Applications used were :-

(i) treatment with either 1 x 10 µl or 1 x 30 µl drops of a 30 ppm solution,

(ii) treatment with either 3 x 10 µl or 1 x 30 µl drops of a 30 ppm solution, and

(iii) treatment with either 5 x 10 µl of a 4 ppm solution, 1 x 10 µl of a 20 ppm solution, 5 x 10 µl of a 20 ppm solution or 1 x 10 µl of a 100 ppm solution.

All treatments were replicated five times and were performed according to a randomized block design. Treatments in (iii) were of flamprop-methyl formulated in water containing 10% (v/v) acetone. In all studies uptake was assessed after 48 hours by extraction with hexane/acetone to recover compound external to the leaf tissues.

2.8 Foliar uptake by cereals in unregulated environments

The foliar uptake by wheat of flamprop-methyl was studied in a series of five experiments, conducted over a period of 15 months, using glasshouse plants maintained in a glasshouse for the duration of the studies. Treatment was of one 10 µl drop of a 30 ppm aqueous solution applied to the second leaf of plants at the emerging fourth leaf stage. In each study the maximum temperature to which the plants were subjected within each 24 hour period post application was recorded. Data were obtained at maximum daily temperatures of between (i) 0°-5°C,

(ii) 15°-18°C, (iii) 20°-25°C, (iv) 25°-35°C and (v) at maximum daily temperatures greater than 35°C. Data were also obtained at maximum daily temperatures of between 20°-25°C on the foliar uptake of flamprop-methyl by barley and wild oat. In all studies, recovery of flamprop-methyl and quantitative analysis of the extracts were performed according to the techniques described earlier.

RESULTS AND DISCUSSION

3.1 Experimental rechnique

The foliar uptake of flamprop-methyl was studied following topical application of the herbicide as a discrete drop of an aqueous solution and redistribution of the compound determined by selective recovery using solvent extraction techniques (Figure 7). Redistribution of the surface deposit of flamprop-methyl was studied using a water wash and/or extraction of the surface with hexane/acetone.

Table 1 shows the recovery of flamprop-methyl from treated leaves effected by repeated water washings. It can be seen that recovery was confined to the first extract and that subsequent washings failed to extract material out of the leaf. The precise delineation with regard to the distribution of flamprop-methyl at the leaf surface made by a water wash supported the assumption that material recovered by this wash was not extracted from the plant but was dissolved off the leaf surface.

A variety of solvents were investigated with a view to recovery of flamprop-methyl from the epicuticular wax layer. Although chloroform has been used to extract the wax layer in studies of epicuticular wax composition, the use of this solvent to recover selectivity a herbicide from the waxes can be criticised on the grounds that, with a solvent of relatively high polarity, material might be simultaneously extracted from underlying layers. Chlorinated solvents were also unsuitable when the extract was to be quantified using ECD. Preliminary studies showed that a hexane solution containing 10% (v/v) acetone effected recovery of the herbicide when treated

Table 1 - Recovery of ^{14}C-flamprop-methyl from treated leaves[1] by repeated washing with water, expressed as cpm.

Extract	Recoveries[2]		
	0 days	2 days	4 days after treatment
1st water wash	15000	7800	3628
2nd water wash	-	328	213
3rd water wash	-	152	136
4th water wash	-	104	59
5th water wash	-	63	78
6th water wash	-	48	42
7th water wash	-	29	23
8th water wash	-	25	13
9th water wash	-	12	9
10th water wash	-	22	18
11th water wash	-	16	30
Hexane/acetone immersion	-	2200	2950

(1) Application of 1 x 10 ul of a 30 ppm aqueous solution equiv. to 15000 cpm.

(2) Each value represents the mean of three replicates.

leaves were immersed for 30 s. Wax extraction by this solvent was similar to that achieved by chloroform extraction in terms of both the mass extracted and the composition of the wax as determined by GLC.

Analysis of extracts by either GLC-ECD or LSC permitted determination of herbicide levels at 0.1% recovery. With GLC-ECD the limit of detection was about 1.10^{-11} g/µl.

Further recovery of herbicide was effected by maceration of dewaxed leaves in acetone and, when appropriate, by combustion of the tissue residue. These techniques were efficient at recovering applied compound up to ten days post application (Table 2).

It was found that some purification of macerates was a necessary prerequisite to quantitative analysis. Figure 10 shows the improvement in the GLC trace effected by chemical partitioning of the extract. When radioactive labelled herbicide was used the counting efficiency of macerates was markedly improved by decolourization: internal standardisation was used to establish the counting efficiency after decolourization.

In all solvent extracts of wheat treated with ^{14}C-flamprop-methyl the compound was recovered as the ester and no free acid was detected, although preliminary studies had shown that the free acid could be extracted from plant material by the techniques as used. The absence of free acid was seen in the distribution of activity on TLCs of the extracts.

Residual material in the tissue residue from maceration was not extracted by repeated maceration or by the use of hot solvents. It was assumed that the residual activity, quantified by combusion to $^{14}CO_2$, was conjugate of plant sugars and the parent acid flamprop.

Table 2 - % Recovery of flamprop-methyl from wheat ten days after treatment[1]

Extracts	Recoveries				Totals
	Treated leaf	First leaf	Upper leaves	Stem	
Surface[2][3]	42.00	0	0	0	42.00
Maceration[3]	39.50	0.90	1.60	1.80	43.80
Combustion[4]	6.14	0.08	0.27	0.50	6.99
Totals	87.64	0.98	1.87	2.30	92.79

(1) Plants treated with a single 10 ul drop of a 30 ppm aqueous solution to the adaxial surface of the second leaf. Recovery expressed as mean of ten plants.

(2) Combination of water wash and immersion in hexane/acetone.

(3) Attributed to ester from TLC/GLC.

(4) Attributed to flamprop-conjugates.

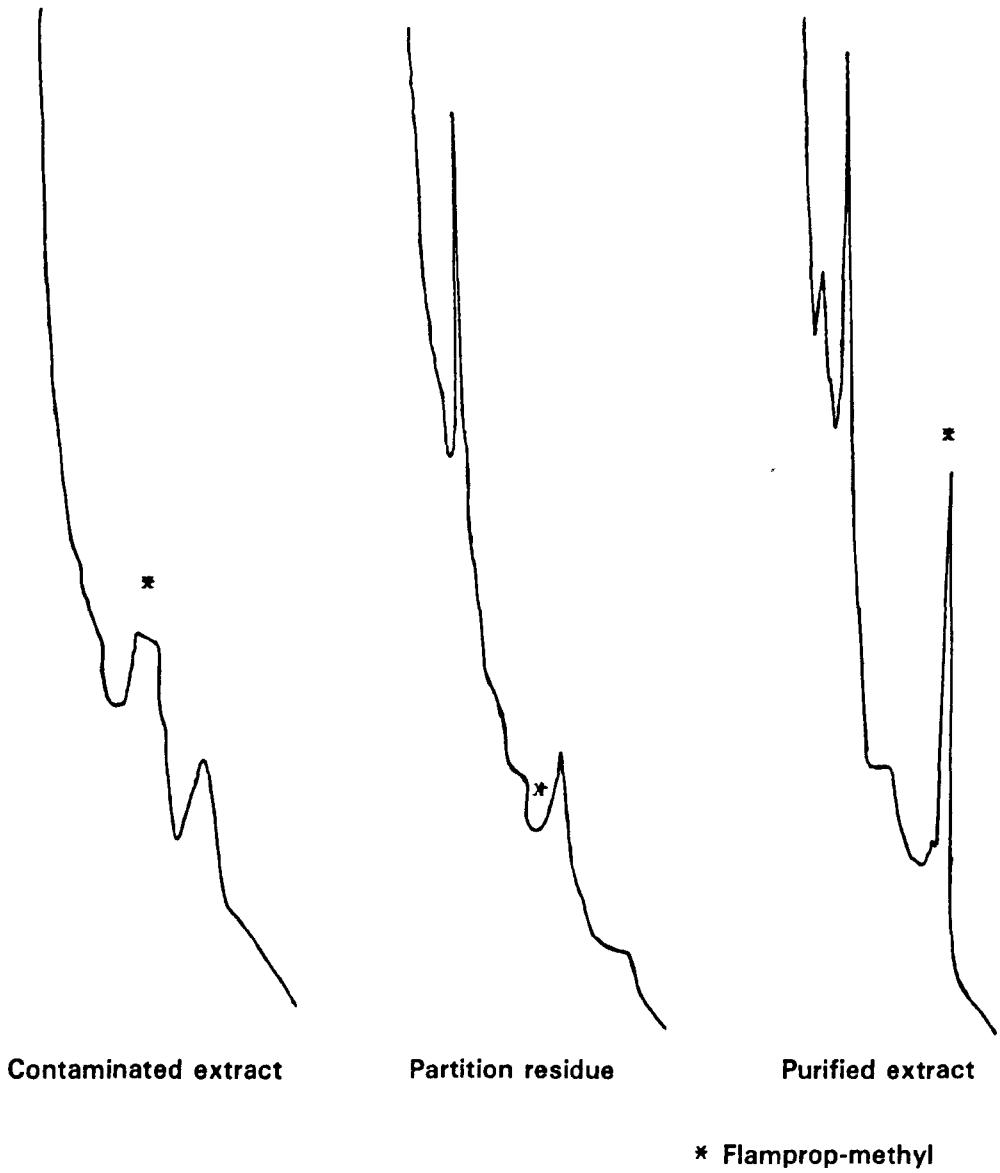


Fig 10 Improvement in GLC analysis following chemical partition of the extract

3.2 Partition studies

Figure 11 shows the redistribution of flamprop-methyl between topically applied drops and the leaf surface in terms of (i) recovery of herbicide from the residual drop by absorbtion into glassfibre paper, and (ii) recovery in a water wash of the leaf surface over the time taken for 10 µl drops to dry. These data were obtained by application of an aqueous herbicide solution (30 ppm) to both turgid and flaccid leaves of wheat, barley and oat and to wax surfaces formed on glass plate. No significant difference was found between substrata with regards to herbicide partition.

It can be seen that the movement of flamprop-methyl between the aqueous drop and the surface was largely confined to the period just prior to the drying of the drop (Figure 11: between 80 and 100 mins). At intermediate times the drop retained more than 80% (m/m) of the herbicide content at zero time, although in a reduced volume. After 75 mins between 90% and 97.5% of the initial content was retained in a volume of about 2 µl.

Figure 11 also shows that transcuticular movement of flamprop-methyl was only initiated subsequent to drying of topically applied drops, as evidenced by the total recovery of the applied herbicide by a water wash at this time. This result is similar to that reported by Farrington as typical with aqueous solutions of relatively non-polar compounds.

3.3 Surface deposits

Evaporation of the solvent from a spray drop can result in the deposition of the active ingredients onto the leaf as a surface residue. The physical form of the residue will, in the absence of additives,

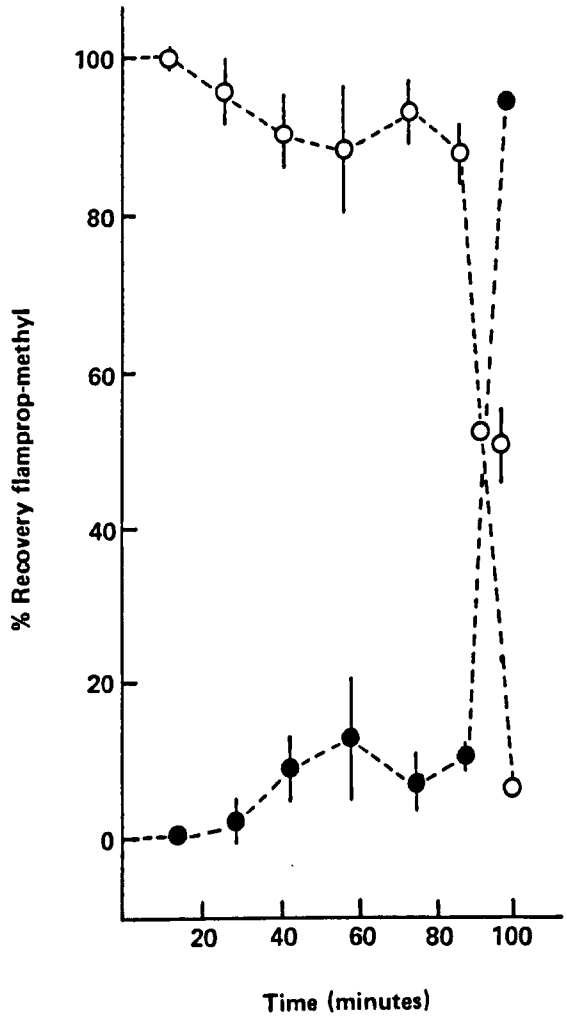


Fig 11

Redistribution of flamprop-methyl between topically applied drops and the leaf surface: recovery of material from the residual drop (○—○) and from the leaf surface by a water wash (●—●). Vertical bars denote variation (3 reps)

reflect the physical characteristics of the toxicant. The surface deposit of flamprop-methyl was examined by light microscopy with reflected light using a polarizer to view the leaf surface. The deposit was seen to be crystalline with rectangular faced crystals, about 0.04 x 0.004 mm, randomly distributed over an area of about 1.8 mm^2 following topical application of 10 µl drops of a 30 ppm herbicide solution. The small area over which crystals could be visualised accorded with earlier observations that transfer of the main part of the herbicide between the drop and the surface occurred from the small residual volume remaining after extensive evaporation.

3.4 Stability of surface deposits

Figure 12 shows the contribution of volatilization to the depletion of a surface deposit of flamprop-methyl formed on glass and wax surfaces. It can be seen that compound was lost from glass at a rate of about 10% (m/m) per day, but was not lost from a wax surface and 100% recovery was achieved up to ten days after application. It has previously been found that in considering losses by volatilization a paraffin surface can resemble the leaf surface more closely than does a glass surface. It was subsequently found that the mass balance on the total extraction of treated plants was better than 90% (m/m) after ten days (Table 2). These results show that any depletion of the leaf surface deposit of flamprop-methyl could not be attributed to volatilization. Other studies showed that flamprop-methyl was stable to photochemical and biochemical degradation on the leaf. It was concluded that any depletion of the leaf surface deposit following foliar application of flamprop-methyl was as a result of transcuticular movement, so that the rate of uptake might be inferred from the rate at which the deposit was diminished.

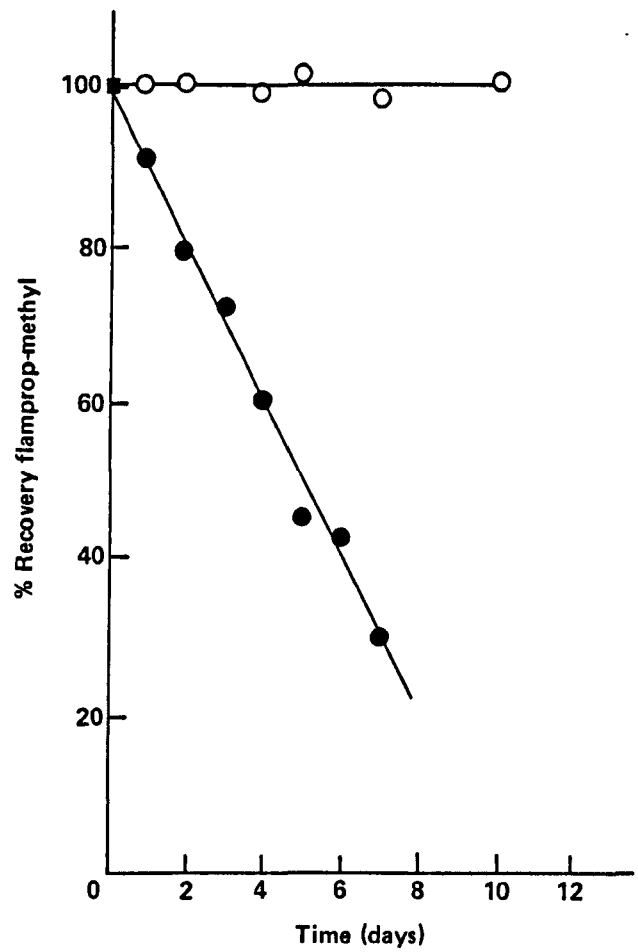


Fig 12

Contribution of volatilization to the depletion of a surface deposit of flamprop-methyl from glass (●) and wax surfaces formed on glass (○)

3.5 Foliar uptake by wheat

Table 3 shows the redistribution of flamprop-methyl between the surface deposit (water wash) and epicuticular waxes (hexane/acetone extract) up to 180 minutes subsequent to topical foliar application of an aqueous solution. It can be seen that over this time frame a large proportion of the applied herbicide was partitioned into the epicuticular waxes but that the total recovery of compound in both extracts was little changed.

Movement between the surface deposit and the leaf was studied up to ten days after treatment (Figure 13). Recovery in the water wash was found to be progressively diminished between 180 mins and ten days at an apparently constant rate which was somewhat slower than the rate of change in recovery over the first 180 mins. Partition into the leaf waxes tended to completion over ten days.

Figure 13 also shows the recovery of flamprop-methyl in a hexane/acetone extract of the water washed leaves up to ten days after treatment and the total recovery from this extract and the water wash over the same period. Recovery of flamprop-methyl from the epicuticular waxes increased to a maximum after about three hours and declined after 24 hours so that minimum recovery was found in this extract about four days after treatment. Subsequently herbicide was further accumulated in the waxes and increased until ten days after treatment when 45% (m/m) of the applied compound was recoverable in this extract. This pattern of recovery was more complicated than the more regular change in the surface deposit. When recovery in the water wash and hexane/acetone extracts were combined and plotted against time (Figure 13: combined extract) the resulting curve indicated that a relatively constant rate of uptake

Table 3 - Percentage recovery of flamprop-methyl from plants treated with 1 x 10 ul of a 30 ppm aqueous solution.

Extracts(1)	Time after treatment			
	0 min	90 min	150 min	180 min
Water wash	100	94.5	67.5	70.4
Hexane/acetone immersion	0	5.5	28.5	27.4
Combined extract	100	100	96.0	97.8

(1) Each value represents the average from three plants.

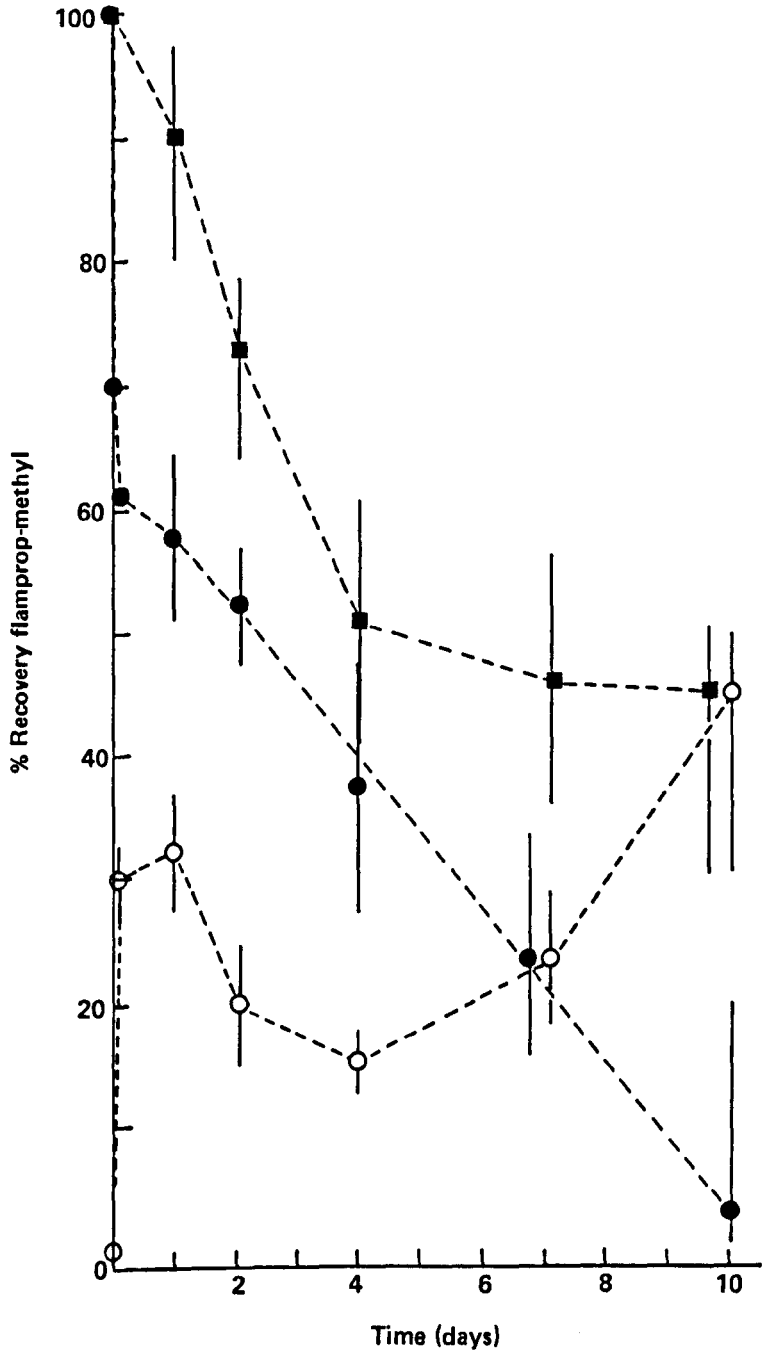


Fig 13 Foliar uptake by wheat of flamprop-methyl

Recovery of flamprop-methyl in water wash extracts (●—●), hexane/acetone extracts of water washed leaves (○—○) and recovery from combined water wash and hexane/acetone extracts (■—■) of treated leaves. Vertical bars denote variation between replicates.

was occurring between zero time (the time at which the drop dried) and four days, but that little change occurred in the redistribution of flamprop-methyl beyond this period. Figure 13 shows that the extent to which flamprop-methyl was lost from the surface deposit via the epicuticular waxes to the underlying tissues was considerably less than was suggested from the observed diminuation of the surface deposit. This discrepancy can be seen to have occurred as a result of the accumulation of herbicide in the wax layer.

The data in Figure 13 may be interpreted in terms of (i) a rapid partition of the herbicide between the surface deposit and the leaf surface waxes over the first three hours post-application, (ii) penetration of the epicuticular wax layer, occurring up to four days on average after treatment, and (iii) a subsequent accumulation of flamprop-methyl in the wax layer as a result of a combination of a continued partition between the surface deposit and the wax layer and an apparent cessation of desorption into the underlying tissues.

The accumulation of herbicide in the wax layer after four days may be attributed to an inhibition of uptake as a consequence of the accumulation of the compound at some site underlying the site of application. It was noted earlier that a compound could not be retained permanently within the cuticle simply because it has a highly unfavourable partition coefficient for desorption, but that partition out of the lipid phase might be prevented by very low concentrations in the underlying apoplast. The data obtained with flamprop-methyl suggested that a similar mechanism could be limiting continued movement into the leaf tissues.

Figure 14 shows the total recovery of flamprop-methyl from treated leaves between zero time and 14 days after treatment. The herbicide lost from the surface deposit and from the epicuticular wax layer was in large part recoverable from the treated leaf by maceration. Total recovery was typically better than 85% (m/m) over 14 days from treated leaves. After ten days the material recovered from the tissues of the treated leaf (Table 2: 45% (m/m)) was found to be mainly ester (39.5% (m/m)) with a small amount of conjugates (6.14% (m/m)). This result is in accord with the mechanism of selectivity of flamprop-methyl proposed by Jeffcoat and Norton Harries and reviewed earlier which suggests that only low levels of free acid should occur in a resistant plant species.

Figure 15 shows the longitudinal movement of flamprop-methyl in treated leaves in terms of the distribution after four and seven days. The results show that movement within the leaf was predominantly acropetal and continued up to seven days after treatment. Accumulation within the treated leaf presumably occurs as a consequence of this transport pattern.

The amount of flamprop-methyl in the leaf tissues immediately underlying the site of application was reduced (Figure 15) between four and seven days after treatment as a consequence of movement towards the leaf tip. Subsequently no further redistribution of compound towards the leaf tip was apparent up to 14 days after treatment and the amount of flamprop-methyl in the tissues underlying the site of application was constant. Correlation between the amount of herbicide in the tissues underlying the site of application and the transcuticular movement of flamprop-methyl was not obvious. It was however suggested

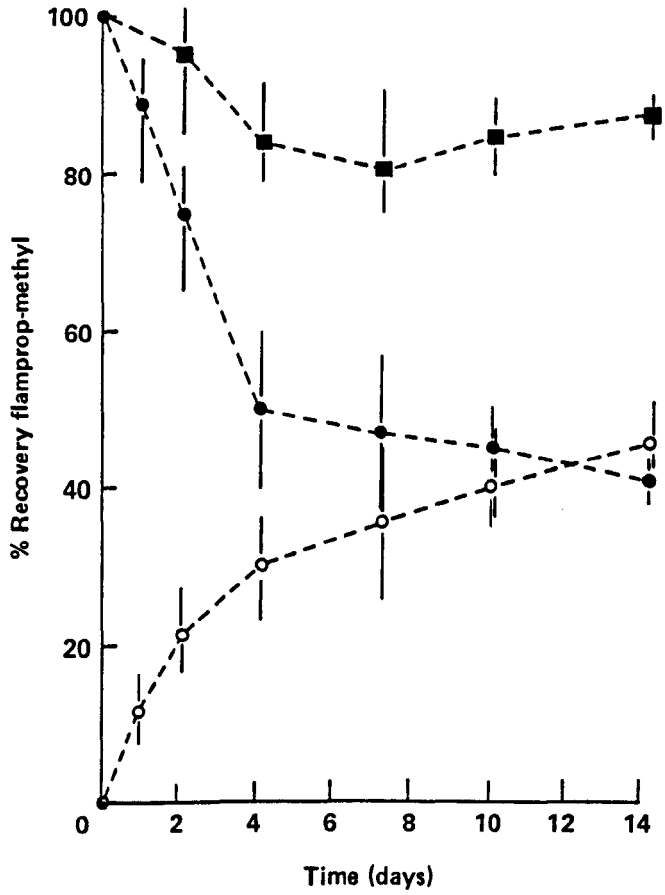


Fig 14 Foliar uptake by wheat of flamprop-methyl

Recovery of flamprop-methyl in combined water wash and hexane/acetone extracts (●– – –●), maceration and combustion studies (○– – –○) and total recovery (■– – –■) from treated leaves. Vertical bars denote variation between replicates.

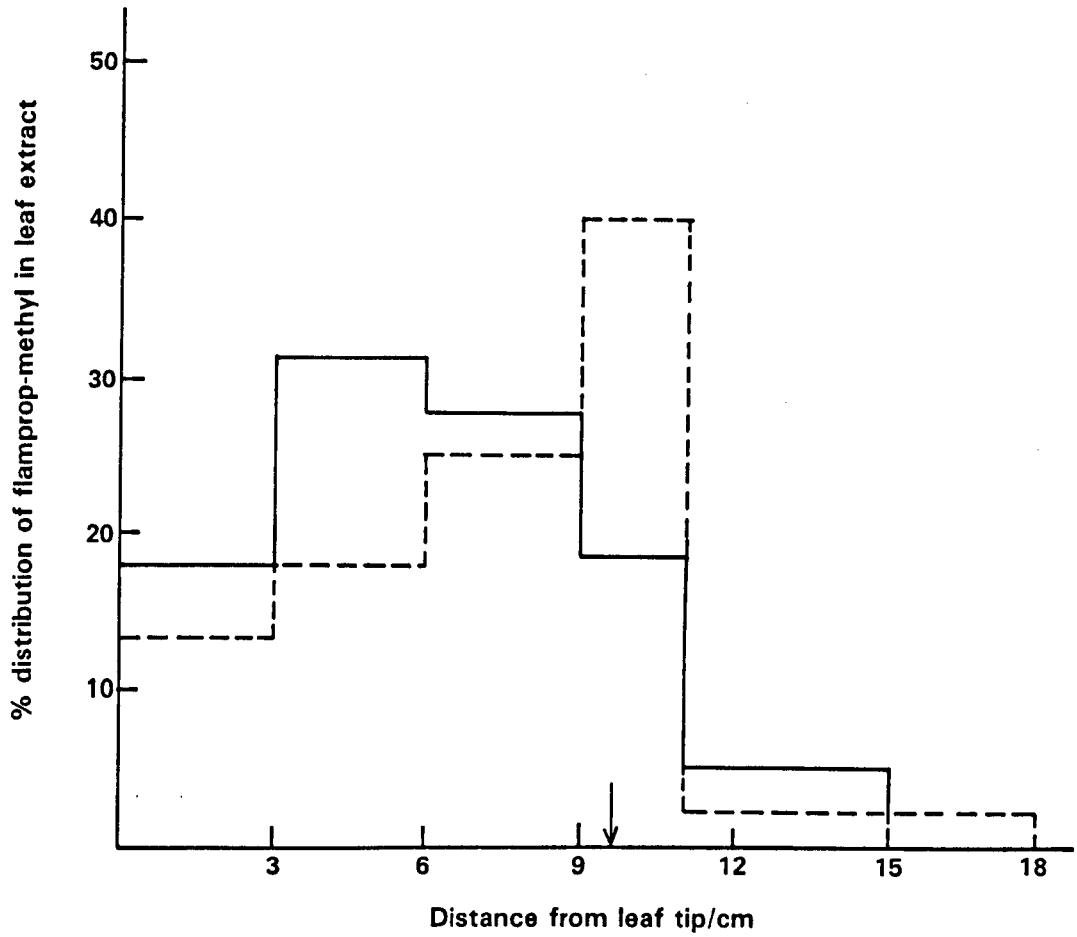


Fig 15 Distribution of activity within the treated leaf after four days, (broken line) and seven days, (solid line) after application to a point 10cm from the leaf tip (↓)

earlier that recovery of material from the leaf tissued lacked the specificity of other extractions in terms of the substratum being extracted. As a consequence, the variation in the amount of flamprop-methyl in the tissues of the mid-segment may not reflect adequately variations in the concentration of the herbicide in particular tissues underlying the cuticle.

To conclude it would appear that, following topical foliar application of aqueous solution, flamprop-methyl was deposited on the leaf surface as a crystalline deposit from which the herbicide moved into the wheat leaf. Flamprop-methyl was adsorbed from this deposit into the epicuticular waxes at a relatively constant rate which was unaffected by subsequent processes occurring within the leaf and tended to completion over about ten days. Transcuticular movement resulted in an accumulation of compound within the acropetal portions of treated leaves; this accumulation was typical of apoplastic transport discussed earlier and may have been responsible for the cessation of transcuticular movement between about four and seven days after treatment.

3.6 Translocation

Although movement of flamprop-methyl within treated leaves was predominantly acropetal, some limited symplastic mobility was suggested by the presence of small amounts of the applied compound outside the treated foliage. It can be seen (Table 2) that small but significant amounts of flamprop-methyl were recoverable from the stem and both upper and lower leaves of plants ten days after treatment. Generally movement out of the treated leaf contributed little to the flamprop-methyl in wheat following foliar application.

3.7 Effect of applied dose

In the studies discussed so far all applications were of 0.3 µg of herbicide delivered as a 10 µl drop of a 30 ppm aqueous solution. It was of interest to establish how transcuticular movement would be affected by variation in the amount of herbicide applied to the leaf. Three variables were identified;

(i) the mass of compound applied,

(ii) the concentration of the applied solution, and

(iii) the area of leaf surface treated with the herbicide solution.

Experimentation was attempted with a view to investigating the effect on foliar uptake of independently changing each of these variables.

(i) Effect of changes in the amount applied with fixed area of application

To vary the amount of compound applied whilst maintaing a fixed area of application requires that either the volume of solution applied or the concentration of that solution must be varied. Practical difficulties are introduced in keeping the area fixed when the volume applied is altered. One method of maintaining a fixed, constant area of contact was discussed earlier when the use of donor solutions was noted. In this work difficulties were encountered with this technique.

Table 4 shows the recovery of flamprop-methyl, by extraction with hexane/acetone and maceration of the dewaxed leaf in acetone, 48 hours after treatment with either one 10 µl drop or one 30 ul drop of a 30 ppm solution of flamprop-methyl. Visual observation suggested that the three-fold variation in the amount applied (0.3 µg vs 0.9 µg) was not accompanied by a similar increase in the area of contact. The results show that transcuticular movement over 48 hours was directly

Table 4 - Recovery of flamprop-methyl 48 hours after treatment with either 1 x 10 ul (low mass) or 1 x 30 ul (high mass) of a 30 ppm aqueous solution

Treatment	Extract	% Recovery					
		Replicates					Mean
Low mass	Surface(1)	74.5	80.3	76.6	73.4	71.1	75.2
Low mass	Maceration	16.4	M	19.5	19.2	M	18.4
Low mass	Combined extract	90.9	M	90.1	92.6	M	98.6
High mass	Surface(1)	84.8	82.0	77.7	70.4	77.3	78.4
High mass	Maceration	13.0	13.2	M	19.1	17.4	15.7
High mass	Combined extract	97.8	95.2	M	89.5	94.7	94.1

(1) Combination of water wash and immersion in hexane/acetone

LSD (P=0.05) for surface extract (5 obs vs 5 obs) : 6.35

proportional to the amount of herbicide applied, since % uptake was not significantly different (P = 0.05) between the two treatments.

(ii) Foliar uptake from a fixed amount of herbicide

The relationship between the amount of compound applied to the leaf surface and the extent of foliar uptake may be considered in terms of the amount per se, and in terms of the amount of compound per unit surface area of the leaf. The effect of the latter was investigated by treating plants with 0.9 µg of flamprop-methyl applied as either one 30 µl drop or three 10 µl drops of a 30 ppm solution. The extent of foliar uptake was investigated 48 hours after treatment (Table 5). The recovery of flamprop-methyl in a hexane/acetone extract of treated leaves was significantly greater from leaves treated with a single 30 µl drop of herbicide solution. Total recovery was not significantly different between the two treatments. It would seem that foliar uptake was greater when a fixed volume of solution was applied as a series of smaller drops, although the difference was much smaller than might have been expected from the nominal increase in the area of contact.

The relationship between the amount of compound applied, the area of application and foliar uptake was further investigated in one experiment when both the amount of compound applied and the leaf surface area to which application was made were varied simultaneously. Systematic variation of these terms was achieved by varying both the volume and the concentration of the solution applied. Because of the limited response noted earlier to a (nominal) three-fold variation in area, a five-fold variation in both the amount of flamprop-methyl applied and the area to which application was made was effected between the four treatments investigated in this experiment.

Table 5 - Recovery of flamprop-methyl 48 hours after treatment with either 1 x 30 ul (low area) or 3 x 10 ul (high area) of a 30 ppm aqueous solution.

Treatment	Extract	% Recovery					
		Replicates					Mean
Low area	Surface(1)	80.7	75.6	84.5	82.0	84.1	81.4
Low area	Maceration	18.8	27.4	15.8	13.6	12.6	17.6
Low area	Combined extract	99.5	103.0	100.3	95.6	96.7	99.0
High area	Surface(1)	75.8	76.7	75.7	68.7	76.9	74.7
High area	Maceration	23.1	22.6	24.5	28.9	17.9	23.4
High area	Combined extract	98.9	99.3	100.2	97.6	94.8	98.1

(1) combination of water wash and immersion in hexane/acetone.

LSD (P=0.05) for surface extract (5 obs vs 5 obs) : 6.58

for maceration (5 obs vs 5 obs) : 9.10

This variation necessitated a 25 fold variation in flamprop-methyl concentration between treatments, which in turn necessitated the use of either very dilute solutions (of the order of 1 ppm) or of solutions in which the herbicide concentration exceeded the saturated aqueous solubility of flampro-methyl. Since the use of very dilute solutions would have created analysis problems the required solutions were prepared in water containing 10% (v/v) of acetone.

The effect of acetone on the foliar uptake of flamprop-methyl was investigated in a separate experiment. The results of that study (Table 6) show that uptake was not significantly altered when acetone was incorporated in aqueous herbicide solutions prior to treatment.

Table 7 shows the % recovery of flamprop-methyl, by extraction with hexane/acetone and maceration of the dewaxed leaf in acetone, 48 hours after treatment with either one 10 µl drop or five 10 µl drops of solutions of flamprop-methyl. The concentration of the applied solution was either 4 ppm, 20 ppm or 100 ppm with respect fo flamprop-methyl. It was concluded that

(i) foliar uptake was increased if a fixed volume was applied as multiple drops (Table 7: comparison between treatment 1 and 2, and 3 and 4).

(ii) the effect of application as a series of smaller drops was more pronounced at the lower dose (treatment 3) and

(iii) % uptake was similar when the amount of compound per unit area was kept constant (treatments 1 and 4).

These results show that penetration can be improved by formulating the herbicide at low concentration and applying a correspondingly increased volume of the formulation as a number of small drops. These results

Table 6 - Recovery of flamprop-methyl in combined water wash and hexane/acetone extract at various times after application of a 30 ppm solution formulated in either water or a 10% (v/v) aqueous acetone solution

Time/days	Aqueous formulation						Aqueous acetone formulation					
	Replicates					Mean	Replicates					Mean
2	71.6	85.6	80.4	78.4	84.0	80.0	61.1	76.0	72.2	58.2	85.6	70.6
4	77.7	75.1	57.6	74.8	71.2	71.3	67.7	69.0	71.3	54.0	78.0	67.0
7	39.5	55.3	61.3	40.9	41.7	47.7	52.1	48.2	45.8	32.1	56.0	46.8

LSD (P=0.05) 5 obs vs 5 obs 2 days after treatment : 9.6
4 days after treatment : 16.1
7 days after treatment : 16.7

Table 7 - Recovery of flamprop-methyl 48 hours after treatment with aqueous/acetone solutions of various concentrations and applied at various volume application rates.

Treatment[1]	Extract	% Recovery					
		Replicates					Mean
A High mass, high area	Surface[2]	65.7	76.3	64.5	53.8	65.2	65.1
B High mass, low area	Surface	78.0	80.8	84.0	76.3	57.3	75.3
C Low mass, high area	Surface	30.4	32.2	34.3	37.9	M	32.1
D Low mass, low area	Surface	73.0	73.0	57.9	64.0	65.9	66.8
A High mass, high area	Maceration	20.3	14.1	19.2	41.5	14.7	22.0
B High mass, low area	Maceration	13.3	4.1	8.4	14.3	33.5	14.7
C Low mass, high area	Maceration	53.8	53.4	43.8	54.4	48.2	48.9
D Low mass, low area	Maceration	20.0	18.8	31.9	32.5	21.8	25.0
A High mass, high area	Combined extracts	86.0	90.4	83.7	95.5	79.9	87.1
B High mass, low area	Combined extracts	89.5	84.9	90.6	90.5	90.7	89.2
C Low mass, high area	Combined extracts	84.3	85.6	78.0	83.3	M	81.9
D Low mass, low area	Combined extracts	94.3	91.8	89.8	96.5	87.6	92.0

LSD values (P=0.05) for surface extract : 10.4
maceration extract: 11.9
combined extracts: 5.0

(1) Treatments

A : 20 ppm solution applied as 5 x 10 ul drops per leaf

B : 100 ppm solution applied as 1 x 10 ul drop per leaf

C : 4 ppm solution applied as 5 x 10 ul drops per leaf

D : 20 ppm solution applied as 1 x 10 ul drop per leaf

(2) Combined water wash and hexane/acetone extract

are also in accord with the hypothesis that uptake of flamprop-methyl was limited by accumulation in tissues underlying the site of application, since uptake was most efficient when the mass of compound per unit area was at a minimum.

It was shown earlier that diffusion across some layer will be directly proportional to that concentration gradient $(c_{1,2}-c_{2,3})/x$ across the layer. Factors tending to increase $c_{1,2}$ will promote movement across the layer. It was also suggested earlier that the value of $c_{1,2}$ could be limiting. From above, transcuticular movement over 48 hours was directly proportional to the amount of herbicide applied to unit area of leaf surface, which suggests that $c_{1,2}$ was directly proportional to the amount of compound applied. This has an important consequence with regards to the effect of increased area of foliar uptake; spreading a fixed amount of herbicide over an extended area may reduce $c_{1,2}$ and hence reduce the concentration gradient. Diffusion is however also directly proportional to the cross-sectional area over which movement occurs. This combination of conflicting trends may explain the only small effect of increasing area of contact on foliar uptake found in these studies.

3.8 Influence of environmental factors

In the studies discussed so far plants were maintained in a controlled environment at an ambient temperature of 25°C ± 1°C. The foliar uptake of flamprop-methyl, following application of 0.3 µg as a 30 ppm solution, was also studied in a series of experiments, conducted in glasshouses, at five times over a period of about 15 months. Environmental conditions were subject to considerable variation between these experiments. The data obtained were not considered suitable for

detailed analysis in view of the lack of control over environment. Over any one experiment considerable variation was found in ambient temperatures. Some general trends were noted from these data.

It was found that the rate of foliar uptake by plants under glass was very similar to that found at 25°C (Figure 13) when maximum daily temperatures in the glasshouse were about 25°C (Table 8). The rate of uptake was not changed significantly when plants were grown on post-application under conditions in which maximum daily temperatures reached 30°C (Table 9), but tended to be slightly slower by plants grown on at slightly lower temperatures (Table 10).

Under more extreme conditions the rate of foliar uptake of flamprop-methyl was found to deviate markedly from that established at 25°C. Figure16 shows the recovery of flamprop-methyl over seven days in hexane/acetone extracts of plants grown post-application in environments in which maximum daily temperatures were about 5°C, 25°C and 35°C. It can be seen that uptake was somewhat reduced at the lower temperatures, and markedly enhanced at the higher temperatures, relative to that found at 25°C. This result is similar to those from other (controlled environment) studies.

Much of the work concerned with the influence of environment on foliar uptake and traslocation has been reviewed by Muzik.[173] In general, within physiological limits, foliar uptake appears to increase with increasing temperature. Thus uptake by Prosopis juliflora of 2,45-T was similar at 22°C and 30°C, but was significantly faster at 38°C. Prasad,[174] investigating the foliar uptake of dalapon, found that uptake was increased about four fold when post-treatment was raised from 26°C to 43°C. These results are very similar to those obtained with flamprop-

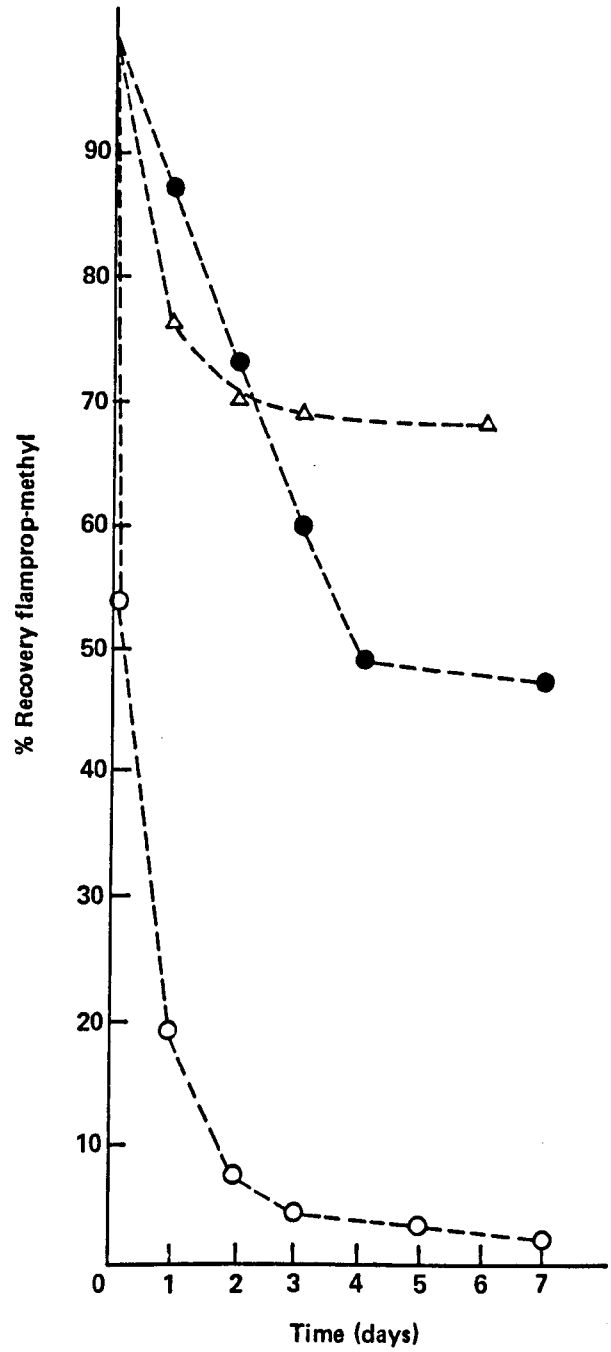


Fig 16 Foliar uptake by wheat of flamprop-methyl; effect of environment

Recovery of flamprop-methyl in a combined water wash and hexane/acetone extract of treated leaves kept under environmental regimes in which the maximum daily temperatures were between 0° – 5°C (△), between 20° – 25°C (●) and were greater than 35°C (○).

Table 8 - Uptake in unregulated environments. Recovery of flamprop-methyl at various times over a period of seven days during which maximum daily temperatures were between 20° and 25°C

Time/days	% Recovery surface extract			
	Replicates			Mean
0	100	100	100	100
1	84.9	87.5	88.1	86.8
2	75.0	74.6	71.3	73.6
3	57.0	62.5	61.3	60.3
4	40.5	51.2	54.8	48.8
7	38.0	54.6	47.4	46.7

Table 9 - Uptake in unregulated environments

Recovery of flamprop-methyl at various times over a period of seven days during which maximum daily temperatures fluctuated between 25° and 30°C

Time/days	% Recovery surface extract			
	Replicates			Mean
0	100	100	100	100
1	71.8	80.0	86.7	79.5
2	56.3	68.4	61.0	61.9
3	40.5	51.9	52.5	48.3
4	42.5	43.0	46.8	44.1
7	42.5	44.1	47.3	44.6

Table 10 - Uptake in unregulated environments

Recovery of flamprop-methyl at various times over a period of seven days during which maximum daily temperatures fluctuated between 15° and 18°C

Time/days	% Recovery surface extract			
	Replicates			Mean
0	100	100	100	100
1	91.5	88.2	85.2	88.3
2	81.3	73.6	69.0	74.6
3	60.0	61.6	65.7	62.4
5	47.0	52.7	59.2	53.0
7	45.8	49.5	51.0	48.8

methyl. The mechanism(s) responsible for these temperature effects have not been identified. Sargent[1,2] has discussed physiological explanations of the influence of temperature. It is known that temperature has a major effect on the rate of respiration and can influence photosynthesis and metabolic activity. Norris and Bukovac[89] examined the permeation of NAA through isolated pear leaf cuticles as influenced by temperature and concluded that the strong correlation between permeability and temperature could not be attributed to any effect on metabolic processes. These authors concluded that the diffusional resistance of the membrane was directly effected by ambient temperature.

The redistribution of flamprop-methyl following topical application to plants grown post-treatment in an environment in which maximum daily temperatures exceeded 35°C is shown in more detail in Table 11. It can be seen that the effect of high temperatures was apparent at very short times post-application and was manifest as reduced recoveries in both water wash and hexane/acetone extracts. It would seem that desorption into the apoplast was enhanced at elevated temperatures since flamprop-methyl did not accumulate in the epicuticular waxes. The diffusional resistance of this layer was also presumably lowered since uptake appeared to be almost completed within 7 days.

3.9 Foliar uptake by barley and oat

Foliar uptake of flamprop-methyl following topical application was compared between wheat, barley and oat in glasshouse experiments. The results (Table 12) show that partition into the epicuticular waxes and transcuticular movement were comparable between the three plant species. It may be concluded that selectivity of action between species is not contributed to by preferential uptake.

Table 11 - Uptake in unregulated environments.
Recovery of flamprop-methyl at various times over a period of seven days during which maximum daily temperatures exceeded 35°C. Mean values (5 reps)

Time/days	% Recovery Surface extract	% Recovery hexane/acetone	% Recovery Combined extracts
0	100	0	100
0.1	44.0	10.0	54.0
1	9.1	10.1	19.2
2	4.5	2.5	7.0
3	2.1	2.2	4.3
5	1.1	1.9	3.0
7	0.9	1.5	2.4

Table 12 - Foliar uptake by barley and wild oat.
Recovery of flamprop-methyl from wheat, barley and wild oat at various times over a period of 7 days.

Time/days	% Recovery(1)								
	Water wash			Hexane/acetone extract			Combined extract		
	WH	BA	OA	WH	BA	OA	WH	BA	OA
0	100	100	100	0	0	0	100	100	100
1	61.4	60.0	62.6	27.5	26.2	27.5	88.9	86.2	90.1
2	48.0	51.3	49.0	19.6	4.1	14.2	67.6	55.4	63.2
3	41.3	41.3	43.9	21.0	14.1	17.8	62.3	55.4	61.7
4	42.6	43.0	42.8	11.4	12.4	10.1	54.0	55.4	52.9
7	27.0	27.2	23.2	27.1	22.3	23.5	54.1	49.5	46.7

(1) mean values (5 reps) for each species

CHAPTER TWO

Physical properties of polyoxyethylene non-ionic surfactants

I N D E X

SUMMARY

The surface properties of aqueous solutions of selected polyoxyethylene non-ionic surfactants have been studied prior to investigating the effect of these compounds on the foliar uptake by cereals of flamprop-methyl. Two types of non-ionic surfactant were included in this study, the alkylphenol ethoxylates as exemplified by the Triton X products (ex Rohm and Haas) and alcohol ethoxylates as exemplified by the Brij products (ex Atlas Chemical Industries).

Products from both sources were analysed using instrumental and separational methods; the results of these analyses supported the manufacturers description of the compounds.

Surface and interfacial tensions of aqueous solutions of the surfactants were determined at various concentrations. Maximum reduction of surface tension in the alkylphenol ethoxylate solutions was found with Triton X-35 and Triton X-45. Maximum reduction of surface tension in the alcohol ethoxylate solutions was found with the tetraoxyethylene dodecyl ether (Brij 30) and was reduced with increasing oxyethylene content with any one hydrophobe. At constant oxyethylene content it was shown that surface activity was dependent on the nature of the hydrophobe. Critical micelle concentrations were determined from plots of surface tension vs concentration for alkylphenol and alcohol ethoxylates.

The spreading and wetting properties of aqueous solutions of alkylphenol ethoxylates were investigated by the Draves test and in terms of calculated spreading coefficients.

INTRODUCTION

Surfactants can be defined[1-3] as substances that can alter the energy relationships at interfaces, thereby causing a reduction of surface tension (at gas/liquid interfaces) and interfacial tension (at liquid/liquid and liquid/solid interfaces). The phenomena of surface and interfacial tension are explicable[4-10] in terms of the short range Van der Waals forces of attraction that exist between molecules and the unbalanced attractive forces that a molecule located at the interface experience.

As a result of this inbalance in attractive forces an interfacial surface will tend to contract spontaneously. The surface tension may be defined as the work required to increase the area of such a surface isothermally and reversibly by unit amount. Surface active agents (surfactants) cause a reduction of the surface and interfacial tension by packing into the interface; the tendancy of surface active molecules to pack into an interface favour expansion of the interface and this must be balanced against the tendency for the interface to contract under normal surface tension forces.

The packing or orientation of surfactant molecules at an interface occurs because of the amphiphilic character of the molecules which contain both polar (hydrophilic) and non-polar (hydrophobic-lipophilic) groups. Interfacial adsorption by location of the hydrophile in a aqueous phase and the hydrophobe in vapour or oil phase is energetically more favourable than complete solution in either phase. This adsorption is in the form of a oriented monomolecular layer and is a dynamic phenomenon[11] opposed by the tendency towards complete mixing due to

the thermal motion of the molecules. The formation of the monolayer is not an instantaneous process and is governed by the rate of diffusion of the surfactant to the interface[11] and hence by the structure of the surfactant.

In addition to interfacial adsorption, surfactant solutions differ from solutions of other solutes in their ability to solubilize in aqueous solutions solutes that are otherwise insoluble or only sparingly soluble.[7] The mechanism of solubilization depends on the interaction of the solute with aggregates of the surfactant termed micelles, which are formed when the surfactant concentration exceeds a definite value termed the critical micelle concentration. The structure of the micelle and solubilization are well described in standard texts.[4-10]

Non-ionic surfactants

Surfactants are generally classified[7] as to ionic activity as anionic, cationic or non-ionic depending on the electrocharge of the surface active group. The non-ionic compounds are broadly classified as a hydrophile-hydrophobe structure devoid of ionizing potential. As such they are generally not subject to hydrolysis by acidic or alkaline aqueous solutions and do not form salts with metal ions, which makes them equally effective in both hard and soft waters. Depending on the chemical composition of the hydrophobe the non-ionic surfactants can be further classified as hydrocarbon, silicone or fluorocarbon.[12]

The hydrophobe is balanced by a non-ionized hydrophile. Non-ionic surfactants commonly derive their hydrophilic characteristics from the presence of a number of weakly hydrophilic (commonly oxyethylene) groups to form molecules with the general structure (1)

$$R - (OCH_2CH_2)_n OH \quad (1)$$

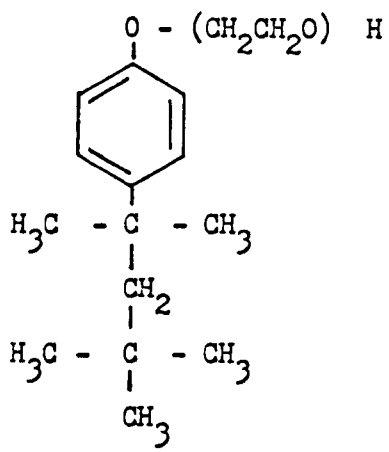
The balance between the hydrophilic and lipophilic characteristics of such a molecule can be systematically varied by varying the value of n. HLB (Hydrophile - Lipophile Balance) is a semi-empirical means of expressing quantitatively the balance between the hydrophilic and lipophilic characteristics of the surfactant.[13] This is represented by an arbitary scale (0 - 20) in which the least hydrophilic materials have low HLB and increasing HLB corresponds to increasingly hydrophilic character. HLB can be calculated from surfactant composition; for materials where only ethylene oxide is used to produce the hydrophilic moiety the HLB can be determined[14] as E/5 where E is the weight percent of oxyethylene content. The determination of HLB values has been shown[14] to be possible by a variety of techniques which depend, more or less, on some fundamental property of the molecule.

Polyoxyethylene alkyl phenols

Polyoxyethylene alkyl phenols, as exemplified by structure (2), also known as alkylarylpolyether alcohols, are prepared by the reaction of the alkyl phenol with ethylene oxide in a condensation reaction in which ethylene oxide adds to the hydroxyl radical by ring cleavage with regeneration of the hydroxyl group.[15,16] This reaction is an addition without termination and in a mechanism of this kind the growth of all adducts proceeds under conditions affording equal opportunities for all the hydroxyl groups to react.[7] The reaction gives rise to a mixture of products with respect to the length of the polyoxyethylene chain.

It has been shown[17] that the first mole of ethylene oxide is added with relative ease. Addition of the second and additional moles of ethylene oxide requires more vigourous conditions and the residues

of the hydrophile then add such that the distribution of chain lengths approximates to the Poisson distribution. This distribution appears[18] to describe the composition of the p-alkylphenolethoxylate mixtures fairly accurately providing the mean oxyethylene content of the sample is not too high. Representative values for surfactants derived from p-t-octylphenol are shown in Table 1.



Methods have been reported for the determination of the general shape of the distribution curves by chromatographic separation on silicic acid,[19] by measurement of foaming properties,[20] by circular Thin Layer Chromatography[21] and by Gas Liquid Chromatography.[22]

Gibbons[23] has made a distinction between materials which are heterodisperse and those which are polydisperse. A heterodisperse material is defined as one in which the variation in structure and hence properties of the components is more or less discontinuous or presents a broad diffuse distribution. A polydisperse material has a gradual variation in structure clustered about a mean, and can be expected to exhibit properties not intrinsically different from those of a single species with the mean structure. Becher[14] has shown that commercial ethoxylated non-ionic surfactants behave as polydisperse

Table 1 - Poisson distribution (Wt.%) Polyoxyethylene Octylphenol[7]

x	Mean oxyethylene content (n)			
	5	9	12	20
1	1.07	0.01		
2	5.06	0.13	0.01	
3	11.62	0.60	0.05	
4	17.52	1.81	0.19	
5	19.54	4.05	0.59	
6	17.24	7.15	1.43	
7	12.57	10.42	2.88	0.02
8	7.79	12.94	4.91	0.05
9	4.29	13.96	7.28	0.13
10	2.00	13.32	9.55	0.30
11	0.86	11.38	11.22	0.60
12	0.33	8.80	11.94	1.11
13	0.11	6.22	11.60	1.86
14	0.04	4.04	10.37	2.86
15	0.02	2.43	8.59	4.10
16		1.36	6.62	5.45
17		0.71	4.77	6.78
18		0.35	3.22	7.93
19		0.16	2.06	8.74
20		0.07	1.24	9.11
21		0.04	0.71	9.01
22		0.02	0.38	8.46
23			0.20	7.58
24			0.10	6.50
25			0.05	5.32
26			0.02	4.18
27				3.16
28				2.29

Cont.

Table 1 - cont

x	Mean oxyethylene content (n)			
	5	9	12	20
29				1.60
30				1.08
31				0.71
32				0.45
33				0.27
34				0.16
35				0.10
36				0.05
37				0.03
38				0.02

materials exhibiting properties close to those of the homogeneous compounds. The concept of the surfactant behaving as a single compound has been adopted below in rationalizing the effects of these compounds on leaf permeability.

1.4 Polyoxyethylene alcohols

Polyoxyethylene alcohols (3), also known as alkylpolyether alcohols are prepared by the reaction of a primary, secondary or tertiary alcohol with ethylene oxide: further discussion will be confined to the products formed with primary alcohols.

$$(C_yH_{2y+1}) - (OCH_2CH_2)_n\ OH \qquad (3)$$

The reaction is broadly similar to that discussed earlier for alkylphenol but the distribution of chain lengths does not follow Poisson distribution.[24] Under basic conditions (commonly used in the manufacture of both types of surfactant[7]) the primary alcohol reacts more rapidly with ethylene oxide than do phenols. The rate constants for the addition of ethylene oxide to the primary alcohol are comparable to those for the addition of ethylene oxide to the adducts, which are also primary alcohols,[16] After the addition of one mole of ethylene oxide a large mole fraction of the starting alcohol remains. This onset of chain growth before all the starting material has reacted is in contrast to the kinetics described above for the reaction between an alkylphenol and ethylene oxide.

The composition of the mixtures resulting from the base-catalysed oxyethylation of a primary alcohol is approximated to[15] by a number of distribution equations. Figure 1 shows Weibull and Nycander and Poisson distribution superimposed on the ratio of products from the reaction of lauryl alcohol with six moles of ethylene oxide. It can be

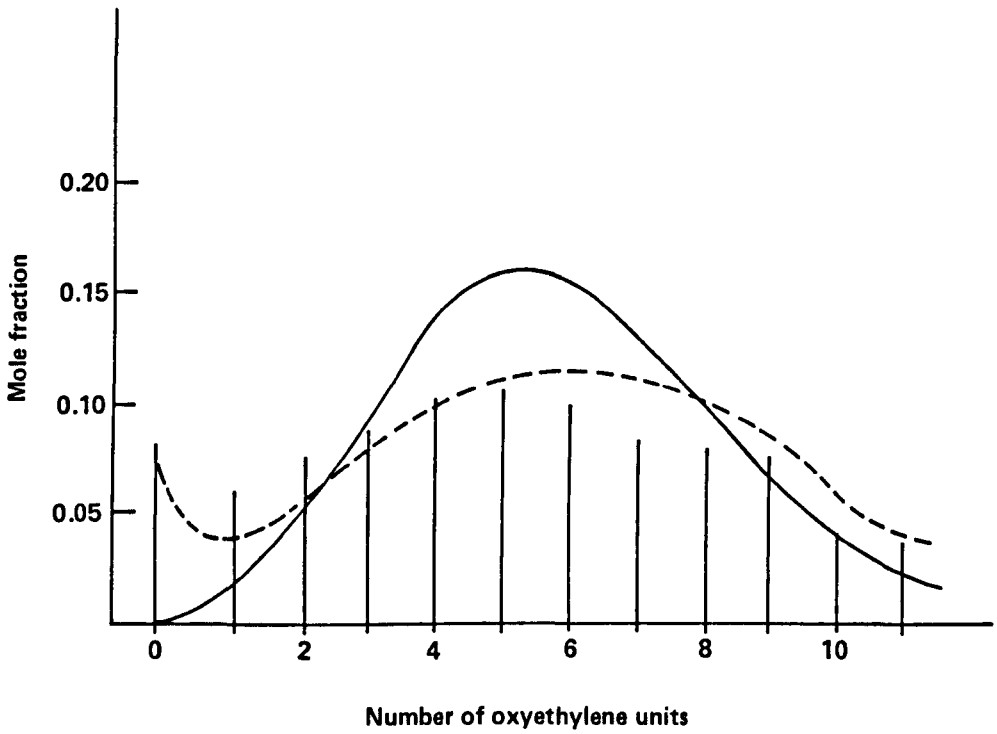


Fig 1

Products of the reaction of lauryl alcohol with six moles of ethylene oxide (16). The solid line is the Poisson distribution, the broken line is the Weibull-Nycander distribution $r = 3$

seen that the distribution is more diffuse than expected from the Poisson distribution and contains considerably more short chain material than would be present in a comparable polyoxyethylene alkyl phenol.

2. EXPERIMENTAL

The Triton X series of non-ionic polyoxyethylene surfactants (based on p-t-octylphenol as the hydrophobe) were obtained ex Rohm and Haas as technical grade materials. The Brij surfactants (assorted hydrophobes) were obtained ex Atlas Chemical Industries. Products from both sources were used as received.

2.1 Structural analysis

All compounds were examined by Infra-red spectroscopy, nuclear magnetic resonance (NMR) and mass spectrometry. Infra-red studies were performed using a spectrophotometer: spectra were obtained as thin films of samples dried at 105°C and were compared with reference spectra.[25] Representative spectra are included as Figures 2 and 3 for polyoxyethylene alkyl ether and polyoxyethylene alkyl phenols respectively. NMR analyses were performed on a Varian Associates HR-60 high resolution instrument according to the method of Greff and Flanagan.[26]

Mass spectrometry was used to investigate the oligomer distribution in commercial products. Spectra were obtained using a Finnigan 3200 quadrupole instrument by chemical ionization (NH_4^+ reagent) and monitoring positive ions only. Sample was introduced via a solid probe programmed between 65° and 320°C. Scan rate was 65-800 amu in 2 s.

The distribution of oligomers was also investigated by Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC).

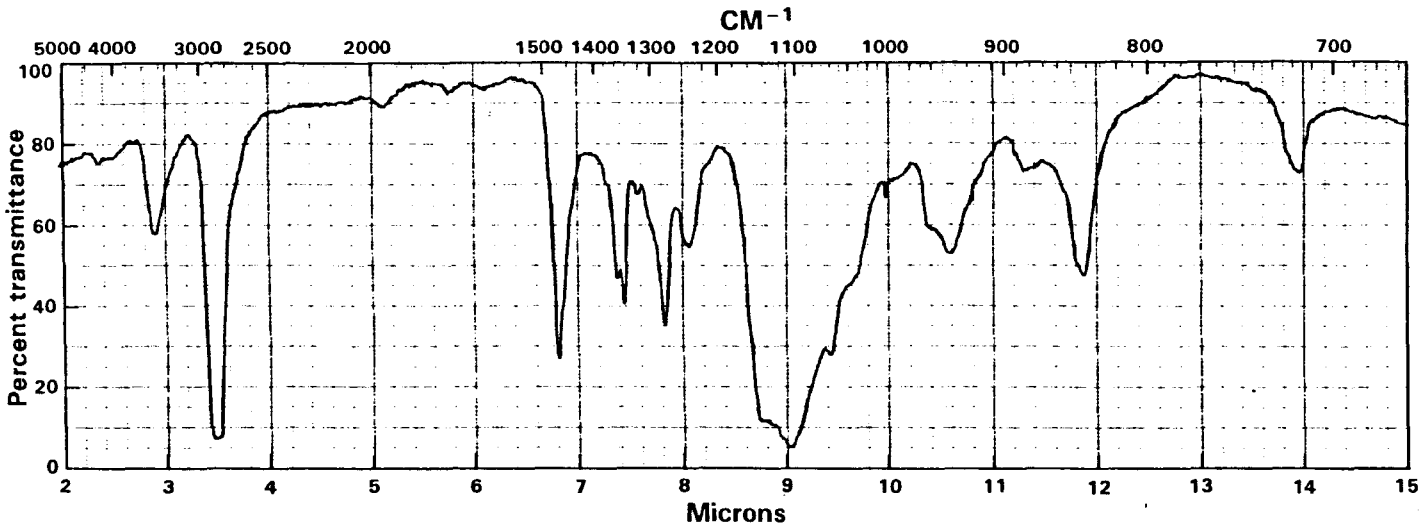


Fig 2 Infrared spectrum of polyoxyethylene (10) stearyl ether (BRIJ 76)

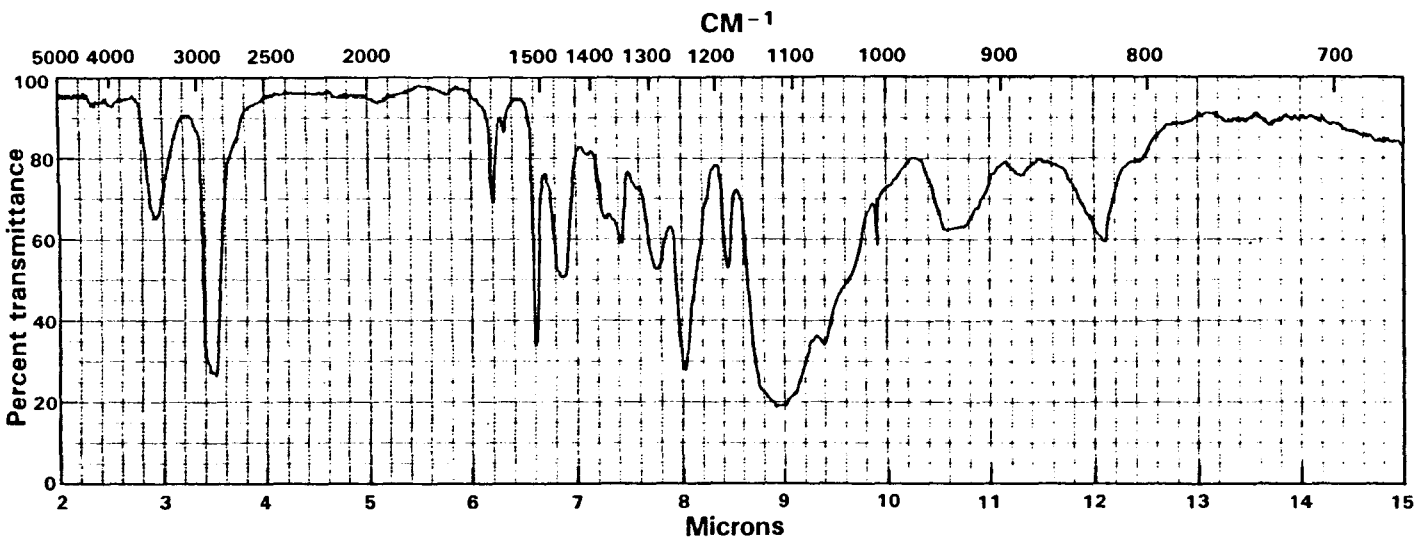


Fig 3 Infrared spectrum of Triton X 100

HPLC analysis was limited to polyoxyethylene alkylphenols and is described below (Chapter Three).

TLC was performed on Silica gel G using water saturated butan-2-one as eluent and developing with Dragendorffs reagent[27] according to the method of Crummert.[28]

Instrumental and separational methods of analysis served to support the manufacturers description of each compound.

2.2 Physical properties

The surface activity of surfactant solutions was investigated with aqueous solutions freshly prepared using water triply distilled from alkaline permanganate (surface tension 71.8 $mN.m^{-1}$). All measurements were made at 25°C. Surface and interfacial tensions of aqueous solutions were measured with a Du Nouy Tensiometer (Cambridge Scientific Instruments Ltd.) by the ring method, according to Reilly and Rae.[29] The ring method can be applied to determining interfacial tensions[30] provided that the lower liquid preferentially wets the platinum ring. Water, with overlying toluene or Acroprime 90, satisfies this condition. Wetting power was measured by the Draves test according to AATCC 17-1952.

3. RESULTS AND DISCUSSION

3.1 Triton X compounds

The Triton X series of p-t-octylphenoxyethoxyethanols consists of ten compounds in which the mean oxyethylene content varies between 1 and 70 moles (Table 2). The physical form of these compounds varies with the oxyethylene content so that at low values of n the surfactants are liquid at room temperature and above about 12.5 moles of oxyethylene

Table 2 - Properties of the Triton X surfactants

TRITON X	15	35	45	114	100	102	165	305	405	705
MEAN EO CONTENT	1	3	5	7.5	9.5	12.5	16	30	40	70
MOL WT	251	339	427	537	625	757	911	1527	1967	3287
HLB	3.6	7.8	10.4	12.4	13.5	14.6	15.8	17.3	17.9	18.7
MOLECULAR AREA										
(1)	23	38	42	50	54		131		88	
(2)					55	65	72	101		

(1) Surface area at air/water interface of Triton X molecules taken from data reported by Rohm and Haas[34] and by Crook et al[35].

NB Value for Triton X 165 appears too large relative to other values.

(2) Surface area at air/water interface of nonylphenol ethoxylates as reported by Hsiao et al[36]

are waxy solids.

Water solubility is dependent[7] on the hydrophilic nature of the ether linkages in the polyoxyethylene chain and the extent of hydration. These ether linkages are readily hydrated at room temperature and water solubility then depends on the number of hydrated linkages; in general at least four to six oxyethylene units are required to produce a 'water soluble' surfactant.[7] Water solubility increases with increasing oxyethylene content. Difficulties were encountered in the present study when attempting to prepare aqueous solutions of Triton X 15 and Triton X 45 above 100 ppm, and solutions of Triton X 45 above 1000 ppm; the remaining compounds were found readily soluble up to 10,000 ppm.

Solubility in non-polar organic solvents is generally the reverse of water solubility for oxyethylene surfactants. The Triton X compounds are all soluble in alcohols but are not appreciably soluble in aromatic hydrocarbons when the mean oxyethylene content exceded 12.5 moles. Above 5 moles of oxyethylene the compounds are not appreciably soluble in aliphatic hydrocarbons. Triton X 45 exhibits unusual solubility behaviour in aliphatic hydrocarbons in being insoluble in dilute solution but soluble above some minimum concentration.[15]

The viscosity of aqueous solutions of Triton X compounds has been studied by Greenwald and Brown;[33] a summary of the dilution effect on viscosity with selected compounds is given in Table 3. The viscosity of aqueous solutions can be seen to increase to a maximum on approaching a concentration between 50% and 70% from either a lower or a higher concentration.

Table 3 - Viscosity (centipoises) of Triton X solutions at 25°C[33]

Triton	Active concentration (wt%) in water					
	10%	30%	50%	70%	90%	100%
X 114	100	280	500	27000	320	260
X 100	2	80	Gel	530	280	270
X 102	3	20	Gel	Gel	310	410

3.2 Surface properties

Table 4 shows the surface tension of aqueous Triton X solutions of flamprop-methyl at selected surfactant concentrations. These data show good agreement with literature values for p-t-octylphenol ethoxylates of comparable oxyethylene content.[34,35] It can be seen that the maximum surface tension reduction in the Triton X series was found with Triton X 35 and Triton X 45. For those compounds in which the mean oxyethylene content was greater than that in Triton X 114 it was apparent that there is a rise in the surface tension with increasing concentration above the concentration of maximum surface tension reduction (CMC). This phenomenon became more apparent as the molecular weight distribution broadened.

The existence of a limiting surface tension has been noted previously[35] and has been discussed in terms of the preferential adsorption of the more surface active species, extant in the normal distribution, at the air/water interface. At surfactant concentrations at or just above the CMC, the air/water interface is thought to consist primarily of shorter chain length molecules. As the surfactant concentration further increases and more micelles are formed then hydrophobic short chain adducts can be extracted from the interface and solubilised in the micelle: sites at the interface vacated by hydrophobic molecules are subsequently occupied by less surface active hydrophilic molecules.

Table 5 shows the CMC values for the Triton X compounds as determined from surface tension vs concentration curves and tabulates values of the surface tensions at the CMC and at the highest concentrations studied.

Table 4 - Surface tension (Du Nuov) ($mN.m^{-1}$) of aqueous flamprop-methyl solutions of Triton X surfactants at 25°C, recorded to within ± 0.5 $mN.m^{-1}$.

Concn ppm	Triton X Compound									
	15	35	45	114	100	102	165	305	405	705
3		53								
4	53		51							
8	46.5		45							
10		49	42			52	56	57		59
12	43.5				48					
16			38.5	44						
17		36.5								
20				42			54		58.5	
24		33								
30		30.5			42		52.5			
40	36.5	27.5	30.5	36			51.5			
50				34						
60	35.5				38		49			
68		27.5								
80			28				48			
100				30	36.5	39	46.5	50	54	54
125	34.5				32.5					
140							45			
160									52.5	
200									50.5	
250					30		41			
300	34.5	27.5								
350							37.5			
400			28							
500				29	31		37		44	
690									41.5	
800							37.5			
1000				29		34		37	39	
1200					31.5					

Table 4 - continued

Concn ppm	Triton X Compound									
	15	35	45	114	100	102	165	305	405	705
1400									37	
1800									36.5	
2000							38.5			
4000			28							
5000				29						
6000									43	
10000			28	29	32	35	38.5	42	44	42

Table 5 - Surface tension and critical micelle concentration at 25°C of Triton X solutions of flamprop-methyl

Triton X	15	35	45	114	100	102	165	305	405	705
Cmc(1) 10^4/mols.dm^{-3}	5.0	1.1	1.2	2.0	3.0	3.6	4.3	5.7	8.1	ND
/ppm	125	40	50	100	180	275	390	870	1600	ND
γ_{cmc}/mN.m^{-1} (2)	34.5	27.5	28	29	30	34	37	37	37	ND
$\gamma_{Limiting}$ (3) mN.m^{-1}	34.5	27.5	28	29	32	35	37.5	42	44	42

(1) Concentration at which minimum surface tension was attained.

(2) Surface tension at the cmc.

(3) Surface tension at 10,000 ppm surfactant.

Table 6 shows the interfacial tension of aqueous Triton X solutions of flamprop-methyl as measured between the solutions and either toluene or the highly refined aliphatic mineral oil Acroprime 90. This table also includes data after Crook et al.,[35] between solutions of the surfactants in distilled water and isooctane. With Acroprime 90 the lowest interfacial tensions were found with Triton X 114 and Triton X 100; with toluene lowest interfacial tensions were associated with more hydrophilic compounds. Interfacial tension data (vs isooctane) determined by Crook[35] showed little variation at any one concentration. The maximum reduction of interfacial tension showed more variation between surfactants and lowest values for interfacial tension were reported with the most hydrophobic compounds.

Table 7 shows partition coefficients determined by Crook et al.,[37] for Triton X compounds in the system isooctane/water. These data show that the compounds with a mean oxyethylene content less than nine moles are more soluble in isooctane and that the compounds with a mean oxyethylene content at and above nine moles are more soluble in water.

3.3 Spreading and Wetting

The spreading coefficient is a measure of the effectiveness of a surfactant in aiding the spreading of two immiscible phases, one on the other. In these studies this term was calculated from :

$$Sa = Tb - (I\,ab + Ta) \quad (31)$$

Where Sa is the spreading coefficient, Tb is the surface tension of the oil phase, Iab is the interfacial tension between the two phases and Ta is the surface tension of the aqueous (surfactant) phase. A more positive value of Sa shows greater ease in spreading.

Table 8 shows calculated spreading coefficients for the systems

Table 6 - Interfacial tensions ($mN.m^{-1}$) of aqueous Triton X solutions of flamprop-methyl at 25°C

Oil Phase	Acroprime 90				Toluene				Isooctane
Surfactant concn/ppm	10^4	10^3	10^2	10^1	10^4	10^3	10^2	10^1	cmc[1]
Triton X									
35			22.5	32.0			34.0	36.0	0.6
45	1.5	7.0	14.5	29.5	18.5	23.0	28.5	34.0	1.1
114	1.5	3.0	11.5	23.5	14.0	18.5	25.0	32.0	3.0
100	1.0	2.5	10.0	21.5	10.5	15.5	21.5	28.5	4.0
102	5.0	5.5	13.0	21.5	6.0	12.5	18.5	24.5	5.2
165	6.5	7.0	11.5	25.5	5.5	11.5	17.0	23.0	6.8
305	6.5	8.5	9.0	15.0	4.5	5.5	10.0	13.0	10.0
405	13.0	11.0	15.0	26.0					13.0
705	6.0	5.0	7.0	12.0					

(1) Data taken from reference 35: Interfacial tension at cmc.

Table 7 - Partition coefficients (isooctane/water) of normal distribution octyl phenol ethoxylates taken from Crook et al, 1965[37]

n(1)	2	3	4	5	6	7	8	9	10	16	40
(2)	$4.1.10^{-3}$	$1.4.10^{-2}$	$4.8.10^{-2}$	$1.3.10^{-1}$	$2.5.10^{-1}$	$4.5.10^{-1}$	$8.2.10^{-1}$	1.6	1.9	31.3	47.2

(1) mean oxyethylene content

(2) $K = C_W/C_O$ where C_W and C_O are the molar concentrations of the surfactant in water and iso octane respectively.

Table 8 - Calculated spreading coefficients for aqueous solutions of the Triton X surfactants, at 25°C

Oil phase	Acroprime 90				Toluene			
Surfactant concn/ppm	10^4	10^3	10^2	10^1	10^4	10^3	10^2	10^1
Triton X								
35			-22.0	-34.5			-35.0	-40.0
45	0.0	-5.5	-14.0	-40.0	-18.5	-23.0	-29.0	-46.0
114	-1.0	-2.5	-12.0	-38.0	-15.0	-19.5	-27.0	-48.0
100	-1.5	-3.0	-11.5	-38.0	-12.5	-17.5	-24.5	-46.5
102	-7.5	-8.0	-15.5	-32.0	-10.0	-16.5	-22.5	-46.5
165	-11.0	-12.5	-15.0	-15.0	-11.5	-18.5	-22.0	-48.0
305	-18.0	-17.0	-17.5	-49.0	-17.5	-15.5	-20.0	-42.0
Water				-94.5				-80.1

aqueous surfactant/Acroprime 90 and aqueous surfactant/toluene. It can be seen that the choice of most effective surfactant depends on both the nature of the oil phase and on surfactant concentration: for the spreading of distilled water and Acroprime 90 one on the other it was apparent that the nature of the surfactant giving most effect moved to lower mean oxyethylene contents with increasing concentrations.

In addition to lowering surface tension it is important for a wetting agent to lower the interfacial tension, and both of these factors are included in the spreading coefficient. Another assessment of wetting ability is provided by the Draves test which measures[32] the concentration of wetting agent necessary to cause sinking of a weighed cotton skein in a given time in an aqueous solution of the wetting agent. In giving an indication of the ability of the test solution to displace air from the skein this test is of possible relevance to the wetting of composite surfaces. The lower the concentration of surfactant needed to cause sinking in a given time the more efficient is the wetting agent. Draves wetting times for the Triton X compounds are given in Table 9: at all times investigated lowest concentrations of surfactant were found using Triton X 100. The most notable feature concerning this data was the marked dissimilarity between those compounds with a mean oxyethylene content equal to or less than 16 moles and those compounds with a mean oxyethylene content between 30 and 70 moles (note 1 to Table 9).

3.4 Brij compounds

As used in these studies the Brij series of polyoxyethylene alcohols contains 7 compounds (Table 10) over which the mean oxyethylene content varies between 2 and 23 moles per mole of hydrophobe. Unlike the

Table 9 - Draves wetting test (25°C) : % concentration Triton X solutions required to cause sinking of a weighed cotton skein at fixed times.

Triton X (1)	Wetting time/s		
	10	25	50
35	0.360	0.100	0.110
45	0.100	0.055	0.034
114	0.096	0.050	0.031
100	0.092	0.048	0.028
102	0.123	0.064	0.045
165	0.780	0.330	0.170

(1) With Triton X - 305, 405 and 705 5% solutions were found to give wetting times greater than 300 s.

Table 10 - Properties of the Brij surfactants

Brij	30	35	56	76	92	96	98
Hydrophobe		(1)		(2)	(3)		(4)	
Mean EO content	4	23	10	10	2	10	20
Mol. Wt	362	1198	682	710	356	708	1148
HLB	9.7	16.9	12.9	12.4	4.9	12.4	15.3

(1) dodecan-1-ol, (C 12) ; lauryl alcohol
(2) hexadecan-1-ol, (C 16) ; cetyl alcohol
(3) octadecan-1-ol, (C 18) ; stearyl alcohol
(4) octadec-9-ene-1-ol, (C 18) ; oleyl alcohol

Triton X compounds the Brij compounds do not share a common hydrophobe and are based on one of four hydrophobes, viz., octadecanol (stearyl alcohol), hexadecanol (cetyl alcohol), dodecanol (lauryl alcohol) and octadec-9-en-1-ol (oleyl alcohol). The hydrophobes are derived from natural sources which presumably accentuates the range of related compounds present following oxyethylation.

Because both Triton X and Brij compounds derive hydrophilic character from oxyethylene chains the trends in the physico-chemical properties identified earlier are generally applicable to the Brij compounds. Surface tension data for aqueous solutions of the Brij compounds are given in Table 11; maximum surface tension reduction can be seen to occur with the tetraoxyethylene dodecyl ether Brij 30, and was lessened with increasing oxyethylene content with any one hydrophobe. At constant oxyethylene content it was apparent that surface activity was also dependent on the nature of the hydrophobe. At an oxyethylene content of 10 moles per mole of hydrophobe Brij 96 showed significantly more activity than did Brij 56 at all concentrations; both compounds were more active than Brij 76. Table 12 shows the CMC values for Brij solutions as determined from surface tension vs concentration curves and tabulates values of the surface tensions at the CMC and at the highest concentrations studied.

Table 11 - Surface tension (Du Nuoy)($mN.m^{-1}$) of aqueous Brij solutions of flamprop-methyl at 25°C

Concn ppm	Brij compounds						
	30	35	56	76	92	96	98
10	38.8 f(1)	48.7 f	45.0 h	51.5 e	44.9 g	45.0 g	47.0 g
30					38.0 h	38.0	
50					33.2 b	36.7 c	
70					33.3 b	36.3 b	
100	30.5	42.4 b	37.5	42.9 f	33.5 c(2)	35.5 g	45.5 e
200						35.5 a	
400						35.0 e	
600						34.0 e	
800						33.8 b	
1000	30.5 e(2)	42.6 c	36.9 a	41.2 b	ND	34.0 e	44.5
5000			37.0(4)				
10000	29.5 c(5)	44.5 a	ND	42.0(3)	ND	34.2 b	43.4 c

(1) letters refer to variation (±) in tabulated values; a, 0.1; b, 0.2; c, 0.3; d, 0.4; e, 0.5; f, 0.6; g, 1.0; h, 2.0.

(2) slightly cloudy

(3) gel-like

(4) insoluble at 10,000 ppm

(5) opaque

Table 12 - Critical micelle concentration and surface tension at 25°C of Brij solutions of flamprop-methyl

Brij	30	35	56	76	92	96	98
cmc(1)/ppm	35	100	45	53	43	30	30
γ_{cmc}/mN.m^{-1} (2)	30	42	37	42	33	34	45
$\gamma_{Limiting}$ (3) mN.m^{-1}	29	44	37	42	ND	34	44

(1) concentration at which minimum surface tension was attained

(2) surface tension at the cmc

(3) surface tension at 10,000 ppm surfactant.

CHAPTER THREE

Penetration of polyoxyethylene non-ionic surface active agents into wheat

I N D E X

SUMMARY

The foliar uptake by wheat of ethoxylated non-ionic surface active agents has been studied following topical application of aqueous solutions. The permeability of the cereal leaf to these compounds has been demonstrated: the rate of uptake was shown to depend on the mean oxyethylene content of the surfactant, decreasing as the oxyethylene content was increased within the Triton X series of compounds. In qualitative terms the trend in uptake was paralleled by changes in both partition coefficient and (estimated) diffusion coefficient. The distribution of oligomers within any one surfactant was apparently retained during transcuticular movement. Movement of the surfactant across the epicuticular waxes resulted in an accumulation of the penetrant in the tissues underlying the site of application. Movement of the surfactant in treated leaves was acropetal.

1. INTRODUCTION

The foliar uptake of surfactant molecules has received little attention in previous studies of the influence of these materials in enhancing the activity of pesticides. This is surprising in view of the fact that many of the mechanisms postulated to explain the influence of surfactants on herbicide uptake and activity depend on the movement of the surfactant into the cuticular membrane and the underlying leaf tissues. It has been suggested that the phytotoxicity of some surfactants is in itself circumstantial evidence of penetration of the outer lipid layers of the leaf[1]. Studies of surfactant phytotoxicity do not however readily lend themselves to the development of a quantitative understanding of the fate of materials deposited on the leaf surface.

Studies[2-5] of the uptake of radioactive labelled surfactant have reported little or no movement of the applied activity out of the treated area: it was concluded that surfactants did not readily penetrate the leaf surface[5] and that the site of action of the surfactant was at the point of application and in the immediately underlying tissues.

In contrast to these findings, a limited amount of direct evidence of the penetration of the leaf by surfactants has been reported[6-8]. Price[7] found that following the application of ^{14}C-Lubrol (a polyoxyethylene non-ionic surfactant), 56% of the applied activity penetrated the leaves of wheat over 48 hours. This reference also contained previously unpublished results obtained by Anderson: the extent of foliar uptake was determined from a calorimetric estimation of the surfactant residue on the leaf surface following application of 2000 ppm aqueous solutions of alkylphenol

and alcohol ethoxylates. Uptake varied between 60 and 80%.

It was decided to study the foliar uptake of Triton X surfactants before investigating the effect of these compounds on the foliar uptake of flamprop-methyl. The quantitative analysis of an applied compound in the presence of co-extracted plant material has been discussed above. When the material to be analysed for is a commercial surfactant mixture the procedure is complicated by the range of compounds present. The distribution of oligomers results in a range of solubilities, partition coefficients and polarities which makes more difficult the separation of the applied materials from co-extractants that may interfere with the analysis. With the exception of Anderson, previous investigations appear to have avoided such difficulties by working with radioactive labelled materials.

The methods available for the analysis of non-ionic surfactants have been reviewed by Nadeau and Siggia[9]. Because the identification of penetrant was considered of importance in demonstrating uptake, a chromatographic technique was favoured, (in the absence of radioactive labelled adducts), combining the elements of separation and both qualitative and quantitative analysis of the applied material in the presence of co-extracted endogenous materials.

The techniques of paper chromatography[10], thin layer chromatography[11], and column chromatography[12] have been used to separate the components of commercial non-ionic surfactant samples, but quantitative analysis cannot readily be achieved by these methods. Gas Liquid Chromatography (GLC) has been used to quantitatively study the composition of alkylphenol ethoxylates[13] and the ethylene oxide adducts of fatty alcohols[14]. The technique is not

ideally suited to the investigation of polyoxyethylene adducts because of the high molecular weight and low volatility associated with these compounds. The investigation of Nadeau et al.,[13] was limited to the separation of molar condensates of up to eight units, and was only made quantitative for the first five in the polyoxyethylene nonyl phenol series. Volatility can be improved by derivatizing the alcohol, eg., by the formation of the acetate esters. Using this method Gildenberg and Trowbridge[14] separated peaks for adducts with up to 13 moles of ethylene oxide residues, based on dodecyl alcohol.

High Performance Liquid Chromatography (HPLC) bears a close resemblance to GLC and complements the latter in being able to separate compounds of low volatility, high polarity and high molecular weight[15]. The theory and practice of HPLC has been discussed by Hamilton and Sewell[15]; separations are commonly effected on either silica gel or on bonded phases in which the silanol surface groups on the silica gel are chemically reacted with compounds such as octadecyl trichlorosilane to give a substituted surface with hydrocarbon characteristics. The separation and analysis of polyoxyethylene compounds by LC has been the subject of a number of recent papers[16-23]; Cassidy[23] has stated that the separation and analysis of these compounds remains a problem in spite of these investigations, and has also noted that separations on silica gel are particularly difficult due to irreversible adsorption which leads to pronounced tailing and irreproducible chromatograms. The separation of the oligomers from Triton X 100 has been achieved by gradient elution on a silica column coated with Carbowax 20M, with excellent resolution of the components of the surfactant[23].

Separation of the Triton X compounds into their component oligomers has also been effected using isocratic elution on a reversed phase (bonded) column[24]; in particular it has been found that using Lichrosorb RP 8 the extent to which the sample is separated can be varied, by appropriate choice of eluent, to allow either a quantitative analysis of the total surfactant present - by elution of the mixture as a single peak - or analysis of the composition of the surfactant by separation into the component oligomers.

2. EXPERIMENTAL

2.1 Uptake studies with tetraoxyethylene octyl ether

Tetraoxyethylene octyl ether was obtained ex Liverpool Polytechnic as a solution in acetone (5.6 $mg.cm^{-3}$) with a specific activity of 0.72 $Ci.mole^{-1}$. A series of dilutions was prepared in distilled water, following evaporation of acetone from aliquots of the solution, at 100, 1000 and 10,000 ppm concentrations of the surfactant. Each solution was separately applied to the adaxial surface of the second leaf of wheat (T. vulgare cv Sappo) at the emerging fourth leaf stage: application was by microsyringe of a single 10 µl drop to an area 10 cm from the leaf tip. Ten plants were treated with each solution.

Treated leaves were harvested after 24 hours and residual activity was recovered from the site of application by (i) a water wash, (2 x 500 µl), and (ii), extraction by immersion in chloroform

(3 x 305; 15 cm^3). The dewaxed leaves were divided transversely into an acropetal segment, (9.5 cm measured from leaf tip), a one cm mid segment and a basipetal segment, (the remainder). Common segments of leaves were combined and extracted by maceration in acetone. Acetone and chloroform extracts were evaporated to dryness and the residues dissolved in scintillation solution and quantified by Liquid Scintillation Counting. Water washings were counted directly after dilution with scintillation solution.

Liquid Scintillation Counting

Disintegrations from tritium were measured in channel one which was set with a gain of 50% and with the discriminator set at 80-1000; channels two and three were set for quench correction using the ESR method described earlier. The water wash and chloroform extract were counted for five minutes to determine the level of activity and for 60 s. with the external standard in position to determine the counting efficiency. Samples obtained by maceration were counted for up to 30 minutes to determine the recovered activity, for 60 s. with the external standard in position, and then for 10 minutes after the addition of an accurately known mass of tritated hexadecane standard (Specific activity 2.27 $\mu Ci.g^{-1}$). From the measured counts, corrected for background and converted to disintegrations per minute (dpm) at 100% efficiency, the percentage recovery of the applied material in the various extracts was calculated.

2.2 Uptake studies with Triton X 100

Triton X 100 was obtained ex Rohm and Haas as technical grade material and was used as received.

a) Experiment 1

Triton X 100 was dissolved in distilled water to give 100, 1000 and 10,000 ppm solutions, each of which was separately applied to wheat, (T. vulgare cv Sappo), at the emerging fourth leaf stage. Fifty four plants were treated with each solution. At zero time, and after 24, 48, 72, 144 and 168 hours, three replicates (each of three leaves) were harvested from each treatment and extracted by water washing (3 x 500 µl). The aqueous extracts were analysed by HPLC.

b) Experiment 2

A 10,000 ppm solution of Triton X 100 was applied to wheat at the emerging fourth leaf stage. Twelve plants were treated with the solution. Treated leaves were harvested as triplicates at zero time and at 24 hour intervals up to three days. At each sampling the leaves were divided into an acropetal, mid and basipetal segment. The combined mid segments from three leaves were extracted by (i) washing with water, (3 x 500 µl), (ii) chloroform extraction, (3 x 500 µl), (iii) a 90 s. immersion in chloroform, (1500 µl), and (iv) dried and immersed in distilled water, (1500 µl) for up to six hours. This procedure was repeated with acropetal and basipetal segments. All extracts were analysed by HPLC.

HPLC

Chromatography was performed on a 200 mm x 4.6 mm i.d Lichrosorb RP8 10 µm column equipped with a Rheodyne injection valve. The mobile phase was supplied by an Altex pump (110 A) at a flow rate of 1 $cm^3.min^{-1}$. The effluent stream from the column was analysed with a Pye Unicam LC 3 UV detector and absorption monitored at a wavelength of 230 nm.

The total surfactant content of an extract was determined by eluting a 10 µl aliquot with methanol-water, (80:20); the total surfactant content was calculated from the detector response, (Figure 1), using a calibration curve of detector response as a function of surfactant concentration, (Figure 2).

The distribution of oligomers within an extract was determined by eluting a 10 µl aliquot with acetonitrile-water (95:5); the chromatogram was compared with that obtained with a standard solution, (Figure 3), on the basis of the relative contribution made by the component peaks.

2.3 Uptake studies with the Triton X series of surfactants

Triton X - 15, 35, 45, 114, 100, 102, 165, 305, 405 and 705 were obtained ex Rohm and Haas as technical grade material and were used as received; aqueous solutions were prepared in distilled water at a nominal concentration of 10,000 ppm, (dissolution of materials with a mean oxyethylene content at or below five moles was aided by sonication and cooling to 1°C), and, using the techniques described previously, each solution was separately applied to wheat (T. vulgare cv. Sappo). Fifteen plants were treated with each solution. Leaves were harvested at zero time and after 1, 2, 3 and 6 days, and were extracted by a water wash; at each sampling three leaves were combined for each analytical sample. Water washings were analysed by the HPLC method described above.

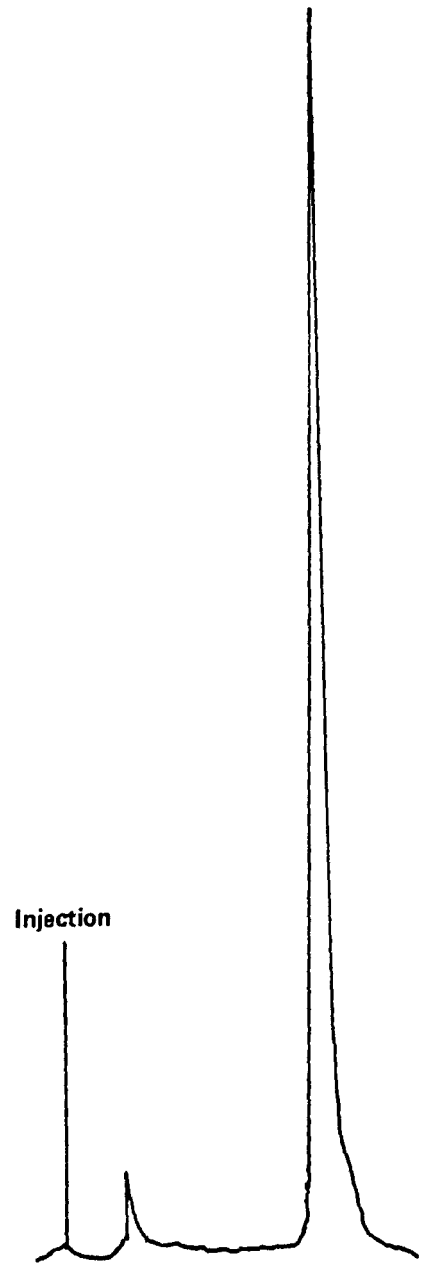


Fig 1 High performance liquid chromatogram of Triton X 100

Detector response to injection of 10 μl of a 500 ppm aqueous solution of Triton X 100 onto a 200 mm x 4.6 mm ID 10 μm Lichrosorb RP8 column eluted with methanol/water (80:20) at 1 $cm^3.min^{-1}$.

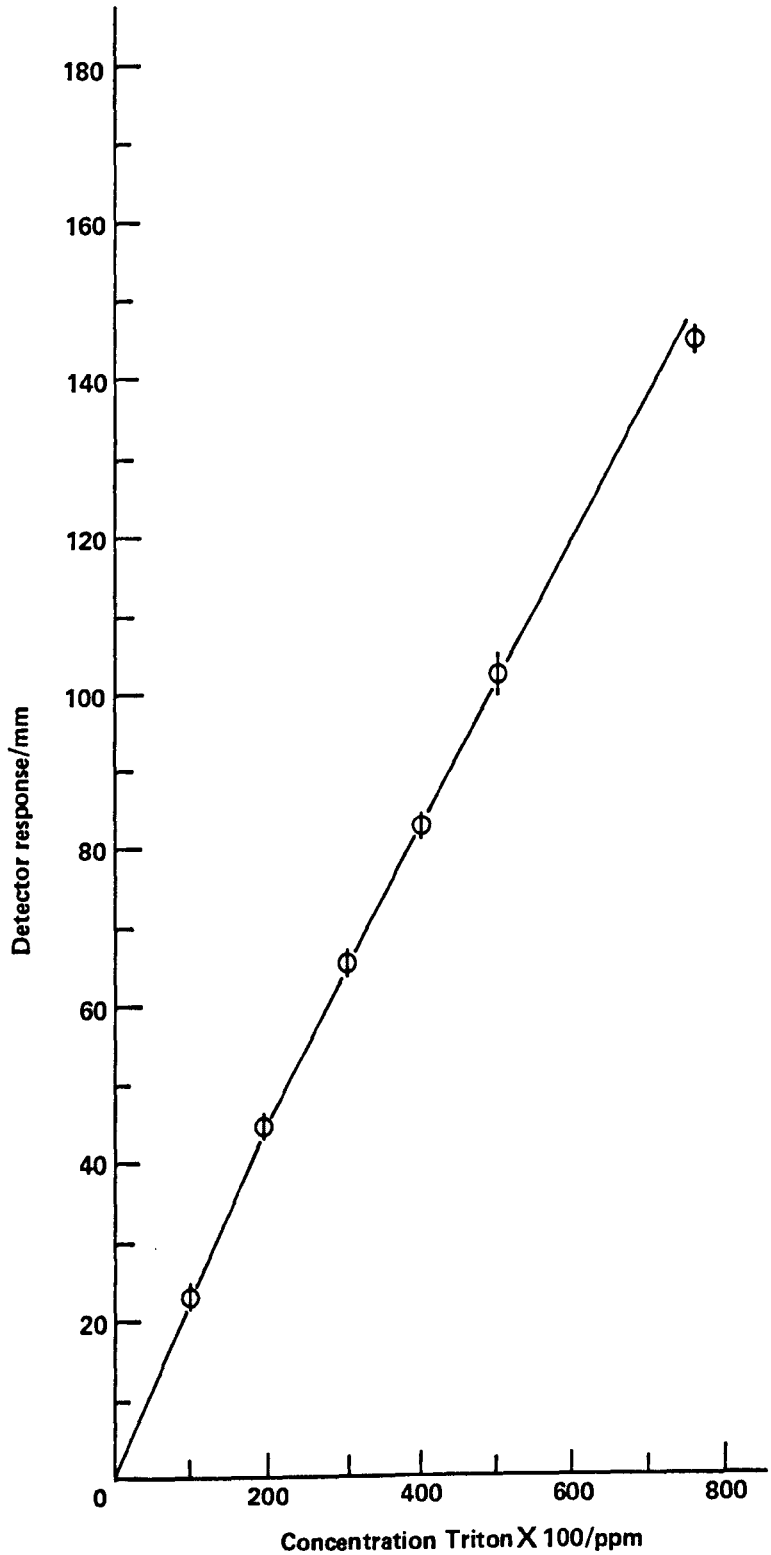


Fig 2 LC Calibration curve

Detector response (Pye Unicam LC3) vs surfactant concentration for Triton X 100

3. RESULTS

3.1 Chromatography

From HPLC studies of standard samples it was concluded that :

(i) 230 nm was the optimum wavelength for the detection of octylphenolethoxylates in the column effluent, (the absorption spectra[25] exhibit maxima at 275 nm and at 224 nm).

(ii) 2 ppm was the limit of detection (twice baseline noise) for Triton X 100 when a 10 µl aliquot was eluted with methanol-water. The volume of the applied sample could be varied using the appropriate loop with the Rheodyne valve between 10 and 500 µl. Use of the larger volume loops can improve the detection of solute in the effluent stream, but in practice the 10 µl and 20 µl loops were preferred on the criterion of improved chromatography.

(iii) the detection limit precluded effective investigation of foliar uptake of Triton X 100 following application of dilute solutions (10-100 ppm) as a consequence of the dilution factor introduced by the recovery procedure, (Table 1).

(iv) relative to Triton X 100, a considerable reduction in UV detector response was found with increasing oxyethylene content, (Table 2), attributable to (a), the reduction in the percentage contribution to the molecule made by the chromophore with increasing oxyethylene content, and (b), to the simultaneous reduction in the number of moles of compound contributing to the absorption at equi-concentrations.

Table 2 shows that the lower molecular weight compounds, (Triton X-15 and -35), also gave a reduced detector response relative

Table 1 - Surfactant concentration/ppm in extracts* assuming certain recoveries

Conc. applied ppm	% Recovery		
	1	10	100
10,000	2	20	200
1,000	0.2	2	20
100	0.02	0.2	2
10	0.002	0.02	0.2

* based on the application of a 10 µl droplet and the use of 500 µl of solvent for recovery of material from the leaf surface.

Table 2 - Relative response of UV detector to equi-concentrations solutions of Triton X compounds

Triton X compound	15	35	45	114	100	102	165	305	405	705
Molarity(1)	20	15	11.5	10.2	9.3	8	5.5	3.3	2.5	1.5
Detector response(2)	45	95	130	116	100	75	59	35	25	8

(1) Relative molarities of 500 ppm solutions of the surfactants

(2) Detector response as determined with Pye Unicam LC 3 UV detector and expressed relative to Triton X 100

to that found for Triton X 100. This was attributed to the limited aqueous solubility of the lower homologues: the data for these compounds probably represented the detector response of saturated solutions. Uptake studies with Triton X 35 and Triton X 15 were limited to a consideration of saturated solutions. As a consequence of reduced sensitivity for higher molecular weight compounds a further limitation was imposed such that for these compounds uptake studies could only be attempted following application of concentrated solutions.

(v) no separation on the basis of ethylene oxide content was discernible when Triton X compounds were eluted with methanol-water, (i.e., Figure 1), and the retention volume (6 cm^3) was found to be independent of the oxyethylene content.

(vi) a partial fractionation into the component oligomers was obtained when Triton X compounds were eluted with acetonitrile-water.

The extent to which the mixture of oligomers could be separated varied with the nominal range of chain lengths for the sample. Figure 3 shows the series of peaks obtained when Triton X 100 was eluted with acetonitrile-water. Preliminary studies indicated that this oligomer spectrum had a reproducible ratio of components which was retained on dilution but that the chromatographic separation was very sensitive to the composition of the eluent. Resolution was rapidly lost if the composition of the eluent varied slightly from that reported above. This, and the absence of any separation when methanol - water was used to elute the samples, may be due to the effect of the eluent on the conformation of the oxyethylene chain. Melander et al.,[26] have shown that the retention behaviour of the alkylphenol polyoxyethylene

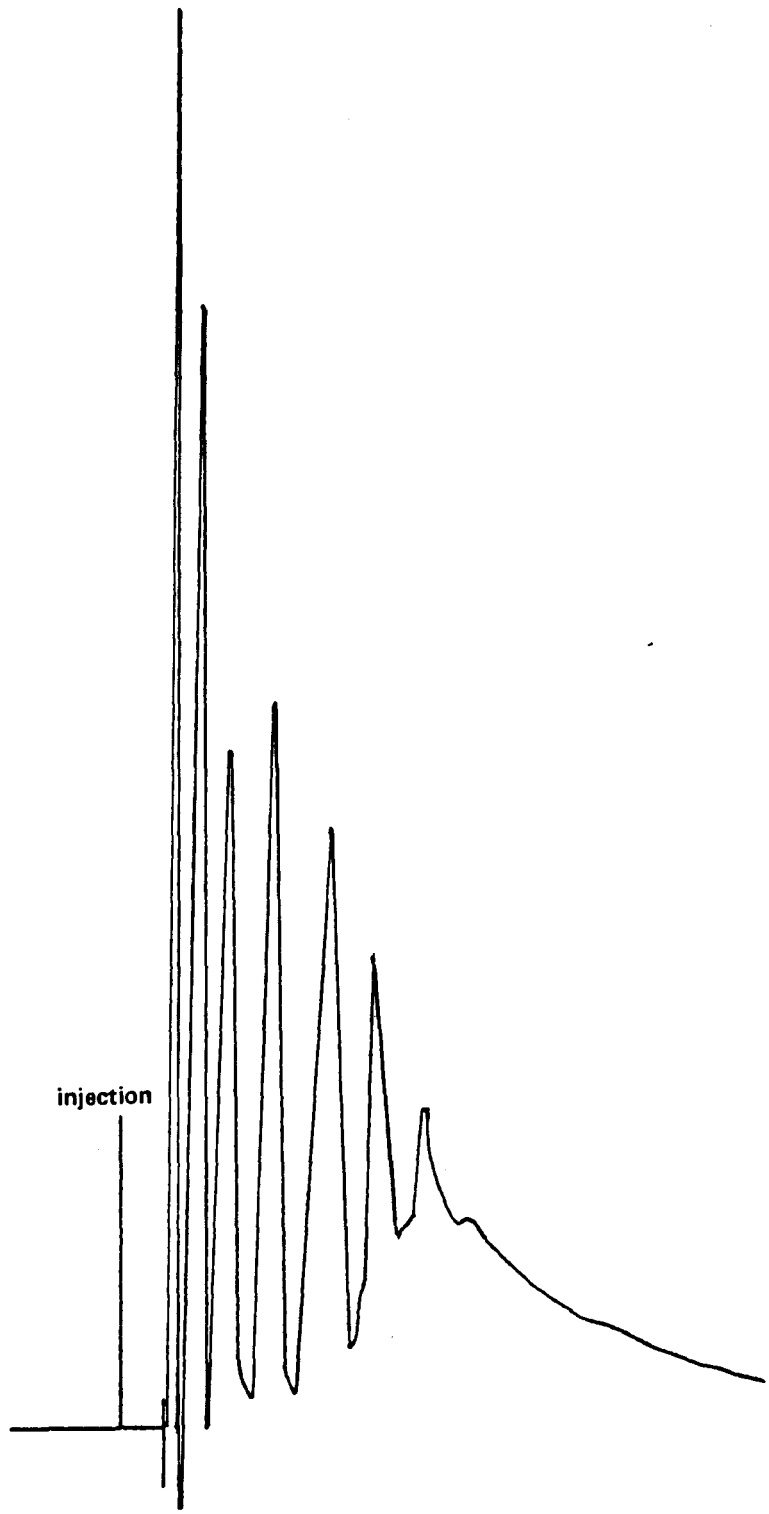


Fig 3 High performance liquid chromatogram of Triton X 100

Detector response to injection of 10 μl of a 500 ppm aqueous solution of Triton X 100 onto a 200 mm x 4.6 mm ID 10 μm Lichrosorb RP8 column eluted with acetonitrile/water (95:5) at 1 $cm^3.min^{-1}$

compounds depends on the solvent composition and that this dependency can be related to the existence of a zigzag and meander conformation of the hydrophilic chain, the two conformers having significantly different retention factors in the same chromatographic system. The relative concentration of the conformers depends on the eluent composition and the number of ethylene oxide residues in the molecule.

The distribution of chain lengths in Figure 3 differed from the expected Poisson distribution if it was assumed that each peak on the chromatogram represented a single oligomer.

The difference in the form of the distribution was attributed to a loss of resolution of the individual chain lengths outside an upper and lower limit. The inability of the technique as used to effect separation of the higher oligomers was illustrated in the chromatograms obtained from Triton X 102 (Figure 4 - mean oxyethylene content 12.5), and Triton X 165 (Figure 5 - mean oxyethylene content 16); comparison of these chromatograms with Figure 3 shows that the series of peaks found for Triton X 100 was replaced to varying degrees by a continuous curve. For Triton X 305 the chromatogram was a continuous curve and further increasing the oxyethylene content resulted in the maximum on this curve being displaced to longer elution volumes.

The large contribution made to the chromatogram in Figure 3 by the first eluted peak was contrary to that expected on the basis of a Poisson distribution, and suggested that the short chain oligomers in the sample had not been separated, the peak representing the combined response of this material. This interpretation is supported by the chromatograms obtained with the lower Triton X homologues; Figure 6 shows that for Triton X 114 (mean oxyethylene content 7.5) the first

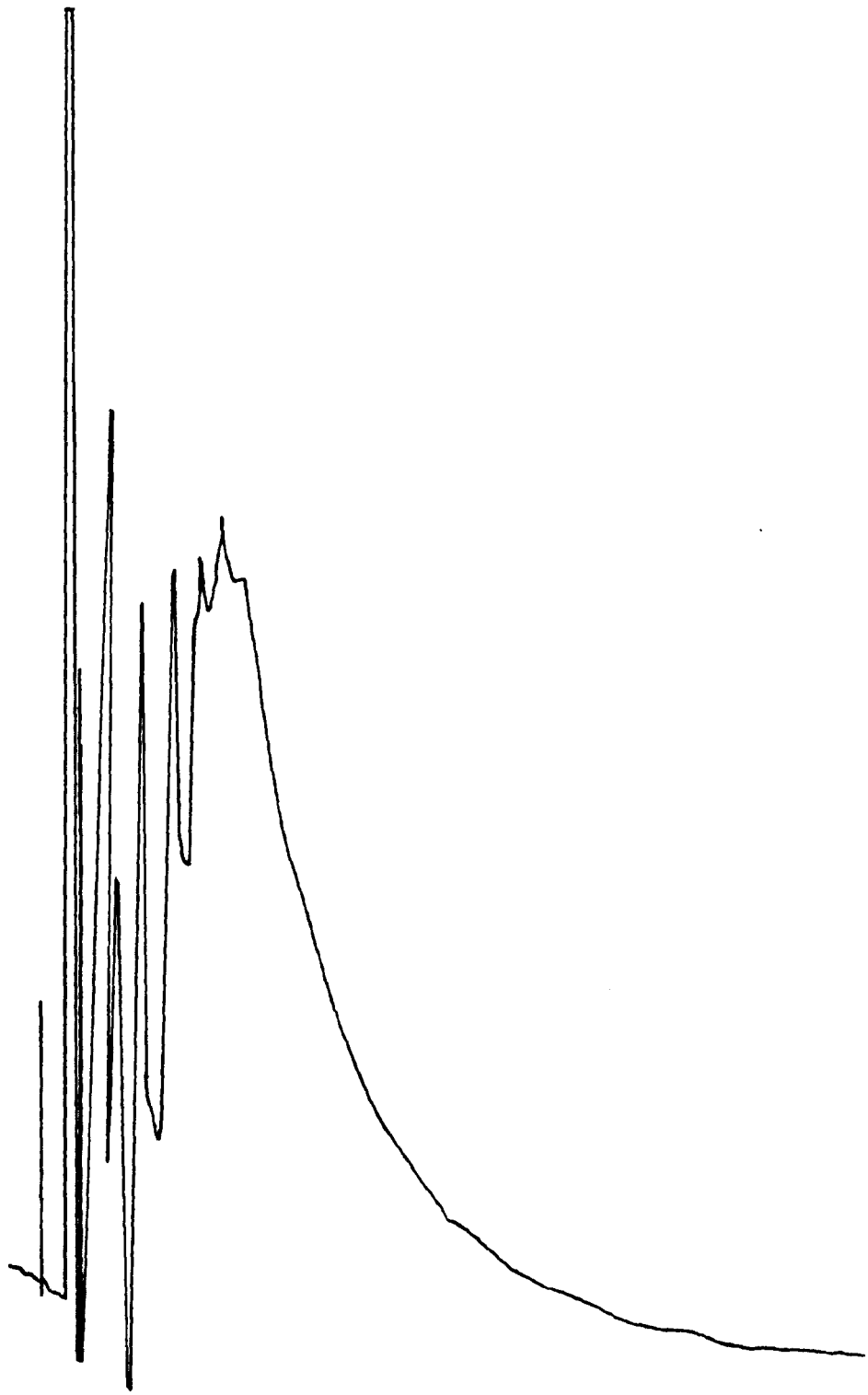


Fig 4 High performance liquid chromatogram of Triton X 102

Detector response to injection of 10 μl of a 500 ppm aqueous solution of Triton X 102 onto a 200 mm x 4.6 mm ID 10 μm Lichrosorb RP8 column eluted with acetonitrile/water (95:5) at 1 $cm^3.min^{-1}$

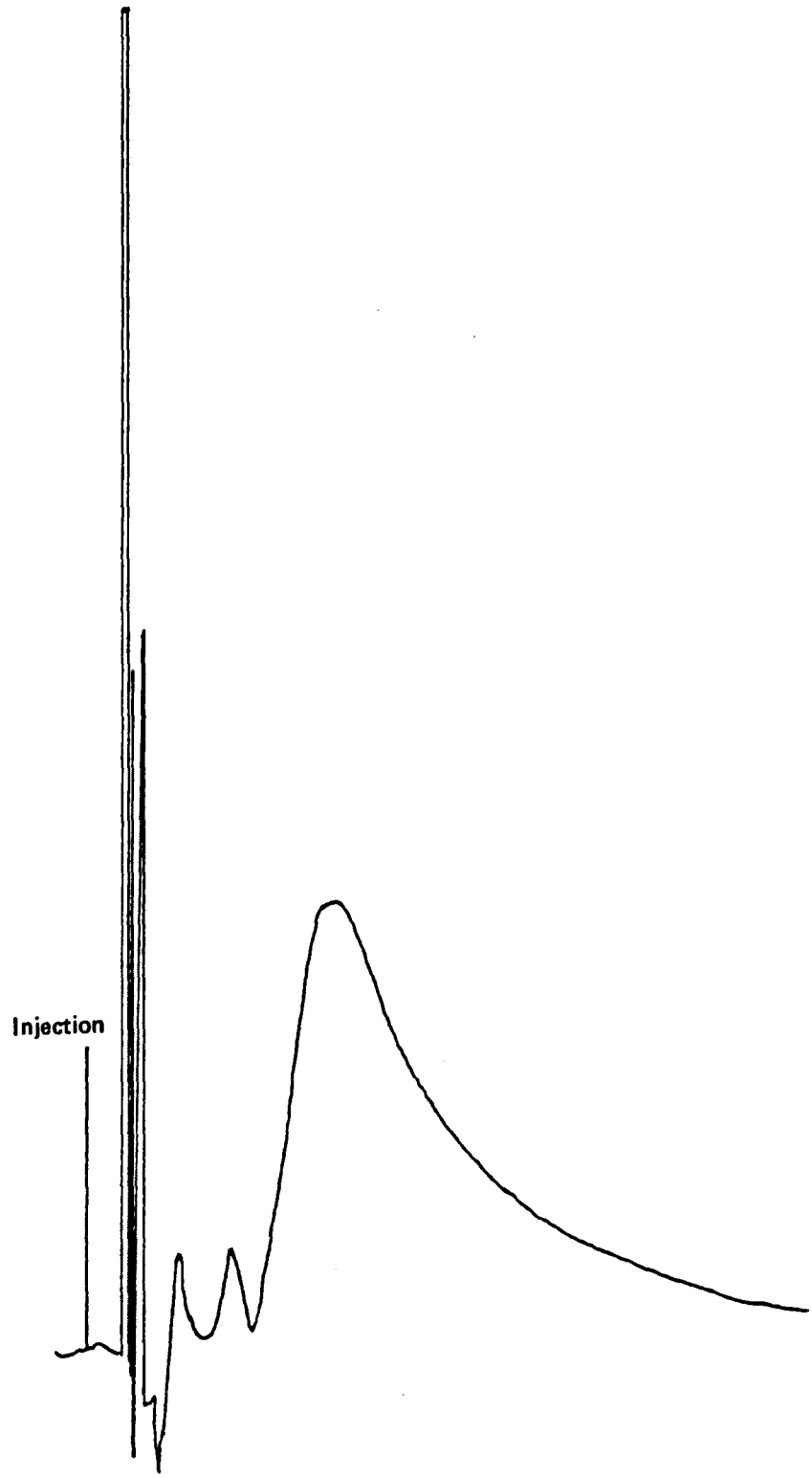


Fig 5 High performance liquid chromatogram of Triton X 165

Detector response to injection of 10 μl of a 500 ppm aqueous solution of Triton X 165 onto a 200 mm x 4.6 mm ID 10 μm Lichrosorb RP8 column eluted with acetonitrile/water (95:5) at 1 $cm^3.min^{-1}$

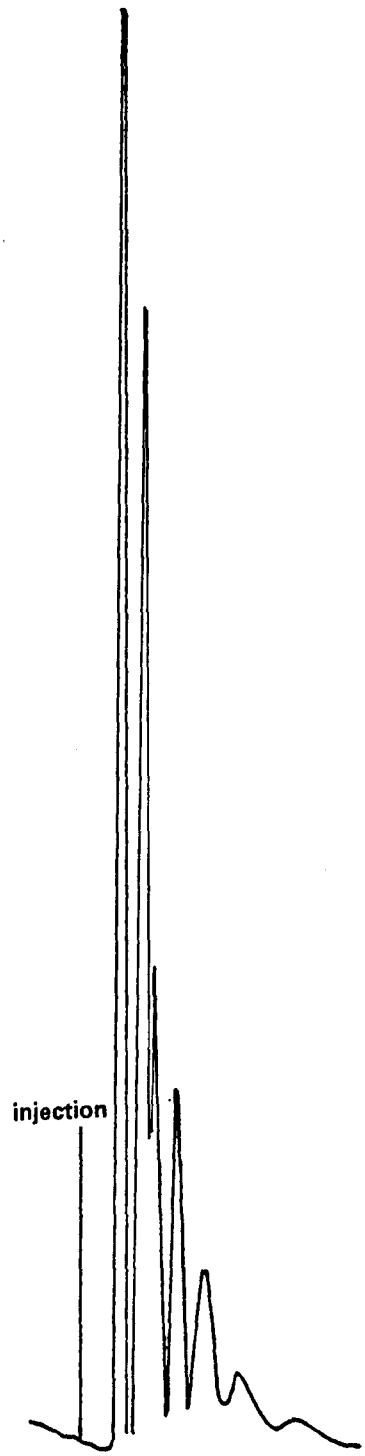


Fig 6 High performance liquid chromatogram of Triton X 114

Detector response to injection of 10 μl of a 500 ppm aqueous solution of Triton X 114 onto a 200 mm x 4.6 mm ID 10 μm Lichrosorb RP8 column eluted with acetonitrile/water (95:5) at 1 $cm^3.min^{-1}$

peak contributed still more to the total chromatogram, and chromatography of Triton X 45 gave only a single peak with a very small retention volume.

3.2 Foliar uptake of Triton X 100

Table 3 shows the recovery made by a water wash of Triton X 100 following application of an aqueous solution of the surfactant to wheat. It can be seen that uptake did not occur during the time taken for the drop to dry, (100% recovery of surfactant at zero time), and that the recovery of the surfactant was gradually diminished over a period of 168 hours to less than 10% of that applied.

Recovery of Triton X 100 from a non porous surface, (glass), treated with the same solution and in the same manner as used in the plant studies, was found to be unchanged over 168 hours; these data suggest that volatilization and photochemical degradation were unlikely to have contributed to the depletion of the foliar residue of Triton X 100.

Table 4 shows the recoveries, made by a series of extractions of the mid segment of treated leaves, of Triton X 100 following application of an aqueous solution of the surfactant to wheat. It was found that the recovery of surfactant in a first water wash agreed almost exactly with the value determined above at zero time and at 24 hours. The recovery made by a water wash after 48 and 72 hours varied slightly between the two experiments; (after 48 hours the recovery found in the second experiment was lower than that expected from the results of the first experiment, (the respective values being 50% and 66%), whilst after 72 hours the results were found to be in the reverse order, (i.e. 50%, Table 4 as against 23% in Table 3)). These differences were attributed to inherent variation in the plants

Table 3 - Percentage recovery of Triton X 100

Time/hrs	% Recovery			
	Replicates			Mean
0	100	100	100	100
24	80	70	85	78
48	60	65	72	66
72	25	45	25	32
144	8	40	5	18
168	8	8	8	8

Percentage recoveries of Triton X 100 made by a water wash of leaves treated with 1 x 10 µl of a 10,000 ppm aqueous solution. Each value represents the mean of three determinations.

Table 4 - Percentage recovery of Triton X 100

	Extracts	Time/hours			
		0	24	48	72
1	First water wash	100	80	50	50
2	Second water wash	0	0	0	0
3	First chloroform extract	-	2.7	4.9	5.0
4	Second chloroform extract	-	0	1.7	2.9
5	Final water extract	-	4.9	14.6	15.0
6	Mass balance	100	87.6	71.2	72.9

Percentage recoveries of Triton X 100 made in a series of extracts of leaves treated with 1 x 10 µl of a 10,000 ppm aqueous solution. Each value represents the mean of three replicates.

used, a feature evident from the difference between replicates found in the data from the first experiment, (Table 3; recoveries after 72 hours varying between 25% and 45%, and after 144 hours varying between 5% and 40%).

At all times of sampling it was found that a repetition of the water wash failed to recover additional surfactant although subsequent extraction with chloroform recovered measurable amounts of material, (Table 4; results at 24, 48 and 72 hours). These data support the hypothesis that material recovered by a water wash was not extracted from the leaf but was dissolved off the leaf surface.

Chloroform extractions recovered a maximum of 7.9% of the surfactant applied after 72 hours, (Table 4), with the greatest recovery being found in the first extract. Little or no additional recovery was achieved by increasing the number of extractions or by increasing the period of immersion. (When the period of immersion was increased beyond about 10 minutes the co-extraction of plant material prevented identification of the surfactant).

The final aqueous extraction was performed on the dewaxed plant segments and achieved the greatest recovery of Triton X 100 after the first wash, recovering a maximum of 1% of the applied material after 72 hours, (Table 4). Material was leached out of the dewaxed plant segments over the six hour period allowed and the total recovery appeared unchanged with longer immersions. This extraction was shown to depend on the prior removal of the lipids by treating leaves by the same method but omitting the chloroform extraction; in these studies the aqueous extraction recovered only trace amounts of surfactant.

The combined recoveries of surfactant made by chloroform and the final water extraction increased over the time frame of the experiment

from 7.6% after 24 hours to 22.9% after 72 hours (Table 4; summation of extracts 3-6). This increase did not equate with the depletion in the surface residue measured over this period and the total recovery of surfactant from the mid segment was found to fall from 87.6% after 24 hours to about 72% after 72 hours. The limited mobility of surface active agents reported by Smith and Foy[5] and discussed above suggested that the material absent from the mid segment should still be retained in the treated leaves after 72 hours, assuming the surfactant to be intact and not metabolized into more mobile compounds.

The analysis of the acropetal and basipetal segments of treated leaves was attempted by the same series of extractions as above. Water washes and chloroform extractions of this plant material did not recover measurable amounts of Triton X 100. Prolonged immersion of the dewaxed material in water extracted a considerable amount of endogenous materials which prevented identification and quantification of surfactant in these extracts.

It was concluded from these studies that Triton X 100 moves through the leaf surface following foliar application, penetrating the epicuticular wax layer and accumulating within the leaf tissues. It was not possible to draw any conclusions on the ultimate fate of the penetrant from these data.

3.3 Effect of surfactant concentration

The data collected above (Tables 3 and 4) relates to application of a 10,000 ppm solution of Triton X 100; these studies were repeated with 5000 ppm and 1000 ppm solutions of the surfactant. Following application of 1 x 10 µl of a 5000 ppm solution of Triton X 100 to wheat leaves it was found that a water wash of the treated leaf recovered 100% of the applied material at zero time, falling to 58%

after 24 hours, 25% after 48 hours and undetectable (less than 1 µg) after 72 hours. Following application of 1 x 10 µl of a 1000 ppm solution of Triton X 100 to wheat leaves it was found that surfactant could not be detected in a water wash of the treated leaf after 24 hours. In conjunction with the data presented in Tables 3 and 4 these observations suggest that Triton X 100 penetrates the wheat leaf at a rate of about 20 µg per day over a period of about 3 days.

3.4 Oligomer distribution

Plant extracts evaluated in terms of the total surfactant content were also analysed for the distribution of chain lengths contributing to the total mass, as described above. In all samples it was found that the oligomer 'spectrum' obtained by HPLC differed from the spectrum of the solution applied to the leaf only in the intensity: the ratio of the component peaks (Figure 3) remained unchanged. From this it would seem that the percentage uptake of any one oligomer must occur at the same rate as that of the remaining oligomers, so that in absolute terms the mass of oligomer penetrant was directly proportional to the mass applied and varied in accord with the weight distribution of the oligomers. The data would not support the hypothesis of the preferential absorption of one oligomer with respect to the range of chain lengths present.

3.5 Foliar uptake of Triton X compounds

The effect of polyoxyethylene chain length on the rate of foliar uptake of polyoxyethylene non-ionic surfactants was further investigated by extending the above observations to consider the Triton X series of surfactants. The movement of these compounds into wheat was studied following the topical application of 1 x 10 µl of a 10,000 ppm aqueous solution: the investigation was limited to a consideration of the rate

of depletion of the surface deposit, measured as above by a water wash of the leaves, and samples were taken after 24, 48, 72 and 144 hours.

Table 5 shows the recoveries of surfactant for the Triton X series made by a water wash of treated leaves. It can be seen that uptake decreased as the mean oxyethylene content of the surfactants increased, a trend that culminated in the absence of any discernible uptake of Triton X 705 over a period of 144 hours (Table 5: treatment number 10). Where the mean oxyethylene content was less than 9.5, (Table 5: treatment number 1-4) foliar uptake was completed within 24 hours. The compounds with mean oxyethylene contents between 9.5 (Triton X 100) and 70 (Triton X 705) showed a progressive reduction in the penetration achieved at any one time as the oxyethylene content increased.

The effect of mean oxyethylene content on foliar uptake was very pronounced between Triton X 114 and Triton X 100, (Table 5: treatment number 4 and 5 respectively). The distribution of oligomers in these materials are subject to considerable overlap as a consequence of the proximity of the respective mean values, (7.5 for Triton X 114, 9.5 for Triton X 100). Table 5 shows that, in contrast to compositional similarities, foliar uptake of these compounds was markedly dissimilar; whereas uptake of Triton X 114 was complete within 24 hours the uptake of Triton X 100 had progressed to only 20% of the applied dose at this time.

Table 6 shows the recovery of surfactant, expressed in moles, made by a water wash of wheat foliage treated with an aqueous solution of the surfactant by topical application. It can be seen that the trends established above in terms of percentage uptake are more pronounced when the data is recalculated to take account of the

Table 5 - Percentage recovery of Triton X compounds

Trt. No.	Triton X	Time/hours				
		0	24	48	72	144
1	15	100	0	-	-	-
2	35	100	0	-	-	-
3	45	100	0	-	-	-
4	114	100	trace	0	-	-
5	100	100	80	50	25	0
6	102	100	ND	55	45	20
7	165	100	ND	62	52	55
8	305	100	ND	80	80	45
9	405	100	ND	88	83	65
10	705	100	ND	100	100	100

Percentage recovery of Triton X compounds made by a water wash of leaves treated with 1 x 10 µl of a 10,000 ppm aqueous solution of the surfactant. Each value represents the mean of three replicates.

Table 6 - Recovery[1] of Triton X compounds

Trt. No.	Triton X	Time/hours				
		0	24	48	72	144
1	15	0.40	0	-	-	-
2	35	0.30	0	-	-	-
3	45	0.23	0	-	-	-
4	114	0.19	trace	0	-	-
5	100	0.16	0.13	0.08	0.04	0
6	102	0.13	ND	0.07	0.06	0.03
7	165	0.11	ND	0.07	0.06	0.06
8	305	0.07	ND	0.05	0.05	0.03
9	405	0.05	ND	0.04	0.04	0.03
10	705	0.03	ND	0.03	0.03	0.03

[1]Recovery of surfactant, expressed in moles.10^6, made by a water wash of leaves treated with 1 x 10 ul of a 10,000 ppm aqueous of the surfactant.

Data recalculated from Table 5 on the basis of the mean molecular weights as reported by Rohm and Haas in reference 34 in Chapter 2.

difference in molecular weight between the surfactants. Thus, the rate of uptake of Triton X 114 was, at about $1.9.10^{-7}$ moles.day^{-1}, about five times greater than the rate of uptake of Triton X 100, ($0.4.10^{-7}$ moles.day^{-1}), and about 50 times greater than the rate of uptake of Triton X 405 which varied between 3.10^{-9} and 7.10^{-9} moles.day^{-1}.

The data presented in Tables 5 and 6 suggested that foliar uptake of the Triton X compounds can be negatively correlated with the mean oxyethylene content of the surfactant. However, it was found that when the extracts were analysed for the distribution of chain lengths, no discernible change in oligomer distribution, relative to that present in the original mixtures, accompanied uptake: as above only the overall intensity of the chromatogram was altered with foliar uptake.

3.6 Translocation

HPLC analysis of plant extracts was found to quantify adequately the depletion of a surface deposit of a Triton X surfactant and to permit investigation of the residue of surfactant in chloroform extracts of the epicuticular wax layer, but could not be used to demonstrate positively transport of the penetrant out of the leaf segment to which the original application was made. This study was prevented by the low value of the ratio of surfactant to interfering co-extracted plant material in the extracts of acro- and basi-petal segments of treated leaves.

Table 4 shows that about 30% of the applied surfactant was not detected in the combined extracts from the mid segment of treated leaves; this result may be explained in terms of transport away from the area of uptake, although alternative mechanisms may be invoked, i.e. metabolic degradation and/or irreversible binding.

The uptake of ^{3}H-tetraoxyethylene octyl ether was investigated following the topical application of 10 ul drops of 100, 1000 and 10,000 ppm aqueous solutions to wheat. Recovery was as above, save that a maceration of the plant material was used in the final recovery step, (analysis of the radioactive labelled surfactant was less sensitive to the presence of co-extracted material), and was restricted to 24 hours after application in view of the rapid uptake found with the lower molecular weight Triton X compounds, (Table 5: treatment number 1-4). It was found that:

(i) At all applied concentrations of surfactant, water and chloroform extractions of the surface of treated leaves failed to recover any of the applied activity,

(ii) Extraction of the treated segment recovered about 100% of the applied activity from drops of 100 and 1000 ppm solutions, and 55% of the applied activity from the application of a 10,000 ppm solution,

(iii) Extracts of plant material basipetal to the area of application did not contain detectable levels of activity,

(iv) Extracts of plant material acropetal to the area of application contained 35% ± 15% of the activity applied in 10 ul drops of a 10,000 ppm solution, and

(v) Total recovery of activity was about 100% from the application of 100 and 1000 ppm solutions, and 90% ± 15% from the application of a 10,000 ppm solution.

From these data it was apparent that tetraoxyethylene octyl ether penetrated the cereal leaf following topical application and moved acropetally within the treated leaf. From the extent of movement within a 24 hour period transport was presumably via the xylem stream.

The overall rate of uptake and movement out of the treated segment was more rapid than found with Triton X 100, (Table 4). Movement of the surfactant was more extensive than that found with Tween 20 by Smith and Foy,[5] but tetraoxyethylene octyl ether is a smaller and possibly more mobile molecule than those studied previously. Considerable accumulation of the surfactant was evident in the leaf tissue underlying the area of application of 100 and 1000 ppm solutions.

4. DISCUSSION AND CONCLUSIONS

The permeability of the cereal leaf to ethoxylated non-ionic surfactants has been clearly demonstrated following topical application of aqueous solutions. The rate of uptake has been shown to depend on the mean oxyethylene content of the surfactant and to decrease with increasing oxyethylene content within the Triton X series of ethoxylated octyl phenols. The distribution of oligomers within any one surfactant was apparently retained during movement into the leaf.

The movement of a foliar applied solute through the cuticular membrane has been considered earlier when the concept of a non polar direct pathway and a polar aqueous pathway were considered in terms of the solubility characteristics of the solute. The amphiphilic character of the surfactant molecules would allow both hypothetical routes to be exploited. Because of the absence of any known morphological structure which could mediate movement through the epicuticular waxes, the data obtained in this study have been interpreted in terms of a direct route involving diffusion through

the lipid layer: penetration of the solute is discussed in terms of (i), partition into the wax layer, (ii), diffusion and (iii), partition out of the waxes into the underlying structures.

The partition coefficients of both single species and normal distribution polyoxyethylene non-ionic surfactants have been discussed in Chapter 2, (Table 7), where it was shown that the partition coefficient between isooctane and water, Ko/w, decreases as the oxyethylene content increases. Table 7 , (Chapter 2), shows that as the oxyethylene content increases between 1 and 10 moles then Ko/w decreases by four orders of magnitude, but further increasing the oxyethylene content to 40 moles only decreases Ko/w by one order of magnitude.

The general trend with oxyethylene content in the value of Ko/w is parallel to the trend in foliar uptake, (Tables 5 and 6), so that uptake is most rapid when Ko/w is such that the equilibrium favours the lipid phase. In quantitative terms, however, there is less agreement and the relative magnitude of Ko/w would not seem to explain adequately differences between compounds in the rate of uptake. The difference in the rate of uptake between Triton X 100 and Triton X 165 was an order of magnitude less than the difference between Triton X 100 and Triton X 114 although the differences in Ko/w values (Table 7 Chapter 2) are vice-versa. The absence of any discernable penetration with Triton X 705 was also difficult to explain in terms of relative partition coefficients given the relatively rapid uptake of the Triton X compounds 165, 305 and 405 at similar rates, (Tables 5 and 6).

The diffusion of a solute through a solid phase was discussed in Chapter 1, when the phenomenum was considered in terms of movement through holes formed by the thermal motion of the solid lattice. It

was noted that the probability distribution of hole sizes would have a form such that small holes would predominate. A small molecule would be expected to diffuse more rapidly in a solid phase as a consequence of the availability of holes of requisite size. When the shape of the molecule departs from that of a sphere it has been found[27] that the rate of diffusion is mainly determined by the shortest dimension of the molecule; from experimental observation, the least cross-sectional area of the molecule is best correlated with the diffusion coefficient.

In the most probable random coil configuration the overall variation in the least cross-sectional area of the Triton X series is less than one order of magnitude, (Chapter 2); in a fully extended configuration less variation in cross-sectional area would be expected.

Although there is no adequate theory[28] which allows the accurate prediction of diffusion coefficients in solid phases, some studies have attempted to correlate the diffusion coefficient with the size of the diffusant in terms of the molecular weight. Calculations based on a number of commonly used expressions have been included (Appendix 1). As with the variation in Ko/w discussed above, the general trend in the magnitude of the diffusion coefficient, calculated as in Appendix 1 or based on the relationship with the cross-sectional area of the molecule considered above, qualitatively parallels the variation in foliar uptake (so that both the diffusion coefficient and the rate of foliar uptake decreases as the oxyethylene content is increased) but the quantitative differences in the rate of uptake, and in particular the difference between Triton X 114 and Triton X 100, and the absence of penetration with Triton X 705, is not explained. Calculated values of the respective permeability coefficients

(Appendix 1) were also unable to account in quantitative terms for the relative rates of uptake.

Desorption into the aqueous phase underlying the lipid membrane was shown (Chapter 1) to be a limiting step in the foliar uptake of flamprop-methyl. With the alkyl phenol ethoxylates this step might be expected to be of lesser importance in view of the much higher solubilities of these compounds in aqueous phases. If partition into the apoplast influenced the rate of uptake, then its effect would be to limit the uptake of the more hydrophobic, short chain compounds relative to the compounds with a higher mean oxyethylene content.

Although Table 5 shows that the rate of foliar uptake of the Triton X compounds was apparently influenced by the oxyethylene content it was also noted that, on the basis of HPLC analyses with acetonitrile-water eluent, no fractionation of the component oligomers in any one product occurred on the leaf. Crook et al[29] have demonstrated the fractionation of a normal distribution oxyethylene alkyl phenol between oil and water: fractionation of the polydisperse mixture of chain lengths in the Triton X product would be expected to accompany foliar uptake as material partitioned into the epicuticular wax phase.

In previous discussions of uptake it has been assumed that the wax phase was unaffected by the presence of the diffusant. It is however possible that a synergistic interaction of the components of the mixture applied to the leaf could occur, such that the more redily permeable adducts 'carried' other adducts across the interface as a result of the modification of the wax phase at the interface. If the wax phase was modified as a consequence of the rapid adsorption of short chain oligomers so that little or no variation existed in

the partition coefficients at differing chain lengths a result similar to that observed would be expected. If this occurred, then one might also expect the contribution made by these short chain lengths to the overall distribution of the sample to have an influence on the relative rates of uptake of the various samples. Previous discussion has been based on the assumption that the properties of the material in any one sample would vary gradually and be clustered about a mean corresponding to the properties of a compound with the mean oxyethylene content of the sample. This type of behaviour would be expected for a polydisperse material, and has been found to be exhibited by many of the properties of non-ionic surfactant mixtures[30]. A synergistic interaction of the type postulated above could result in the behaviour of the mixture deviating from that expected for a polydisperse mixture, since the observed effect would depend not on the overall distribution but on the contribution to the total of the material in one of the tails of the distribution curve. The concept of a synergistic interaction between the components of the surfactant mixture can be used to explain certain features of the results which could not be explained previously.

APPENDIX 1

Estimated values of diffusion and permeability coefficients

1. Diffusion coefficients

The diffusion of a sphere in a continuous liquid medium has been considered by the hydrodynamical theory, initiated by Einstein, which applies Stokes law to describe the drag on a large spherical solute molecule moving through a continuum of small solvent molecules. The resulting Stokes - Einstein equation relates the diffusion coefficient (D) to the radius of the solute molecule (r) such that D is inversely proportional to r. The Stokes - Einstein relationship can be reduced to $DM^{1/3}$ = a constant, at constant temperature[27], where M is the molecular weight of the solute. If the solute molecule is small relative to the solvent a better fit to experimental observations is given by $D M^{1/2}$ = a constant[31]. The difference in these expressions has been attributed to the fact that for small solute molecules the solvent can no longer be considered as a continuous medium. Because this discontinuity can be considered analogous to the movement via holes postulated for diffusion in a solid phase, the reciprocal of the square root of M has been used [31] as an estimate of the relative diffusion coefficients in solids.

Lieb and Stein[27] have shown that for diffusion in hydrophobic polymers the best fit to experimental observations was given by $D M^{1.1}$ = a constant (natural rubber) and $D M^{3.8}$ = a constant (polymethylacrylate), and have suggested that relationships of this order are to be expected for biological membranes.

It has been suggested that relationships of the type $D M^{X}$ equals a constant hold over only a restricted range of molecular sizes and

are only applicable to molecules that do not depart too drastically from a spherical shape. Although in general the shape of surfactant molecules tends to approximate to cylindrical rather than spherical, the relationships identified above were used in the absence of alternative equations to calculate values of D for the Triton X compounds. The values of the molecular weight used in these calculations were based on the mean oxyethylene content of each sample. Table 1 shows the variation in the relative values of D over the Triton X series calculated for x = 1/2, 1/3, 1.1 and 3.8. It can be seen that the variation in D is less than one order of magnitude when x = 1/2 or 1/3, and that the extreme values differ by only one order of magnitude when x = 1.1, but that when x = 3.8 considerable variation is predicted over a range of four orders of magnitude.

2. Permeability coefficients

The rate of foliar penetration of the surfactant can be considered as a composite of two independant events, viz. partitioning of the surfactant between the external phase and the wax phase, and diffusion through the wax phase. If the wax is considered as a membrane separating the two phases external and internal, it can be shown[32] that the overall membrane permeability coefficient (P) is related to these events such that $P = K_D D_{mem}/l$, where K_D is the equilibrium distribution coefficient between membrane and the separated phases, D_{mem} the diffusion coefficient within the membrane, and l is the thickness of the membrane. The quantitative evaluation of this expression was simplified by use of the following assumptions;

i) the value of l was assumed to be unity,

ii) the partition coefficients for the compounds between isooctanol and water tabulated in Chapter 2 were used as an approximation to

the distribution coefficient between the membrane and an aqueous phase, and

iii) the relative values of D calculated above (Table 1) were used for D_{mem}. The value of D was arbitarily assumed to be of the order of $10^{-10} cm^2 s^{-1}$ for Triton X 15.

Table 2 shows the permeability coefficients, calculated according to $P = K_D D_{mem}$, for the Triton X compounds. When the variation in the estimated permeability coefficients was compared with the variation in the rate of foliar uptake (Table 3: a comparison facilitated by recalculating all data relative to Triton X 100) it was seen that, with the exception of data relating to Triton X 705, the variation in permeabilities was greater than the experimentally determined variation in the rate of uptake. For the compounds Triton X - 102, 165, 305 and 405 the relative uptake found was better than that predicted by any of the expressions tested. The best agreement between calculated and determined values was found from $P \propto K_D/M^{0.5}$; however relative to Triton X 100 the variation in the calculated permeabilities was an order of magnitude less than the observed variation in uptake for the compounds X- 165, 305 and 405. The marked difference in the observed uptake of Triton X 114 and Triton X 100 was only approached in the relative permeabilities calculated from $P \propto K_D/M^{3.8}$, which also yielded the lowest permeability for Triton X 705. This expression however predicted a difference in permeabilities between Triton X 100 and Triton X 405 of four orders of magnitude when the experimentally determined values differed by less than one order of magnitude.

Table 1 - Estimated relative diffusion coefficients (D)[1]

Triton X	M[2]	$1/M^{0.5}$	$1/M^{0.3}$	$1/M^{1.1}$	$1/M^{3.8}$
15	251	6.3	1.6	2.3	7.6
35	339	5.4	1.4	1.6	2.4
45	427	4.8	1.3	1.3	1.0
114	537	4.3	1.2	1.0	0.4
100	625	4.0	1.2	0.8	0.2
102	757	3.6	1.1	0.7	0.1
165	911	3.3	1.0	0.6	0.06
305	1527	2.6	0.9	0.3	0.008
405	1967	2.3	0.8	0.2	0.003
705	3287	1.7	0.7	0.1	0.0004

(1) Calculated as $D \propto \frac{1}{M} X$

(2) Molecular weight calculated for $C_{14}H_{21}O(CH_2CH_2O)_nH$ where n is the mean oxyethylene content.

Table 2 - Estimated permeability coefficients (P)[1]

Triton X	K_D/M^X		
	$K_D/M^{0.5}$	$K_D/M^{0.3}$	$K_D/M^{3.8}$
15	7.4	1.9	8.9
35	$3.9.10^{-1}$	$1.0.10^{-1}$	$1.7.10^{-1}$
45	$3.7.10^{-2}$	$1.0.10^{-2}$	$7.7.10^{-3}$
114	$6.8.10^{-3}$	$1.9.10^{-3}$	$6.6.10^{-4}$
100	$2.1.10^{-3}$	$6.1.10^{-4}$	$1.2.10^{-4}$
102	$4.8.10^{-4}$	$1.5.10^{-4}$	$1.5.10^{-5}$
165	$1.0.10^{-5}$	$3.2.10^{-5}$	$1.8.10^{-6}$
305	$6.2.10^{-5}$	$2.0.10^{-5}$	$1.9.10^{-7}$
405	$4.9.10^{-5}$	$1.7.10^{-5}$	$6.4.10^{-8}$
705	$3.0.10^{-5}$	$1.2.10^{-5}$	$7.7.10^{-9}$

(1) From $P = \frac{K_D D_{mem}}{l}$ (see text)

then $P \propto K_D D_{mem}$

$\propto \frac{K_D}{M^X}$ given than $D_{mem} \propto \frac{1}{M^X}$

Table 3

Triton X	Permeability coefficients		Time/hours				Time/hours			
	$P=\frac{K_D}{M^{0.5}}$	$P=\frac{K_D}{M^{3.8}}$	24	48	72	144	24	48	72	144
			% Uptake(1)				Molar uptake(2)			
114	3.20	5.50	5.00				6.30			
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
102	0.23	0.13		0.90	0.73	0.80		0.70	0.60	0.65
165	0.05	0.02		0.76	0.64	0.45		0.50	0.44	0.31
305	0.03	1.10^{-3}		0.40	0.27	0.55		0.19	0.13	0.22
405	0.02	5.10^{-4}		0.24	0.23	0.35		0.08	0.08	0.10
705	0.01	6.10^{-5}		0.00	0.00	0.00		0.00	0.00	0.00

(1) % uptake recalculated relative to data for Triton X 100 from Table 5, Chapter 3

(2) Molar uptake recalculated relative to data for Triton X 100 from Table 6, Chapter 3

CHAPTER FOUR

Penetration of flamprop-methyl into wheat : effect of non-ionic surfactants

I N D E X

SUMMARY

The foliar uptake of flamprop-methyl by wheat has been studied following topical application of the herbicide formulated in aqueous surfactant solutions. Non-ionic ethoxylated surfactants have been found to markedly enhance transcuticular movement of the herbicide but do not promote transport of the compound within treated leaves. The extent to which uptake was enhanced was largely influenced by the concentration of the formulation with respect to the surfactant, was much less dependant on either hydrophobe or hydrophile structure and was apparently independent, within experimental limits, of the herbicide concentration. Optimum surfactant concentrations were between 100 and 1000 ppm. Correlation between penetration enhancement and the surface properties of the formulations was non-existent. The effect of the surfactant on penetration is discussed in terms of a surfactant/lipid interaction which facilitated diffusion of the herbicide. At high surfactant concentrations a variety of responses were identified ranging from varying degrees of enhancement through to an inhibition of transcuticular movement: these responses were shown to correlate with the resistance to uptake afforded by a persistent surfactant residue on the leaf surface.

1. INTRODUCTION

It has long been recognised that surface active agents may facilitate and accentuate the emulsifying, dispersing, spreading, wetting, solubilizing and/or other surface modifying properties of herbicidal formulations.[1][2] Surfactants may also be incorporated into herbicidal formulations to assist in specific ways, for instance by facilitating penetration of the toxicant.[3] Previous reports[4-7] have noted that the incorporation of such additives in formulations modified the observed biological activity of the formulations, producing a variety of activity enhancing and inhibitory effects. It has been shown[8] that small variations in the nature of the surfactant may result in the overall effect on toxicant uptake changing from one of enhancement to one of inhibition, although generally addition of a surfactant has been reported to enhance the penetration and/or effectiveness of foliage applied compounds.[2,5,9-22]

The surface active agent may alter the effectiveness of the herbicide in various ways; Foy and Smith[23] identify five sites at which the presence of the surfactant could conceivably influence the activity of a herbicidal spray:

a) within the spray solution

b) on the surface of the leaf

c) within the cuticular layers

d) within or on the surfaces of the cells underlying the cuticle, and

e) within plant tissues removed from the treated area

Since all evidence to date[24] indicates that surfactants do not facilitate herbicide transport per se, the main site of involvement being primarily at the site of application and in the immediately

underlying tissues, then surfactant effects remote from the site of application will not feature in subsequent discussion.

Interactions at the leaf surface resulting in penetration enhancement could involve,[25,26]

i) direct molecular interaction between herbicide and surfactant,

ii) alterations in the partitioning behaviour of the herbicide

iii) increased area of contact between formulation and leaf surface, as a result of improved droplet spread and/or the elimination of air films,

iv) modifications to the physical form of the residue in terms of particle size and crystal shape,[27]

v) reduced volatility

vi) maintaining a liquid state on the leaf surface either by co-solvent action or (since surfactants are hygroscopic in nature and may act in a secondary capacity as humectants) by keeping spray drops moist, and

vii) inducement of stomatal infiltration by a lowering of surface and interfacial tensions.

Direct molecular interaction between the herbicide and formulation components is probably uncommon save when ionic herbicide/surfactant combinations are under consideration. With the further exception of (vii), the beneficial effect on uptake of the interactions identified can be interpreted in terms of the compartmental partition - diffusion model of foliar uptake introduced in Chapter One. Interactions serving to increase the area over which uptake occurs (iii), and/or modifying the partition coefficient can enhance the rate at which transfer occurs by partition and diffusion. It is known[13] that the physical state of

the spray residue can markedly influence the ease of absorption, the energy required for adsorption from a fluid state being less than that required for adsorption from a solid phase. Penetration enhancement may thus be attributed in part to the increased fluidity of the spray deposit, (vi).

With regard to the ability of surfactants to promote this fluidity Heritage[27] investigating the water capacities of surfactants as a function of humidity, reported that 10% (m/m) of water could be retained at only 50 per cent RH. Temple and Hilton[25] found that the solubility of lipophilic herbicides in some surfactants was great enough (about 4 to 15%) to keep all of the herbicide from a saturated spray in solution as the water carrier evaporated. Where uptake is essentially confined to the period during which the spray drop exists on the leaf surface these properties can prevent drying and extend the period of transcuticular movement.[11,13,26]

Most published work has concentrated on the effects of the surface active agent on the wetting and spreading of spray drops, and on the possibility of stomatal infiltration. (One reason for this may be the difficulties involved in for example quantifying the fluidity of a deposit). These studies have been reviewed below in the context of uptake with flamprop-methyl-surfactant formulations.

Specific herbicide-surfactant-plant interactions have been recognised and have necessitated a further consideration of the interactions between surfactant and plant, in particular those interactions which might occur following foliar penetration of the surfactant. Postulated surfactant-plant interactions[28] within the cuticular layer (and/or the underlying cells) involve alteration of the physico-chemical properties of these regions by :

i) the exhaustion of binding or adsorption sites within the cuticle, thereby altering the charge/polarity of the cuticle,[23][29]

ii) reduction of interfacial tensions between relatively polar and apolar submicroscopic regions,[12][25]

iii) alteration of cuticular adsorption pathways

iv) increasing the permeability of the cuticle by disruption of the epicuticular waxes, solubilisation of cuticular components,[29] and/or swelling of the cutin matrix,[30][1]

v) facilitating movement in the region of the cuticle/cell wall and the cell wall/cytoplasm interfaces,[12,25] including increased permeability of the plasmalemma through stimulation of incipient toxicity and general improvement in transport across membranes.

It has been shown[4,30] that certain surfactants are inherently phytotoxic and can cause leaf injury. These phytotoxic materials may enhance or reduce biological activity of the herbicide according to circumstances.[3] A variety of plant processes can be affected by surfactants; recent studies have shown that some compounds may interfere with the cellular energy generating systems that maintain membrane integrity, or have a direct interaction with the membrane thereby influencing permeability.[31,32] Surfactant phytotoxicity has been correlated[31,33] with induced alterations in cell/membrane permeability in isolated plant cells, and with structural alterations in artificial phospholipid membranes. These studies suggest that phytotoxic materials exert their effect through a direct interaction with membranes which renders the membranes incapable of maintaining normal permeability characteristics. Interactions of this type could be of particular

importance where uptake is regulated by symplastic mobility involving penetration of the plasmalemma.

Several studies have shown that damage to the leaf cuticle can increase the rate of foliar uptake of herbicides; this effect has followed injury by aphids,[34] frost,[35] wind[36] and abrasion.[37] Aberg[38] suggested that the chemically induced leaf injury resulting from the foliar application of contact acting chemicals might potentiate the foliar uptake of systemic herbicides by the disruption of the surface structure of the leaf. In practice however the biological activity of systemics has been found[39] to be reduced when combined with a contact acting compound. While treatment with the latter may indeed serve to potentiate penetration of the cuticle the destruction of the underlying tissues is likely to restrict further movement of a systemic compound away from the site of uptake. From this, an important criterion for the viability of a penetrant aid can be established, viz; the adjuvant must be capable of potentiating uptake without adversely affecting the subsequent transport of the herbicide when this is necessary for the expression of biological activity or for the continuation of uptake. Bland and Brian,[24] investigating surfactants and the uptake and movement of paraqat in plants, have shown that the potentiation of both uptake and mobility within the plant can demand conflicting requirements of the surfactant.

If one conceives the process of absorption as a rate process[40,41] the concept of an energy barrier to the transfer of the herbicide between external and internal phases is brought into consideration from the application of classical physico-chemical principles.[42] The absorption rate then depends on the state of the energy barrier, represented physically by the cuticle, and from this additives may be construed as

in some manner interacting with the cuticle, thereby lowering the energy barrier.

It has been shown that a membrane can present three distinct types of resistance to the passage of a solute;

i) resistance encountered to partitioning from the external phase into the membrane,

ii) diffusion resistance within the membrane, and

iii) resistance to partitioning between the membrane and the internal phase.

If the epicuticular waxes are dissolved or solubilized by the applied formulation then the barrier function can be largely diminished, even if the waxes are redeposited as the solvent evaporates, since this layer will no longer exist as a coherent layer underlying the residue. Partition between the external residue and the epicuticular waxes will also be favoured by improved contact between these phases.

Price[43] has noted that surfactants appear to increase the diffusion of other solutes across the cuticle. Diffusion resistance within the cuticle could, as noted earlier, be reduced by a variety of interactions following adsorption of the surfactant into the membrane. Lawrence[44] has shown that the penetration of water into a bimolecular lattice of fatty acids or alkanols results in only small swelling and about 2% (m/m) of water is the saturation value. This can be greatly increased, with improved swelling and the formation of a liquid crystal phase, in the presence of a surfactant. This interaction may be similar to that responsible for the alterations in cell membrane permeability discussed previously. The work of Lawrence is of particular interest since it was shown earlier that the orientation of wax molecules might take the form of bimolecular planes.

Resistance to partitioning between the membrane and the internal (aqueous) phase may be lessened by the absorption of surface active molecules at the interface, lowering interfacial tension, and by factors which serve to increase the solubility of the penetrant in the internal phase. Partition out of the membrane into the aqueous phase would be least favourable when the penetrant is a lipophilic, non-polar molecule.

It follows from the foregoing remarks that surfactant enhancement of foliar uptake could result, in part or whole, from a promotion of herbicide uptake and movement as a consequence of a surfactant induced lowering of various permeability barriers in the leaf cuticle. Enhancement of uptake would seem to depend on:

i) the magnitude of the various resistances per se, which would depend on the physico-chemical properties of the cuticle and hence on the type of plant,

ii) the physico-chemical properties of the solute,

iii) the physico-chemical properties of the surfactant, and

iv) the amount of solute and surfactant per unit cross section of the leaf surface, and the ratio of solute:surfactant.

In these studies the type of plant and the solute were held constant and the structure and amount of surfactant were varied. Holly and Turner[3] have observed that, in view of the published work demonstrating the occurrence of specific interactions between surfactants, herbicides and plants, a great deal of empirical work is rendered necessary to optimize the surfactant requirement with any one herbicide. The studies reported herein were performed with a view to optimization of surfactant structure (from a limited range of non-ionic surfactants) and concentration for the foliar uptake by wheat of flamprop-methyl. Critical evaluation of the relationships between structure, concentration, surface activity and

the physico-chemical properties of aqueous herbicide-surfactant formulations was attempted in the belief that such an approach might permit an insight to be gained into the precise mechanisms involved in herbicide-surfactant-plant interactions.

It was shown earlier that sorption of flamprop-methyl into the wax layer need not be paralleled by penetration of the wax layer. It was decided that the optimum herbicide-surfactant combination would be that which maximised penetration of the wax layer and hence maximised the availability of the herbicide to the apoplast. Because of this the degree to which uptake was modified by formulation was in general investigated by an organic solvent extraction of treated foliage and maceration of the extracted plant material.

2. EXPERIMENTAL

The experimental method used to investigate the effect of surfactant on the foliar uptake of flamprop-methyl was essentially as described earlier when considering uptake from aqueous solutions. Materials were as described in Chapter One (flamprop-methyl, plants) and in Chapter Two (polyoxyethylene non-ionic surface active agents). The general method involved topical application to selected leaves of a droplet of flamprop-methyl formulated in aqueous surfactant and the selective recovery of the herbicide from treated plants over a period of up to ten days after application. Both radioactive labelled and unlabelled herbicide was used in these studies; in both instances an aliquot of a acetone solution of the herbicide of known concentration was evaporated to dryness and the residue dissolved in a known volume

of an aqueous surfactant solution. The concentration of each formulation was determined analytically in all experimentation. Recovery and analysis of extracts were as described earlier.

3. RESULTS

Table 1 shows the recoveries of flamprop-methyl achieved by extraction of the treated leaf by immersion in hexane-acetone, and by maceration of the dewaxed leaf in acetone, 24 hours after application of the herbicide to wheat as a solution in aqueous Triton X solutions. At zero time the total herbicide applied in these solutions was recoverable in a hexane-acetone extract. The total herbicide applied was also recovered after 24 hours, divided between the surface extract and the macerate. It can be seen that, relative to the uptake achieved with the control treatment, (treatment number 36: aqueous solution of flamprop-methyl), the foliar uptake of flamprop-methyl was potentiated by incorporation of any of the Triton X compounds in aqueous formulations of the herbicide. This enhancement of uptake by wheat was evidenced by both the decreased recovery effected by the surface extract and by the corresponding increase in the recovery of herbicide in the macerate, (Table 1). The least effective treatment identified in this experiment was a 10,000 ppm solution of Triton X 165, (Table 1; treatment number 23); based on the recovery of herbicide in the macerate this treatment effected a five fold increase in the amount of flamprop-methyl moved into the leaf. The most effective formulation, a 1000 ppm solution of Triton X- 35, (treatment number 4), effected a thirteen fold increase in the transcuticular movement of the herbicide.

Table 1 : Foliar penetration over 24 hours from solutions of Triton X surface active agents (Experiment 1)

Trt No.	Triton X	Conc.		Extract	% Recovery ^{14}C-flamprop-methyl				
		ppm	molar		Replicates				Mean
1	15	100	39	Surface	48.8	50.7			49.8
1	15	100	39	Leaf	49.6	51.0			50.3
1	15	100	39	Total	98.4	101.7			100.1
2	15	1000	398	Surface	24.3	27.3	26.9	27.0	26.4
2	15	1000	398	Leaf	75.0	70.8	74.1	72.4	73.1
2	15	1000	398	Total	99.3	98.1	101.0	99.4	99.5
3	35	100	29	Surface	38.9	41.2	36.8		39.0
3	35	100	29	Leaf	60.0	58.6	62.7		60.4
3	35	100	29	Total	98.9	99.8	99.5		99.4
4	35	1000	295	Surface	25.9	19.4	19.3	30.2	23.7
4	35	1000	295	Leaf	74.3	82.0	83.5	70.6	77.6
4	35	1000	295	Total	100.2	101.4	102.8	100.8	101.3
5	45	100	23	Surface	49.1	35.6	29.8	38.9	38.4
5	45	100	23	Leaf	49.8	74.2	70.1	60.0	63.5
5	45	100	23	Total	98.9	109.8	99.9	98.9	101.9
6	45	1000	234	Surface	26.3	17.6	26.7	14.5	21.3
6	45	1000	234	Leaf	72.9	83.0	74.2	85.0	78.8
6	45	1000	234	Total	99.2	100.6	100.9	99.5	100.1
7	45	5000	1170	Surface	19.5	24.6	25.3		23.1
7	45	5000	1170	Leaf	79.4	75.3	75.2		76.6
7	45	5000	1170	Total	98.9	99.9	100.5		99.7
8	114	100	19	Surface	31.2	44.9	33.8	55.0	41.2
8	114	100	19	Leaf	68.7	50.3	65.9	45.1	57.5
8	114	100	19	Total	99.9	95.2	99.7	100.1	98.7
9	114	1000	186	Surface	28.8	24.5	21.5		24.9
9	114	1000	186	Leaf	70.5	75.6	78.5		74.9
9	114	1000	186	Total	99.3	100.1	100.0		99.8
10	114	5000	930	Surface	30.6	28.4	27.1		28.7

Table 1 - Cont/

Trt No.	Triton X	Conc.		Extract	% Recovery ^{14}C-flamprop-methyl				
		ppm	molar		Replicates				Mean
10	114	5000	930	Leaf	71.4	70.0	73.0		71.5
10	114	5000	930	Total	102.0	98.4	100.1		100.2
11	114	10000	1860	Surface	31.6	21.9			26.8
11	114	10000	1860	Leaf	68.7	70.9			69.8
11	114	10000	1860	Total	100.3	92.8			96.6
12	100	100	16	Surface	29.0	35.2	30.0		31.4
12	100	100	16	Leaf	70.8	65.3	70.0		68.7
12	100	100	16	Total	99.8	100.5	100.0		100.1
13	100	1000	160	Surface	20.5	25.2	32.6		26.1
13	100	1000	160	Leaf	81.0	74.6	67.5		74.4
13	100	1000	160	Total	101.5	99.8	100.1		100.5
14	100	5000	800	Surface	18.2	19.8	34.1	37.6	27.4
14	100	5000	800	Leaf	84.2	80.0	65.8	64.2	73.6
14	100	5000	800	Total	102.4	99.8	99.9	101.8	101.0
15	100	10000	1600	Surface	54.9	54.1	56.2		55.1
15	100	10000	1600	Leaf	43.0	44.7	44.5		44.1
15	100	10000	1600	Total	97.9	98.8	100.7		99.2
16	102	100	13	Surface	32.8	33.9	31.5		32.7
16	102	100	13	Leaf	66.6	65.3	69.5		67.1
16	102	100	13	Total	99.4	99.2	101.0		99.8
17	102	1000	132	Surface	29.6	34.1			31.9
17	102	1000	132	Leaf	70.0	65.8			67.9
17	102	1000	132	Total	99.6	99.9			99.8
18	102	5000	660	Surface	48.4	53.0	60.7		54.0
18	102	5000	660	Leaf	50.2	47.0	41.3		46.2
18	102	5000	660	Total	98.6	100.0	102.0		100.2
19	102	10000	1320	Surface	56.8	74.1			65.5
19	102	10000	1320	Leaf	43.9	31.4			37.7
19	102	10000	1320	Total	100.7	105.5			103.2
20	165	100	11	Surface	30.0	28.3	31.2		29.8
20	165	100	11	Leaf	70.0	70.0	70.5		70.2

Table 1 - Cont/

Trt No.	Triton X	Conc.		Extract	% Recovery ^{14}C-flamprop-methyl				
		ppm	molar		Replicates				Mean
20	165	100	1320	Total	100.0	98.3	101.7		100.0
21	165	1000	109	Surface	31.0	29.5	27.6	33.0	30.3
21	165	1000	109	Leaf	68.4	70.0	70.3	65.2	68.5
21	165	1000	109	Total	99.4	99.5	97.9	98.2	98.8
22	165	5000	545	Surface	39.3	40.4	45.0		41.2
22	165	5000	545	Leaf	61.1	60.5	55.2		58.9
22	165	5000	545	Total	99.4	100.9	100.2		100.1
23	165	10000	1090	Surface	70.3	70.0			70.2
23	165	10000	1090	Leaf	30.0	34.8			32.4
23	165	10000	1090	Total	100.3	104.8			102.6
24	305	100	6	Surface	33.5	30.7	28.9	32.4	31.4
24	305	100	6	Leaf	65.9	69.4	70.2	70.5	69.0
24	305	100	6	Total	99.4	100.1	99.1	102.9	100.4
25	305	1000	65	Surface	34.0	33.7	36.0		34.6
25	305	1000	65	Leaf	65.7	64.3	64.2		64.7
25	305	1000	65	Total	99.7	98.0	100.2		98.3
26	305	5000	327	Surface	62.2	70.8	52.0		61.7
26	305	5000	327	Leaf	37.7	31.6	45.5		38.3
26	305	5000	327	Total	100.0	102.4	97.5		100.0
27	305	10000	655	Surface	38.8	44.2	65.1		49.4
27	305	10000	655	Leaf	70.4	65.8	33.2		56.5
27	305	10000	655	Total	109.2	110.0	98.3		105.9
28	405	100	5	Surface	35.6	56.4	30.7		40.9
28	405	100	5	Leaf	64.0	45.2	69.8		59.7
28	405	100	5	Total	99.6	101.6	100.5		100.6
29	405	1000	51	Surface	25.4	23.5	34.4	31.5	28.7
29	405	1000	51	Leaf	75.1	75.7	75.1	M	75.3
29	405	1000	51	Total	100.5	99.2	109.5		104.0
30	405	5000	255	Surface	49.7	55.8	51.1		52.2
30	405	5000	255	Leaf	50.0	43.6	52.5		48.7

Table 1 - Cont/

Trt No.	Triton X	Conc.		Extract	% Recovery ^{14}C-flamprop-methyl				
		ppm	molar		Replicates				Mean
30	405	5000	255	Total	99.7	99.4	103.6		100.9
31	405	10000	510	Surface	61.3	51.9			56.6
31	405	10000	510	Leaf	39.7	48.3			43.5
31	405	10000	510	Total	100.0	100.2			100.1
32	705	100	3	Surface	33.4	39.6	32.6		35.2
32	705	100	3	Leaf	67.2	60.5	60.4		62.7
32	705	100	3	Total	100.6	100.1	93.0		97.9
33	705	1000	30	Surface	31.7	35.1	34.0		33.6
33	705	1000	30	Leaf	68.5	66.4	63.0		66.0
33	705	1000	30	Total	100.2	101.5	97.0		99.6
34	705	5000	152	Surface	55.3	50.7	66.7	61.2	58.5
34	705	5000	152	Leaf	44.6	51.4	34.8	38.9	42.4
34	705	5000	152	Total	99.3	102.1	101.5	100.1	100.9
35	705	10000	304	Surface	58.8	51.8	56.0		55.5
35	705	10000	304	Leaf	44.5	50.2	40.6		45.1
35	705	10000	304	Total	103.3	102.0	96.6		100.6
36	Control	Nil	-	Surface	87.6	92.3	91.8		90.6
36	Control	Nil	-	Leaf	5.0	4.8	9.6		6.5
36	Control	Nil	-	Total	92.6	97.1	101.4		97.1

LSD values (P = 0.05)	Surface extract	Leaf extract
For 2 obs vs 2 obs	12.1	13.1
For 2 obs vs 4 obs	10.4	11.4
For 2 obs vs 3 obs	11.0	12.0
For 4 obs vs 4 obs	8.5	9.3
For 4 obs vs 3 obs	9.2	10.0
For 3 obs vs 3 obs	9.8	10.7

Table 2 shows the recovery of flamprop-methyl 24 hours after application of the herbicide to wheat as a solution in aqueous Brij solutions. The results of this experiment parallel those above, with improved uptake being achieved with every formulation. Best results indicated an eighteen fold increase in foliar uptake.

The low oxyethylene content adducts of both the Triton X and the Brij series potentiated foliar uptake most consistently over the concentration range investigated in Experiments 1 and 2. For both series, and with the exceptions of the Brii compounds 96 and 98, the uptake of flamprop-methyl was seen to decrease as the concentration of the surface active compound was increased above 1000 ppm when the oxyethylene content was equal to or greater than 10 moles per mole of hydrophobe. This trend can be exemplified by comparison of uptake between Triton X- 165 solutions, (Table 1: treatments 20-23), for which recoveries in the leaf macerate decreased from 70 per cent at 1000 ppm to 58 at 5000 ppm and 30 at 10,000 ppm, and Triton X- 114 for which uptake was not significantly altered between 1000 and 10,000 ppm. Brij 98 differed from the other compounds studied in that (i), at low concentrations, formulation in solutions of this surfactant was markedly less efficacious at potentiating the foliar uptake of flamprop-methyl than any other surfactant treatment, and (ii) uptake was not significantly reduced with increasing concentration despite the high polyoxyethylene content of this surfactant.

Tables 1 and 2 show that the variation in uptake with oxyethylene content within the two series was relatively limited at lower concentrations. With the Triton X formulations recoveries in the leaf macerate varied between 60 and 70 per cent at 100 ppm, (excluding the somewhat lower data for Triton X-15), and between 66 and 79 per cent at 1000 ppm.

Table 2 : Foliar penetration over 24 hours from solutions of Brij surface active agents (Experiment 2)

Trt No.	Brij	Conc.		Extract	% Recovery ^{14}C-flamprop-methyl			
		ppm	molar		Replicates			Mean
1	92	100	28	Surface	10.0	13.9	14.8	12.9
1	92	100	28	Leaf	90.0	86.0	85.1	87.0
1	92	100	28	Total	100.0	99.9	99.9	99.9
2	92	1000	281	Surface	14.7	12.4	12.0	13.0
2	92	1000	281	Leaf	85.2	87.7	87.9	86.9
2	92	1000	281	Total	99.9	100.1	99.9	99.9
3	92	5000	1405	Surface	15.3	15.2	13.3	14.6
3	92	5000	1405	Leaf	84.6	84.3	86.4	85.1
3	92	5000	1405	Total	99.9	99.5	99.7	99.7
4	92	10000	2810	Surface	10.6	10.0	12.2	10.9
4	92	10000	2810	Leaf	89.4	90.0	87.5	89.0
4	92	10000	2810	Total	100.0	100.0	99.7	99.9
5	30	100	27	Surface	14.8	14.6	16.2	15.2
5	30	100	27	Leaf	84.9	85.0	84.0	84.6
5	30	100	27	Total	99.7	99.6	100.2	99.8
6	30	1000	276	Surface	17.6	13.7	16.2	15.8
6	30	1000	276	Leaf	82.0	87.0	85.2	84.7
6	30	1000	276	Total	99.6	100.7	101.4	100.5
7	30	5000	1380	Surface	12.9	13.6	15.0	13.8
7	30	5000	1380	Leaf	87.0	86.7	84.8	86.2
7	30	5000	1380	Total	99.9	100.3	99.8	100.0
8	30	10000	2760	Surface	12.0	10.1	7.6	9.9
8	30	10000	2760	Leaf	82.0	90.3	92.7	88.3
8	30	10000	2760	Total	94.0	100.4	100.3	98.2
9	96	100	14	Surface	12.3	14.1	17.2	14.5
9	96	100	14	Leaf	87.5	85.5	82.5	85.2
9	96	100	14	Total	99.8	99.6	99.7	99.7
10	96	1000	141	Surface	9.4	11.0	10.5	10.3
10	96	1000	141	Leaf	90.6	89.0	90.2	89.9
10	96	1000	141	Total	100.0	100.0	100.7	100.2

Table 2 - Cont/

Trt No.	Brij	Conc.		Extract	% Recovery ^{14}C-flamprop-methyl			
		ppm	molar		Replicates			Mean
11	96	5000	706	Surface	9.0	9.8	9.8	9.5
11	96	5000	706	Leaf	90.5	90.7	90.2	90.5
11	96	5000	706	Total	99.5	100.5	100.0	100.0
12	96	10000	1410	Surface	7.0	7.4	8.9	7.8
12	96	10000	1410	Leaf	93.0	92.5	91.5	92.3
12	96	10000	1410	Total	100.0	99.9	100.4	100.1
13	56	100	15	Surface	13.9	10.9	14.2	12.9
13	56	100	15	Leaf	86.1	90.0	85.9	87.3
13	56	100	15	Total	99.8	100.9	100.1	100.2
14	56	1000	147	Surface	10.8	9.4	14.0	11.4
14	56	1000	147	Leaf	89.2	91.0	86.0	88.7
14	56	1000	147	Total	100.0	100.4	100.0	100.1
15	56	5000	733	Surface	16.3	15.5	15.0	15.6
15	56	5000	733	Leaf	84.3	84.3	84.9	84.5
15	56	5000	733	Total	100.6	99.8	99.9	100.1
16	56	10000	1470	Surface	19.0	35.0	25.5	26.5
16	56	10000	1470	Leaf	80.0	65.0	75.6	73.5
16	56	10000	1470	Total	99.0	100.0	101.1	100.0
17	76	100	14	Surface	14.1	16.5	14.4	15.0
17	76	100	14	Leaf	85.9	83.3	85.0	84.7
17	76	100	14	Total	100.0	99.8	99.4	99.7
18	76	1000	141	Surface	19.4	23.3	25.0	22.6
18	76	1000	141	Leaf	81.7	76.8	75.0	77.8
18	76	1000	141	Total	101.1	100.1	100.0	100.4
19	76	5000	705	Surface	52.0	60.4	54.9	55.8
19	76	5000	705	Leaf	47.6	40.0	44.6	44.1
19	76	5000	705	Total	99.6	100.4	99.5	99.9
20	76	10000	1408	Surface	68.4	70.0	65.0	67.8
20	76	10000	1408	Leaf	37.9	30.0	35.4	34.4
20	76	10000	1408	Total	106.3	100.0	100.4	102.2

Table 2 - Cont/

Trt No.	Brij	Conc.		Extract	% Recovery ^{14}C-flamprop-methyl			
		ppm	molar		Replicates			Mean
21	35	100	8	Surface	20.0	30.8	25.6	25.5
21	35	100	8	Leaf	79.4	78.7	74.3	77.5
21	35	100	8	Total	99.4	109.5	99.9	103.0
22	35	1000	84	Surface	24.4	43.7	M	34.1
22	35	1000	84	Leaf	75.5	56.4	M	66.0
22	35	1000	84	Total	99.9	100.1		100.1
23	35	5000	417	Surface	58.0	33.7	42.0	44.6
23	35	5000	417	Leaf	41.7	65.8	59.4	55.6
23	35	5000	417	Total	99.7	99.5	101.4	100.2
24	35	10000	835	Surface	42.7	33.0	47.6	41.1
24	35	10000	835	Leaf	59.4	77.0	52.6	63.0
24	35	10000	835	Total	102.1	110.0	100.2	104.1
25	Control	Nil	-	Surface	95.0	89.0	91.6	91.9
25	Control	Nil	-	Leaf	5.0	3.6	4.8	4.5
25	Control	Nil	-	Total	100.0	92.6	96.4	96.4

LSD values (P = 0.05)	Surface extract	Leaf extract
For 3 obs vs 3 obs	7.3	8.1
For 3 obs vs 2 obs	8.2	9.1
For 2 obs vs 2 obs	9.0	10.0

With the Brij formulations recoveries in the leaf macerate varied between 75 and 90 per cent at 100 ppm, (excluding Brij 98), and, with the exception of one further value, between 75 and 91 per cent at 1000 ppm.

Table 3 shows the recoveries of flamprop-methyl, achieved by surface extraction, 24 hours after application to wheat of formulations based on single Brij compounds and on mixtures of two Brij compounds. These data suggested that the enhancement of uptake achieved with any one surfactant was not simply additive when that treatment was combined with a less effective formulation. Uptake from a 1:1 mixture of the Brij compounds 76 and 96 was about what would be expected for Brij 76 in isolation, but the marked effect of Brij 96 as a penetrant aid appeared to have been lost. This result suggests that the overall rate of uptake from mixtures of adjuvants would be dictated by the least effective adjuvant.

3.2 Uptake from dilute surfactant formulations

Figure 1 shows the foliar uptake by wheat of flamprop-methyl over 24 hours following application of the herbicide formulated in aqueous solutions of Triton X 45 between 10 and 1000 ppm. In this experiment uptake was estimated from the residue remaining outside the leaf tissue, based on triplicated determinations; redistribution of the unlabelled herbicide was quantified by GLC. These data show a marked dependence of uptake with surfactant concentration between 10 and 100 ppm.

The above experiment was repeated with flamprop-methyl formulations based on Triton X 100, (Figure 2), Triton X 305, (Figure 3), and Brij 92, (Figure 4). The marked increase in the rate of foliar uptake of flamprop-methyl as seen in Figure 1 with increasing surfactant concentra-

Table 3 : Foliar penetration from solutions of Brij surfactants, (Experiment 3) : Surface recoveries

Trt No.	Brij	Conc/ppm	% Recovery ^{14}C-flamprop-methyl after 24 hours							
			Replicates							Mean
1	96	1000	44.2	35.3	40.8	42.0	36.2	52.5		41.8
2	96	10000	32.0	29.6	56.4	37.7	32.7			37.7
3	76	1000	62.0	59.0	53.1	66.0	49.0			57.8
4	76	10000	75.8	81.3	71.1	74.9	74.0	82.9		76.7
5	98	100	66.0	79.0	51.6	71.5	72.6	50.0	53.8	63.5
6	98	1000	77.2	79.1	55.9	77.3	70.2	61.3	55.6	68.0
7	98	10000	45.8	54.0	45.2	31.0	68.4	60.8	68.5	53.4
8	96 + 76	5000 + 5000	75.8	71.3	71.4	59.0	59.3	63.7	71.6	66.0
9	98 + 76	5000 + 5000	78.9	71.6	63.2	82.2	71.6	84.0	65.0	73.8

LSD Values (P = 0.05)

For 6 obs vs 6 obs	11.2
For 6 obs vs 5 obs	11.8
For 6 obs vs 7 obs	10.8
For 5 obs vs 5 obs	12.3
For 5 obs vs 7 obs	11.4
For 7 obs vs 7 obs	10.4

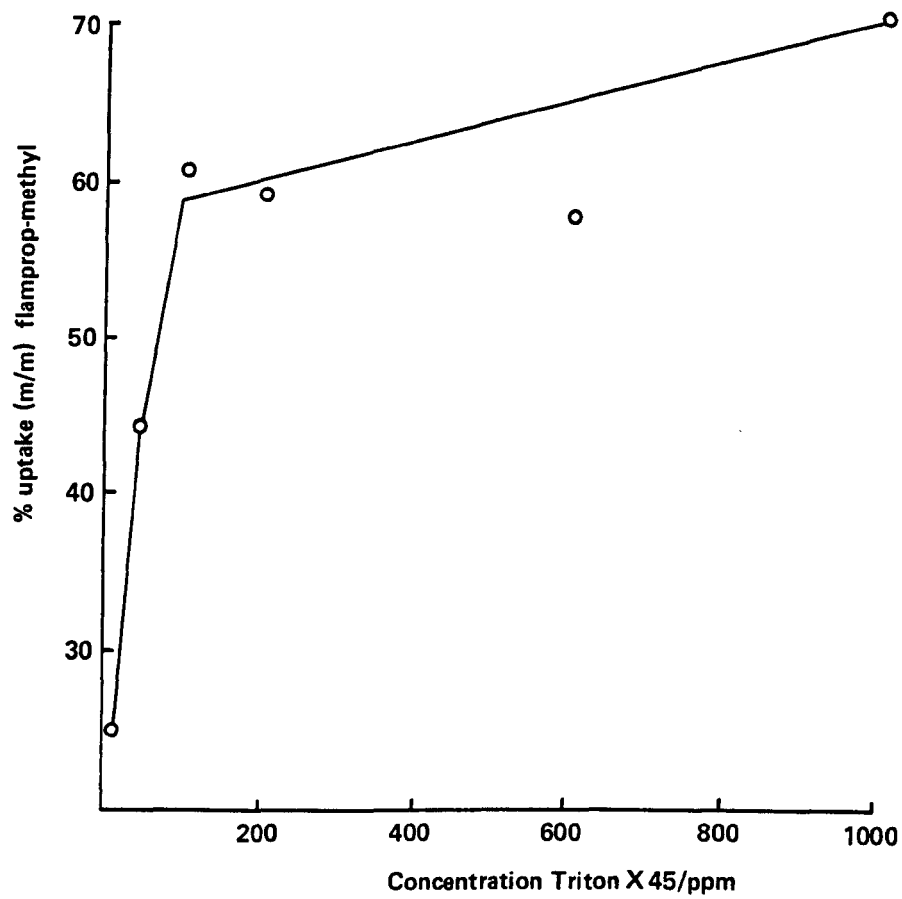


Fig 1 Foliar uptake by wheat of flamprop-methyl 24 hours after the application of the herbicide (0.3 μg) formulated in aqueous solutions of Triton X 45/ ppm

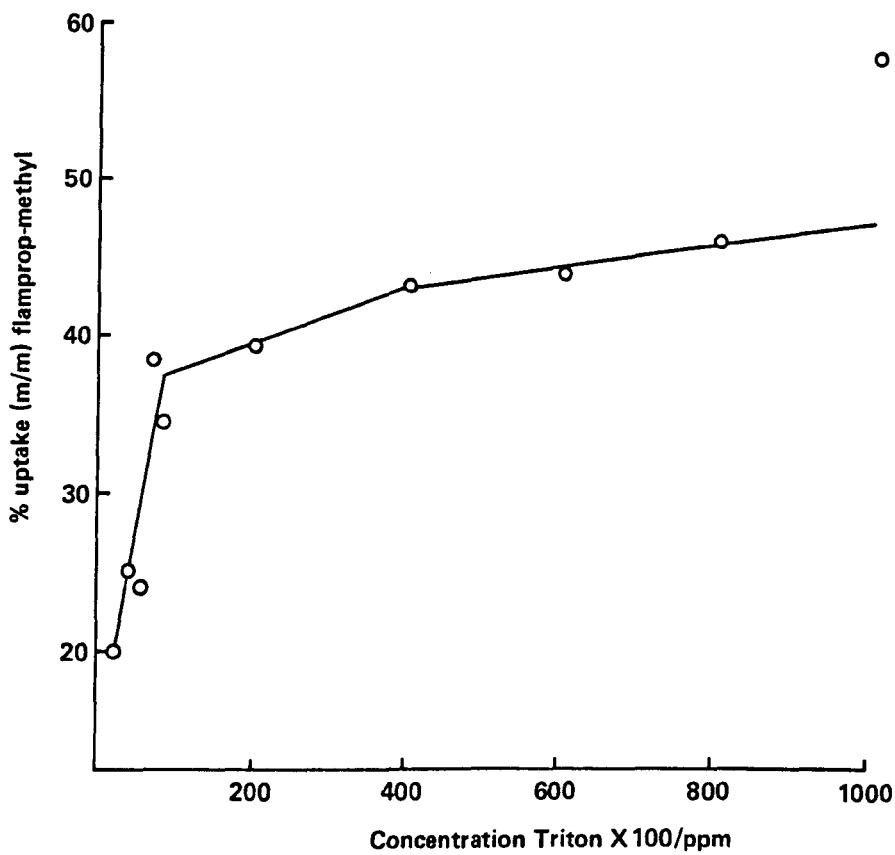


Fig 2 Foliar uptake by wheat of flamprop-methyl 24 hours after the application of the herbicide (0.3 μg) formulated in aqueous solutions of Triton X 100/ppm

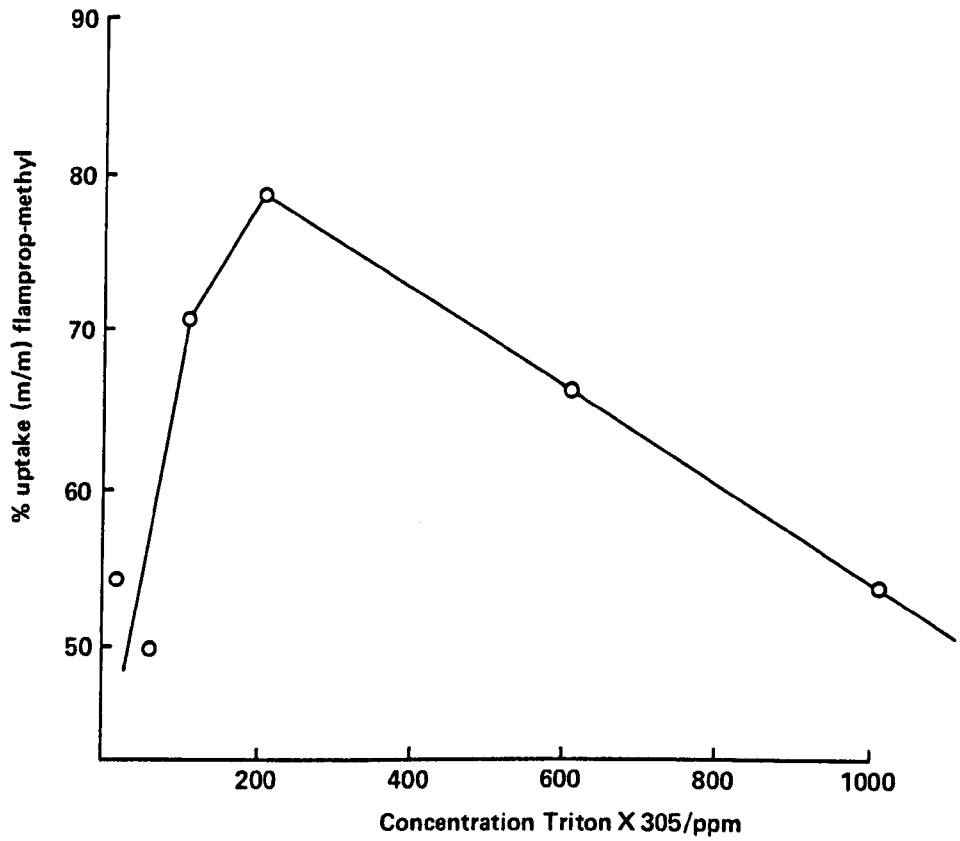


Fig 3 Foliar uptake by wheat of flamprop-methyl 24 hours after the application of the herbicide (0.3 μg) formulated in aqueous solutions of Triton X 305 / ppm

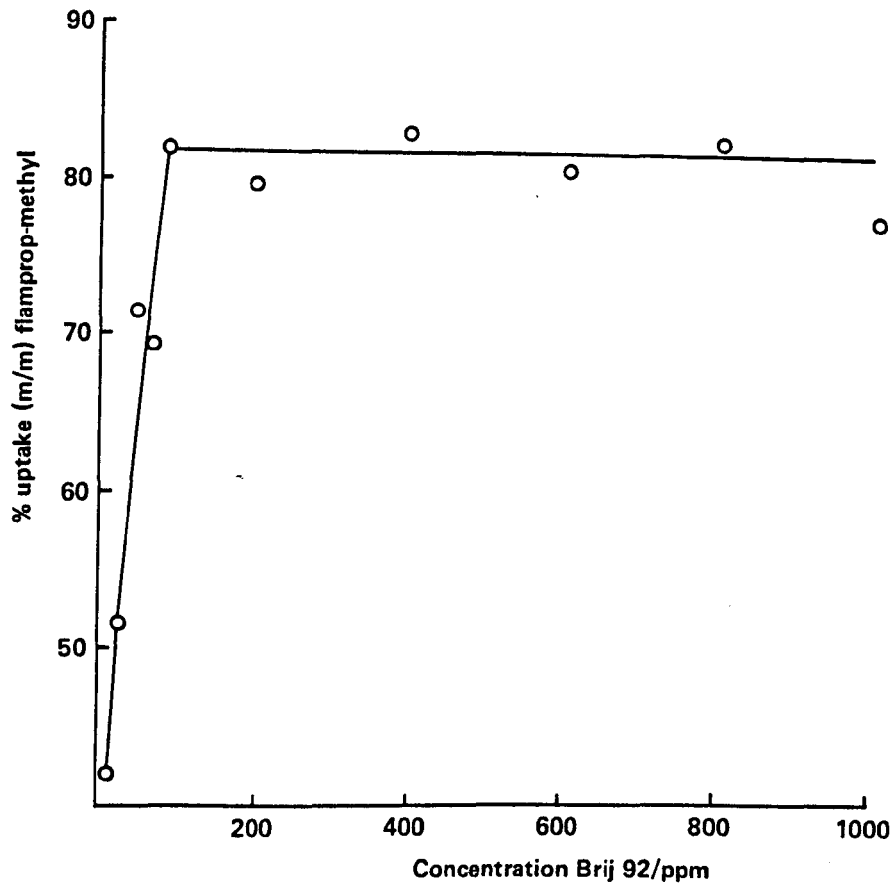


Fig 4 Foliar uptake by wheat of flamprop-methyl 24 hours after the application of the herbicide (0.3 μg) formulated in aqueous solutions of Brij 92 / ppm

tion between 10 and 100 ppm was a common feature of these experiments.

Foliar uptake of flamprop-methyl formulated in aqueous solutions of Brij 96 was investigated in Experiment 8. This experiment was performed over an extended time frame: data were collected over nine days, (Table 4) and the variation of uptake with surfactant concentration was assessed from these data by calculating a time averaged mean value for each treatment which were used to construct a curve (Figure 5) similar to those generated above. Experiment 8 was repeated with flamprop-methyl formulations based on Brij 30, (Table 5), and Brij 35, (Table 6), and time averaged mean values plotted against surfactant concentration, (Figure 6 and 7 respectively). Inspection of these curves revealed a close similarity with Figures 1-4.

The reproducibility of the above curves was assessed by repeating these studies with radioactive labelled herbicide; experiments were restricted to a consideration of the uptake achieved over 24 hours. With one exception the relative response of uptake to formulation identified above was similar for each surfactant. This similarity did not extend to the absolute values, which tended to be displaced between the two sets of data. To avoid repetition the second set of results was normalised with respect to the earlier results and the data combined.

It was found that the curves generated using time averaged mean values were not significantly different from those obtained from 24 hour data with common formulations.

Using Brij 92 the relative values determined in the second experiment differed from the earlier data. In this instance the sampling frequency was common to both experiments. These data were not combined (Figure 8) and were considered in conjunction with other data in subsequent deliberations.

Table 4 : Foliar penetration from solutions of Brij 96 (Experiment 8)

Time days	Brij 96 Concentration/ppm									
	10	30	50	70	90	200	400	600	800	1000
	% Recovery flamprop-methyl(1)									
1	68.2	61.3	36.0	69.5	38.5	32.0	30.9	25.0	19.9	19.3
2	70.1	ND	70.5	35.0	16.4	18.3	15.2	12.3	10.5	22.6
3	60.2	46.0	36.0	41.7	18.3	19.6	13.0	6.2	6.7	21.6
7	53.6	32.8	18.0	13.6	10.6	8.7	8.8	7.7	2.9	12.2
8	32.5	22.0	17.0	11.2	17.3	12.4	10.7	8.3	3.0	25.3
10	33.4	36.8	26.0	16.9	12.0	10.0	10.8	3.4	3.4	12.4
Mean	53.0	42.5	34.0	31.3	18.9	16.8	14.9	10.5	7.7	18.9

(1) Each value represents the average of three plants

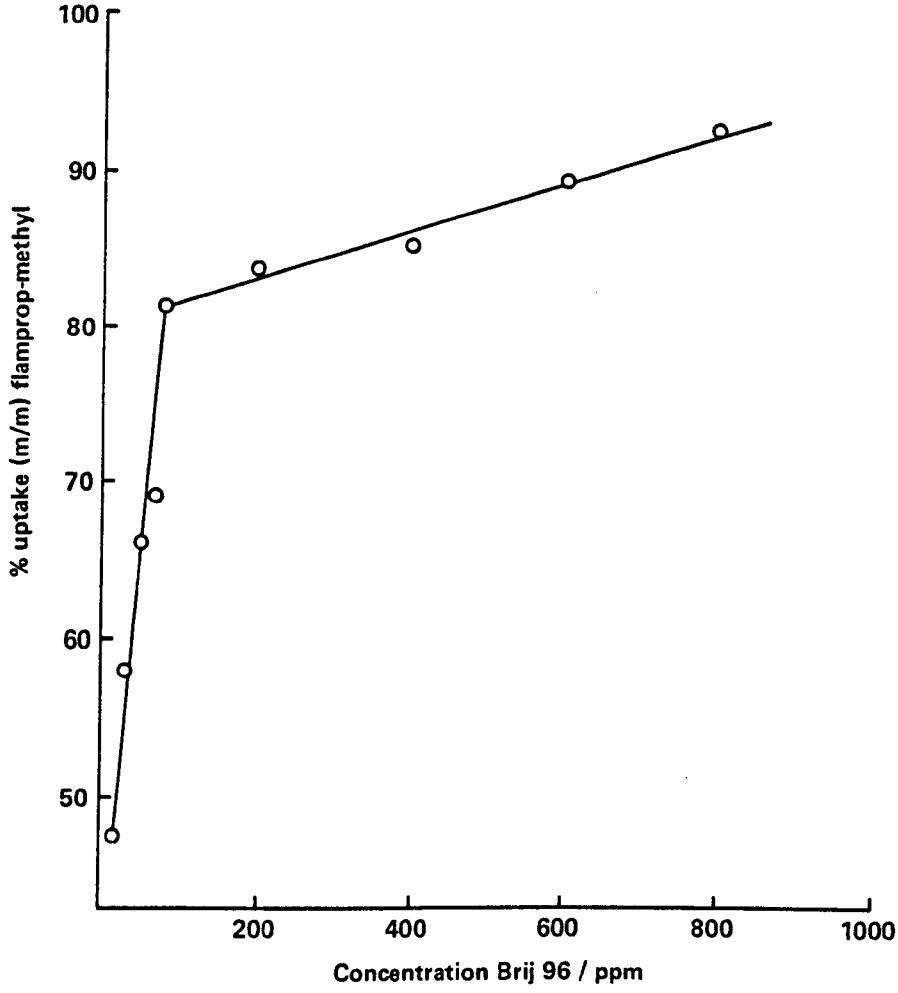


Fig 5 Foliar uptake by wheat of flamprop-methyl; time averaged mean values over 9 days after the application of the herbicide (0.3 μg) formulated in aqueous solutions of Brij 96.

Table 5 : Foliar penetration from solutions of Brij 30 (Experiment 9)

Time days	Brij 30 concentration/ppm									
	10	30	50	70	90	200	400	600	800	1000
	% Recovery flamprop-methyl(1)									
1	70.4	47.9	40.3	48.7	23.5	16.9	20.9	20.3	18.8	19.8
2	39.0	32.2	41.3	27.9	26.0	18.1	16.3	19.7	20.5	17.2
3	69.3	35.7	28.9	33.1	19.1	14.1	10.4	15.5	5.2	14.5
7	25.0	24.0	11.0	13.1	10.3	5.7	7.6	6.3	6.4	6.3
8	34.4	21.0	11.8	11.8	11.0	8.7	7.0	6.9	6.5	6.9
9	29.7	ND	17.5	10.9	5.6	11.9	ND	5.7	9.6	7.4
Mean	50.0	30.0	25.0	24.0	16.0	12.5	11.5	12.0	11.3	12.0

(1) Each value represents the average of three plants.

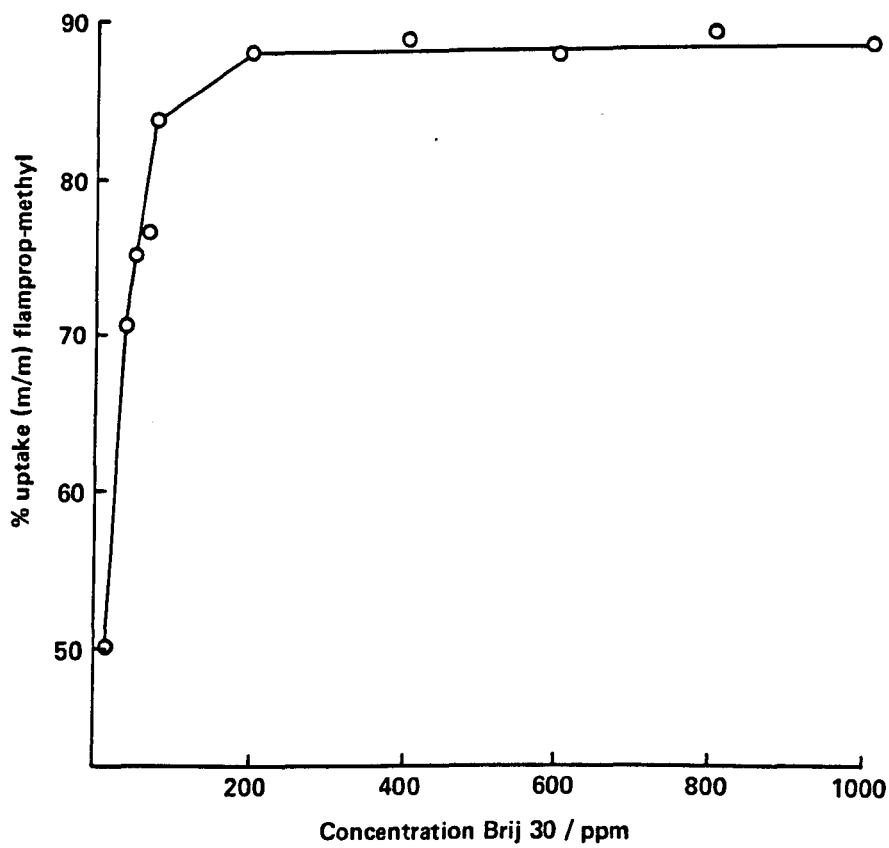


Fig 6 Foliar uptake by wheat of flamprop-methyl; time averaged mean values over 10 days, after the application of the herbicide (0.3 μg) formulated in aqueous solutions of Brij 30

Table 6 : Foliar penetration from solutions of Brij 35 (Experiment 10)

Time days	Brij 35 concentration/ppm									
	10	30	50	70	90	200	400	600	800	1000
	% Recovery flamprop-methyl(1)									
1	63.1	58.8	50.9	53.6	45.3	38.2	48.1	50.0	ND	63.6
2	63.3	51.5	33.9	35.9	28.2	31.3	38.3	38.2	54.5	42.9
3	46.7	22.1	17.6	21.2	13.2	22.2	18.8	31.3	35.9	28.4
5	24.5	15.1	8.8	13.2	6.8	11.9	15.0	26.4	15.7	19.5
6	12.4	8.6	9.6	6.7	10.4	12.9	12.0	18.8	23.9	10.0
Mean	42.0	31.0	24.0	26.0	20.0	23.0	26.0	32.5	32.5	33.0

(1) Each value represents the average of three plants.

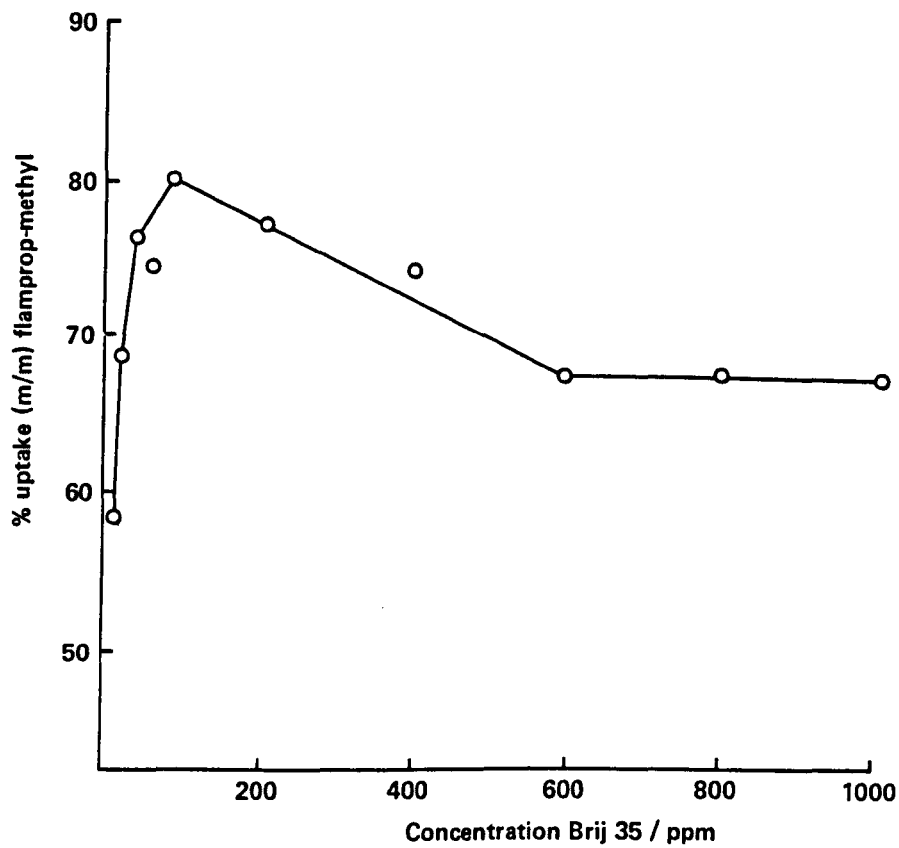


Fig 7 Foliar uptake by wheat of flamprop-methyl; time averaged mean values over 6 days after the application of the herbicide (0.3 μg) in aqueous solutions of Brij 35

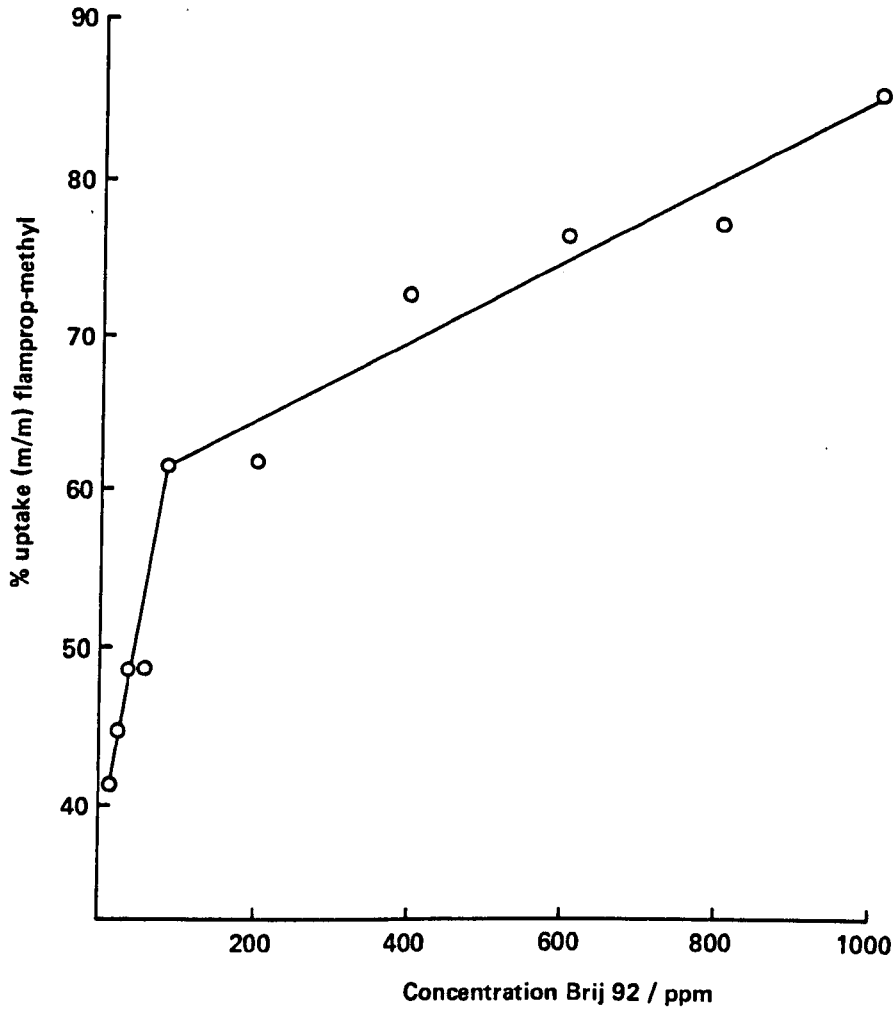


Fig 8 Foliar uptake by wheat of flamprop-methyl 24 hours after the application of the herbicide (0.3 μg) formulated in aqueous solutions of Brij 92

Figures 1-8 suggest that the marked dependency of the uptake by wheat of flamprop-methyl with surfactant concentration in the range 10 to 100 ppm was independent of surfactant structure, (within the limits of this investigation). All curves were found to exhibit a lesser gradient about 100 ppm: above this concentration treatments could be subdivided on the basis of whether further increasing surfactant concentration resulted in further increases in uptake or in a decreased level of uptake enhancement. The latter was found only with surface active agents having a high polyoxyethylene content, ie. Triton X-305 and Brij 25 (Figures 3 and 7 respectively). In all studies the complete form of the curve was evidence of a complexity of interactions between the surfactant and the foliar uptake of flamprop-methyl.

3.3 Rates of uptake

A. Uptake from solutions of alkyl ethoxylates

Tables 4 and 5 show that at concentrations of the Brij surfactants 96 and 30 above 100 ppm the uptake of flamprop-methyl was largely limited to the first 24 hours after application, after which uptake almost ceased. Thus, with formulations containing Brij 96 between 100 and 1000 ppm, herbicide uptake was found to account for between 70 and 80 per cent of the applied dose after 24 hours, increasing to between 85 and 95 per cent after six to nine days.

Below 100 ppm surfactant, herbicide uptake was maintained over a longer period, although to less overall effect than at higher surfactant concentrations. As a consequence of the extended period of active uptake from the more dilute formulations the difference in the total uptake of flamprop-methyl between dilute and more concentrated surfactant solutions was less after six days than after 24 hours.

Table 6 shows that foliar uptake of flamprop-methyl following application in formulations of Brij 35 progressed over a period of up to six days from all treatments up to a surfactant concentration of 1000 ppm. Although the initial rate of uptake was greater with formulations containing between 90 and 200 ppm of Brij 35, (Figure 7), the continued uptake of herbicide from all treatments resulted in little difference in the total uptake of herbicide over six days.

To conclude it would appear that, in the presence of a surface active compound, the uptake of flamprop-methyl was enhanced over periods up to about seven days subsequent to application, provided a sufficient residue of the herbicide was available to take advantage of this potentiation. Uptake did not proceed to completion in any of the treatments investigated, appearing to cease when the surface residue was reduced to about 10 per cent of the original application.

Experiment 11 investigated the kinetics of the foliar uptake of flamprop-methyl over an 18 hour period following application in a 1000 ppm aqueous solution of Brij 96. In this experiment the surface residue of the herbicide was recovered at three hourly intervals and the data were subjected to kinetic analysis. All analyses were based on triplicate determination of each value. Figure 9 shows that herbicide uptake was not linear with time: a linear relationship was however found between the common logarithm of the surface residue and the elapsed time, (Figure 10). Such a relationship is indicative of a first order rate process.

B. Uptake from solutions of alkylphenol ethoxylates

Figures 11 and 12 show the rate of uptake of flamprop-methyl over a seven day period following application of the herbicide formulated in aqueous solutions of Triton X 100. In these experiments uptake was

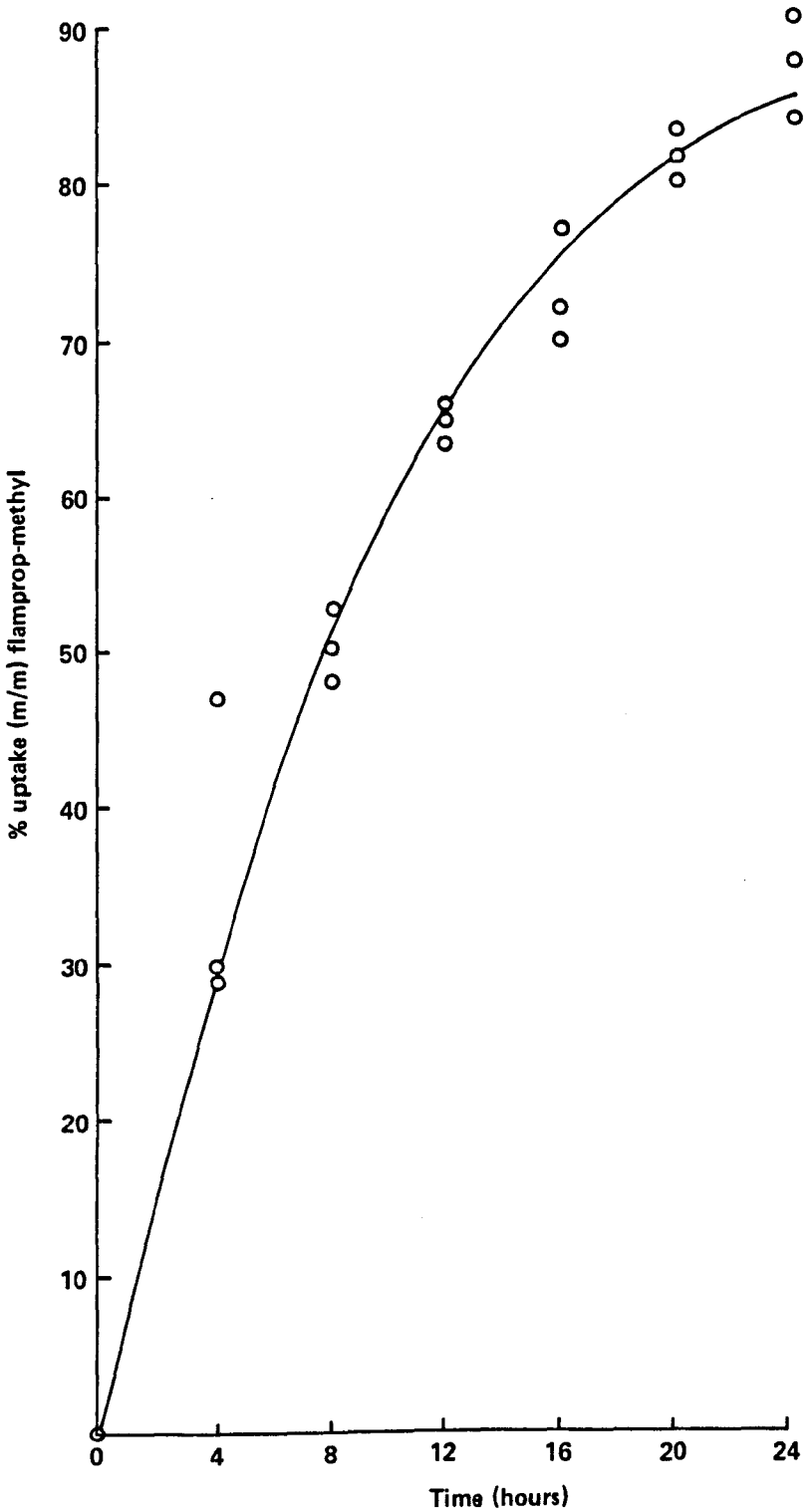


Fig 9 Foliar uptake by wheat of flamprop-methyl over 24 hours after application of the herbicide (0.3 μg) formulated in an aqueous solution of Brij 96 (1000 ppm)

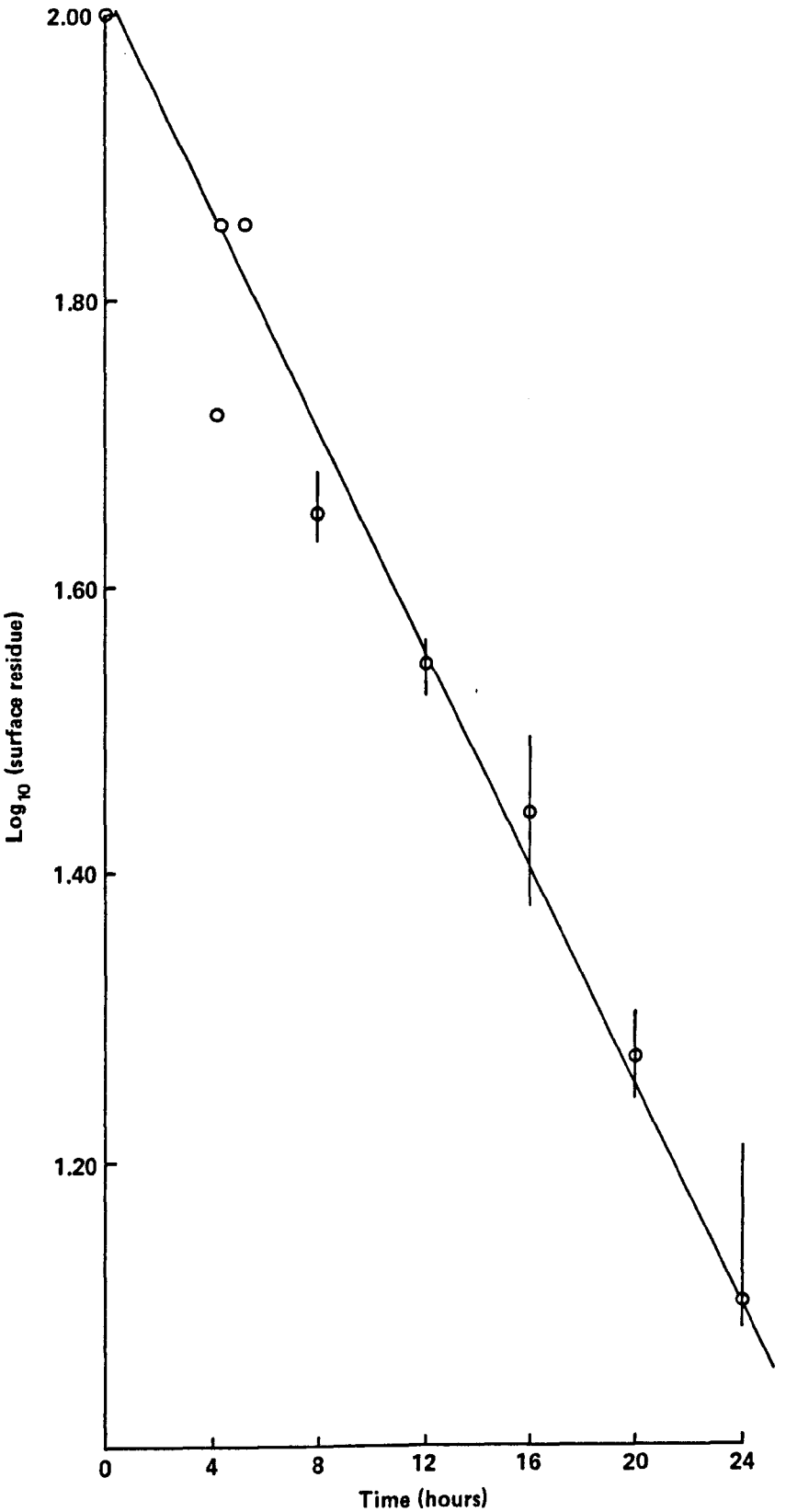


Fig 10 Log_{10} surface residue of flamprop-methyl over 24 hours after treatment with the herbicide formulated in an aqueous solution of Brij 96 (1000 ppm) as a function of time. Vertical bars show variation about mean values (3 reps).

estimated from the residue remaining outside the leaf tissues, based on triplicated determinations: recoveries were quantified by GLC. The results of similar experiments are shown in Figures 13 and 14; in the former uptake was investigated following application of formulations based on a range of Triton X compounds whilst in the latter the polyoxyethylene content of the surfactants was kept constant and the alkyl group was varied.

It can be seen, (Figures 11-14), that many of the features of the enhancement of herbicide uptake identified with alkyl ethoxylate formulations were common to formulations based on alkylphenol ethoxylates:

i) Foliar uptake by wheat of flamprop-methyl was initially more rapid and was seen to be decreased at longer times when the surface deposit was depleted.

ii) Uptake did not proceed to completion.

iii) Continued uptake over the time frame of the experiment from treatments assessed after 24 hours as being less effective tended to minimize differences between treatments at greater elapsed times.

iv) Uptake was inhibited when the herbicide was formulated in concentrated surfactant solutions, an effect well demonstrated in the results obtained with a 50,000 ppm solution of Triton X 100, (Figure 12).

Kinetic analyses of the data presented in Figures 11-14 were less explicit than the result found in Experiment 11. Figure 15 shows that the data used in Figures 11 and 12 were generally consistent with a first order mechanism; data used in Figure 13 were not compatible

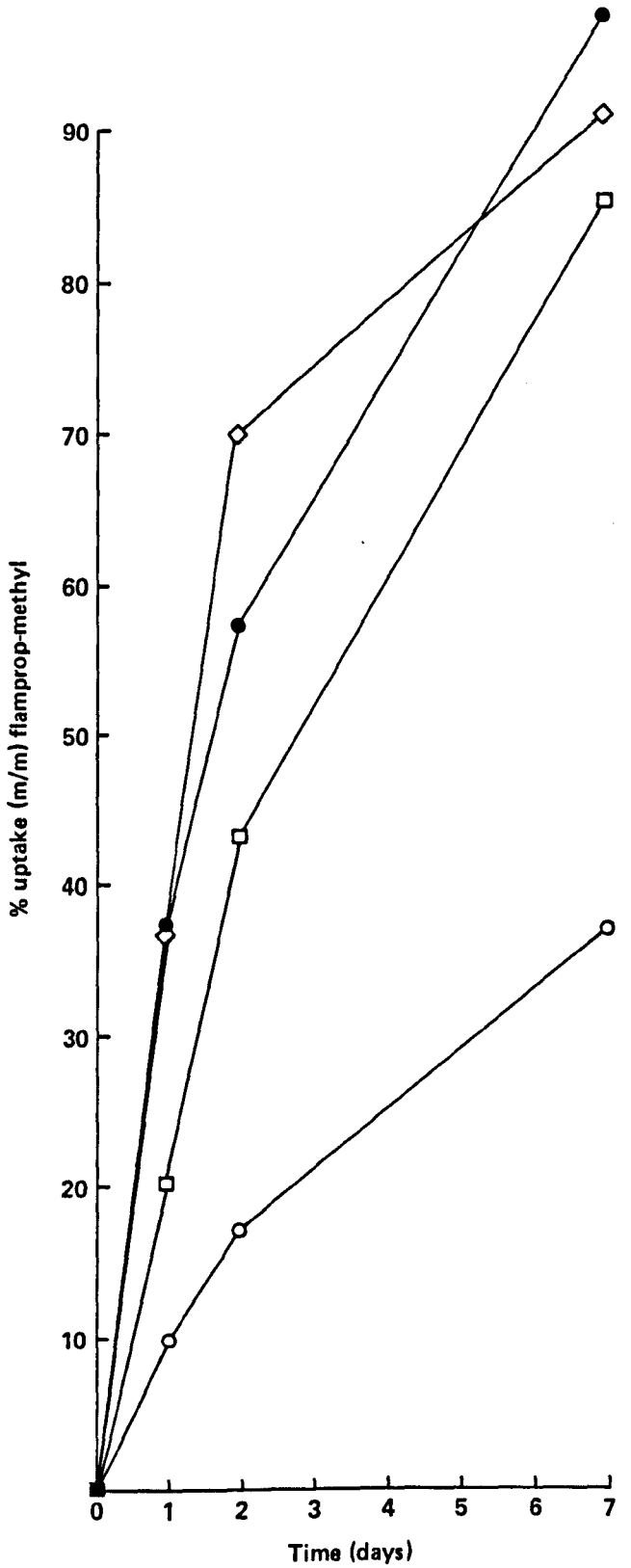


Fig 11 Foliar uptake by wheat of flamprop-methyl over 7 days after application of the herbicide (0.3 μg) formulated in 100 ppm (●——●), 1000 ppm (◇——◇) and 10,000 ppm (□——□) solutions of Triton X 100 and in water (○——○)

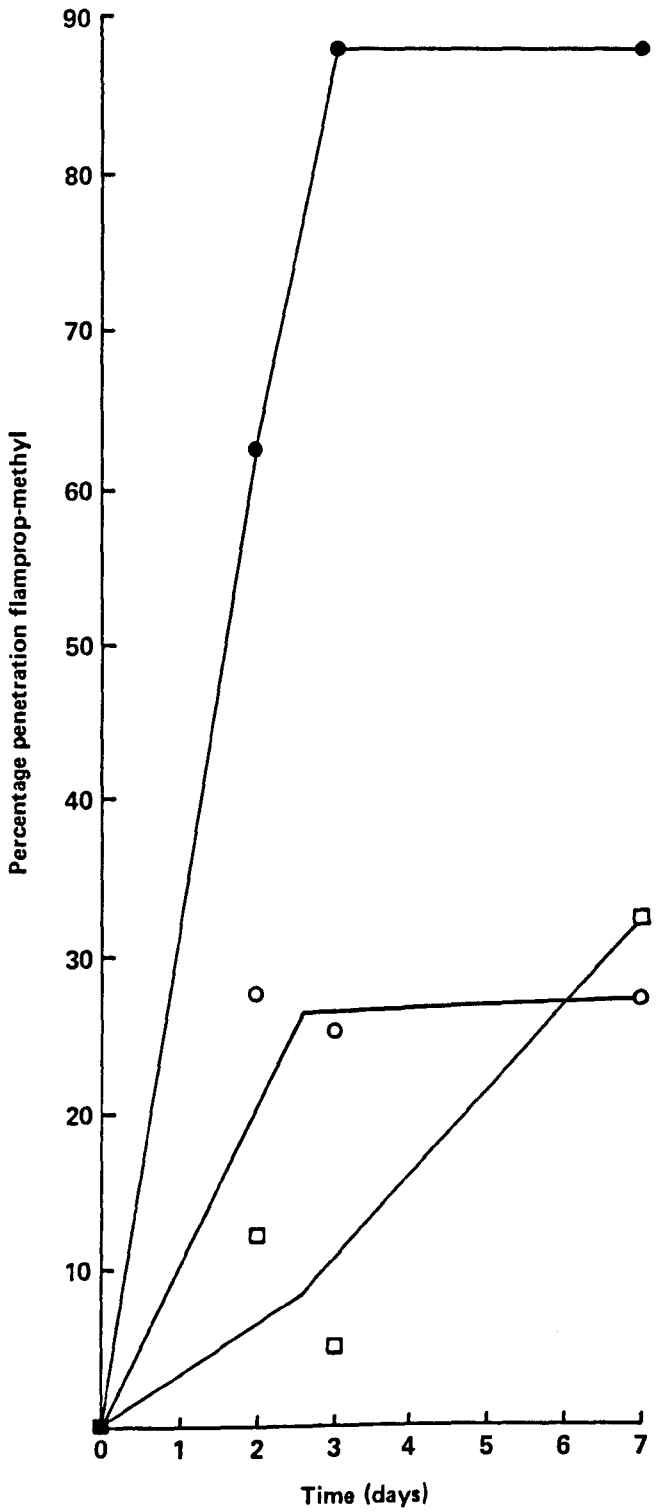


Fig 12 Foliar uptake by wheat of flamprop-methyl over 7 days after application of the herbicide (0.3 μg) formulated in water (o——o) and in 100 ppm (●——●) and 50,000 ppm (□——□) solutions of Triton X 100

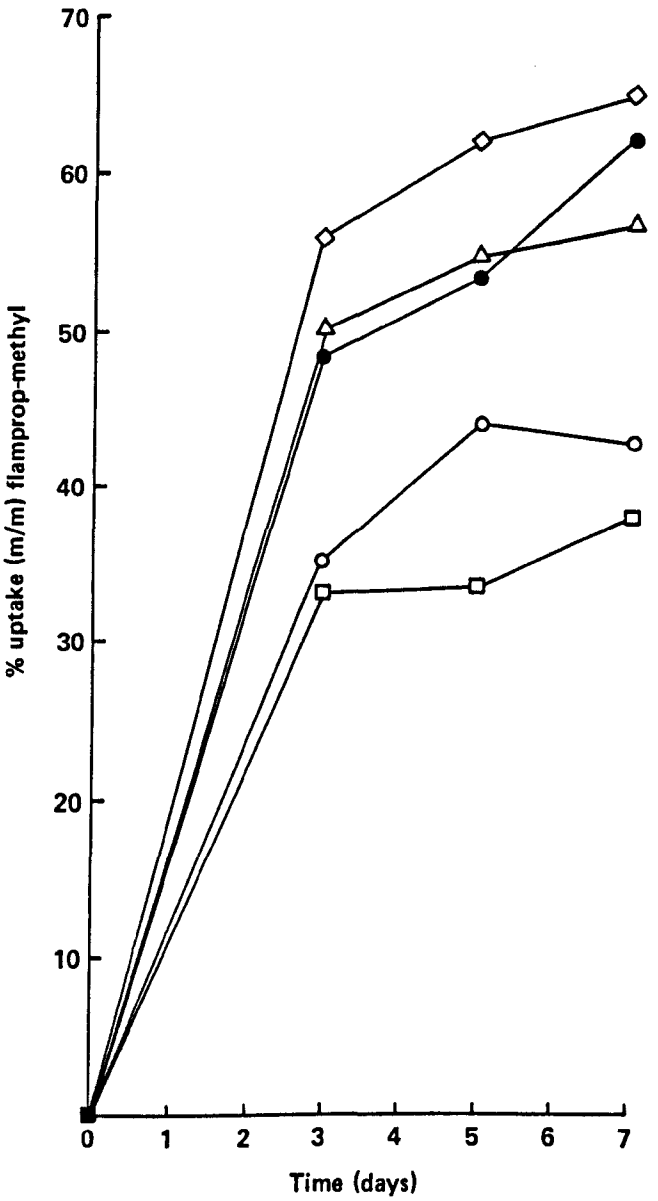


Fig 13 Foliar uptake by wheat of flamprop-methyl over 7 days after application of the herbicide (0.3 μg) formulated in aqueous solutions of Triton X 100 (△——△), Triton X 114 (◇——◇), Triton X 405 (○——○) and ethoxylated castor oil (□——□) at surfactant concentrations of 100 ppm, and in a 300 ppm aqueous solution of Triton X 405 (●——●)

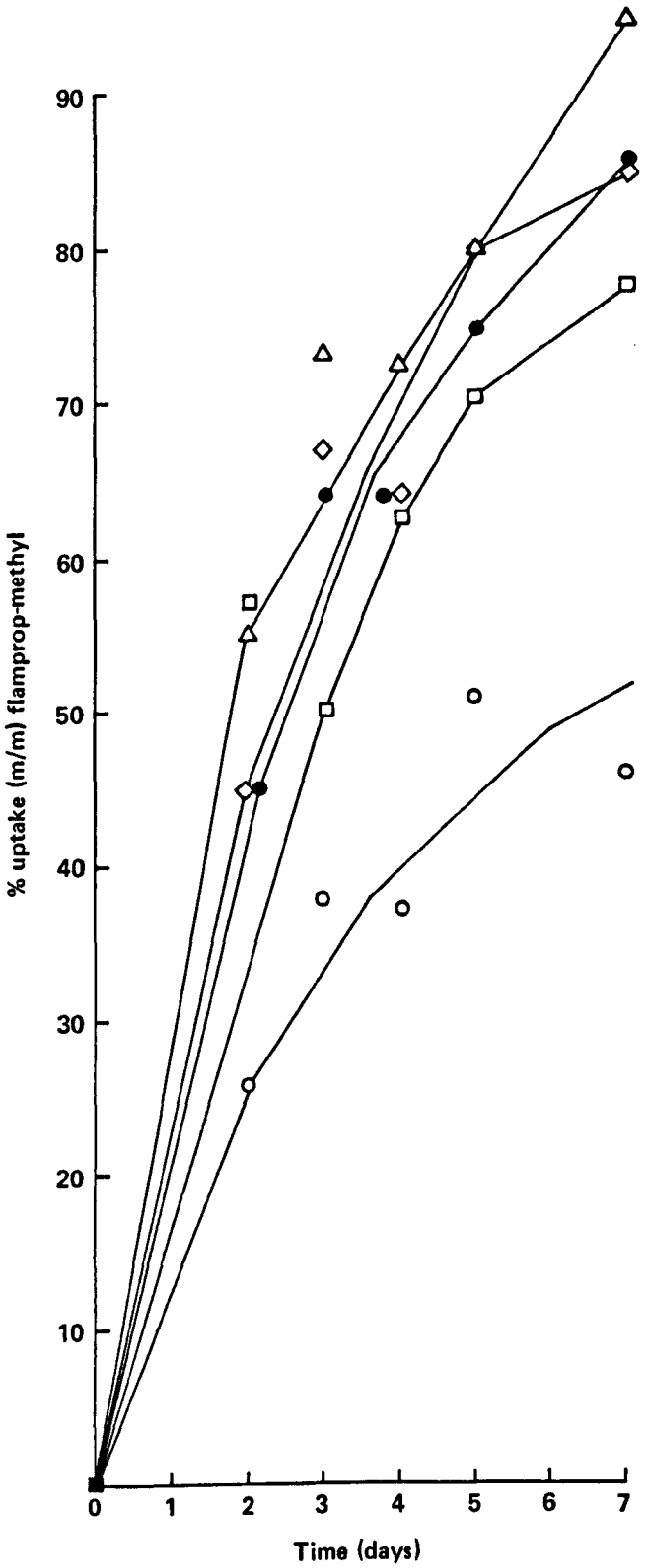


Fig 14 Foliar uptake by wheat of flamprop-methyl over 7 days after application of the herbicide (0.3 μg) formulated as an aqueous solution (o——o) and in 100 ppm aqueous solutions of Ethylam GMF (△——△), Tensopane D 30 (●——●), Lutensol AP 10 (◇——◇) and Triton X 100 (□——□)

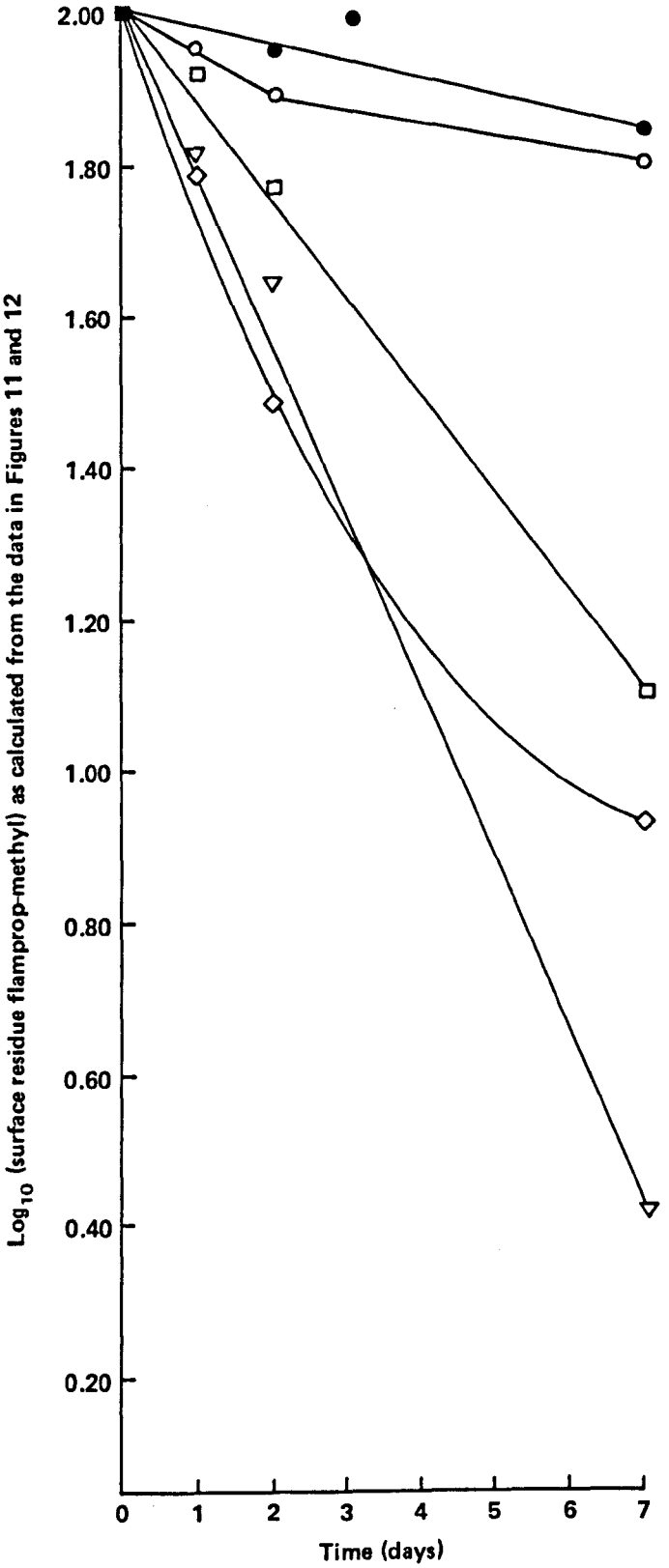


Fig 15 Log_{10} surface residue of flamprop-methyl over 7 days after treatment with the herbicide (0.3 μg) formulated as an aqueous solution (○——○) and in 100 ppm (▽——▽), 1000 ppm (◇——◇), 10,000 ppm (□——□) and 50,000 ppm (●——●) solutions of Triton X 100 as a function of time

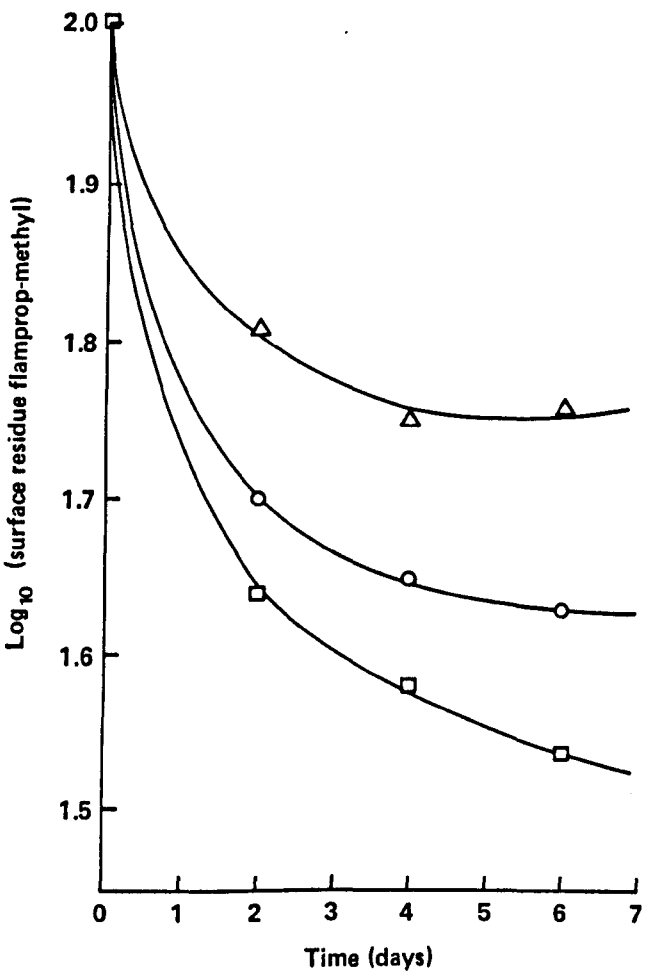


Fig 16 Log_{10} surface residue of flamprop-methyl over 7 days after treatment with the herbicide (0.3 μg) formulated in 100 ppm aqueous solutions of Triton X 114 (□——□) Triton X 100 (○——○) and Triton X 405 (△——△) as a function of time

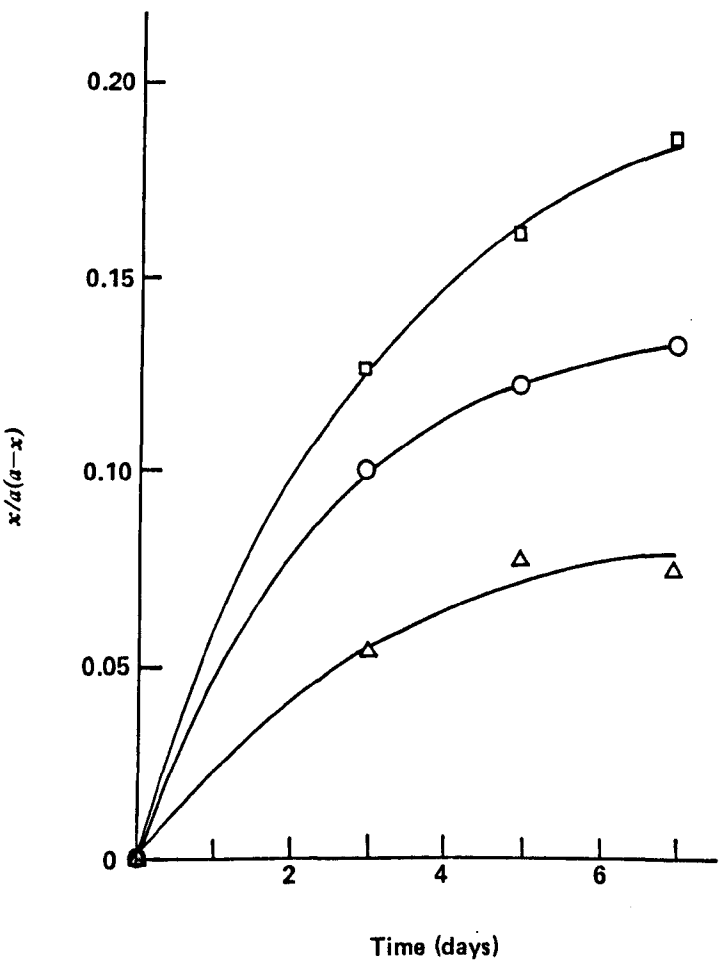


Fig 17

Analysis of data from Figure 13 recalculated as $x/a(a-x)$ where a is the surface residue at zero time and x is the surface residue at any given time after treatment with flamprop-methyl formulated in 100 ppm aqueous solutions of Triton X 114 (□—□), Triton X 100 (○—○) and Triton X 405 (△—△).

with either first order, (Figure 16), or second order, (Figure 17), kinetics.

3.4 Uptake from concentrated surfactant solutions

Table 7 shows the recovery of flamprop-methyl by surface extraction over a period of seven days following application of the herbicide formulated in a 10,000 ppm aqueous solution of Brij 76. Uptake of flamprop-methyl was largely confined to the first 24 hours. Uptake between one and four days was less than that expected in the absence of the surfactant: in this regard the effect of this treatment was similar to that found with Triton X 100 at 50,000 ppm, although with the latter the initial relatively rapid uptake was not seen, (Figure 12). Both treatments give evidence to the possibility that uptake can be adversely affected by formulation in surfactant solutions.

3.5 Effect of surfactant - toxicant ratio

Table 8 shows the recoveries of flamprop-methyl by surface extraction following application of the herbicide in aqueous solutions of Brij surfactants. The treatments included in this study were selected with a view to comparing uptake from formulations with differing surfactant: herbicide ratios and similar surface tensions. These data are considered below in the context of related data. Generally the results obtained with these treatments were in accord with earlier results. Treatments with Brij 35 gave poor agreement with earlier data: Table 8 shows that uptake of flamprop-methyl, as assessed from the depletion of the surface extract, was better at 1000 ppm with Brij 35 than at 100 ppm, (data at 1 and 2 days), although earlier work had shown uptake to be reduced at the higher surfactant concentration, (Figure 7).

Table 7 : Foliar penetration of flamprop-methyl following application in an aqueous solution of Brij 76 (1% m/v) (Experiment 16)

Time days	% Recovery flamprop-methyl				Uptake Calc.
	Replicates			Mean	
0	100	100	100	100	0
1	76.9	65.4	77.8	73.4	26.6
2	61.4	67.0	74.8	67.7	32.3
3	61.4	82.3	79.6	74.4	25.6
4	66.5	75.2	63.2	68.3	31.7
7	54.0	59.8	M	56.9	43.1

Table 8 : Foliar penetration from solutions of Brij surface active agents (Experiment 17)

Trt No.	Brij	Conc.		Time/days							Mean
		ppm	(2)	0	1	2	3	4	6	7	
				% Recovery flamprop-methyl(1)							
1	30	20	5.5	100	71	83	81	80	60	50	70.8
2	30	100	27.6	100	47	34	30	25	18	18	28.7
3	30	500	138.0	100	30	70	14	11	7	6	23.0
4	30	1000	276.0	100	30	29	23	15	8	10	19.2
5	35	100	8.4	100	81	73	70	56	50	65	65.8
6	35	1000	83.5	100	36	35	20	24	6	6	21.2
7	35	1500	125.3	100	60	68	40	16	9	10	33.8
8	96	30	4.2	100	83	80	68	70	70	21	65.3
9	96	100	14.0	100	34	48	39	43	30	30	37.0
10	96	1000	140.0	100	50	18	13	18	9	9	19.5

(1) Each value represents the average of three plants

(2) Molarity of solutions with respect to surfactant x 10^5

LSD value (P = 0.05) 13.0

Comparison with earlier data suggested that the rate of uptake achieved with the 100 ppm Brij 35 treatment in this experiment was much less than was expected.

The effect on the foliar uptake of flamprop-methyl following application in solutions of Brij 96 of varying the ratio of surfactant to herbicide was investigated by systematically varying the concentrations of both surfactant and herbicide in a range of formulations, (Table 9). This experiment was designed to investigate a four fold variation in the mass of herbicide applied at a constant volume application rate; the simultaneous variation of the formulation with regards to the surfactant concentration resulted in a variation in the mole ratio of surfactant to herbicide between 1:4 and 4:1.

Table 9 shows the recovery of herbicide 18 hours after application of Brij 96 formulations. It can be seen, from the calculated mean values of treatments at common concentrations of either herbicide or surfactant, that differences between treatments were attributable to the effect of surfactant concentration. Results from treatments 1-3 confirmed the trends identified in similar formulations in earlier studies. The trend in uptake with surfactant concentration was paralleled at the three herbicide concentrations. Close inspection of individual treatments shows that percentage uptake at any one surfactant concentration was constant and was independent of the herbicide concentration: in absolute terms herbicide uptake was thus directly proprotional to the mass of herbicide applied. No effect attributable to the surfactant: herbicide ratio was evident in these data.

Table 10 shows the effect of surfactant: herbicide ratio on the recovery of flamprop-methyl from leaves treated with a range of

Table 9 : Foliar penetration from solutions of Brij 96; effect of surfactant-toxicant ratio (Experiment 18)

Trt No.	Tox ppm	Brij ppm	Ratio molar	Extract	% Recovery flamprop-methyl after 18 hours					
					Replicates			Mean values		
									(1)	(2)
1	30.0	70.0	1:1	Surface	11.0	8.6	9.3	9.6		10.1
2	30.0	35.0	1:2	Surface	16.5	17.2	21.2	18.3	20.8	14.5
3	30.0	17.5	1:4	Surface	20.2	39.6	43.8	34.5		34.9
4	15.0	70.0	2:1	Surface	9.6	10.3	10.9	10.3		
5	15.0	35.0	1:1	Surface	12.8	11.6	12.6	12.3	19.0	
6	15.0	17.5	1:2	Surface	27.9	30.0	45.0	34.3		
7	7.5	70.0	4:1	Surface	9.5	10.4	11.0	10.3		
8	7.5	35.0	2:1	Surface	11.8	15.7	11.2	12.9	19.7	
9	7.5	17.5	1:1	Surface	36.6	40.0	30.8	35.8		
10	30.0	70.0	1:1	Leaf	88.3	90.7	90.4	89.8		89.4
11	30.0	35.0	1:2	Leaf	82.4	82.6	78.3	81.1	78.2	85.2
12	30.0	17.5	1:4	Leaf	75.2	60.1	56.2	63.8		63.9
13	15.0	70.0	2:1	Leaf	89.5	89.5	88.7	89.2		
14	15.0	35.0	1:1	Leaf	85.3	90.0	87.5	87.6	80.6	
15	15.0	17.5	1:2	Leaf	70.5	70.0	54.7	65.1		
16	7.5	70.0	4:1	Leaf	90.0	89.6	87.8	89.1		
17	7.5	35.0	2:1	Leaf	88.6	83.9	87.8	86.8	79.5	
18	7.5	17.5	1:1	Leaf	61.4	59.3	67.5	62.7		

(1) Mean value of % recovery of any one concentration of toxicant

(2) Mean value of % recovery of any one Brij concentration.

LSD values (P = 0.05)	Surface extract	Leaf extract
Means	9.4	8.5
Means at given toxicant concentrations (1)	4.5	4.1
Means at given Brij concentrations (2)	4.5	4.1

Table 10 : Foliar penetration from solutions of Triton X 100; effect of surfactant-toxicant ratio (Experiment 19)

Trt No.	Tox ppm	Brij ppm	Ratio molar	Extract	% Recovery flamprop-methyl after 18 hours					
					Replicates			Mean value		
									(1)	(2)
1	30.0	70.0	1.3:1	Surface	71.3	68.2	60.0	66.5		67.0
2	30.0	35.0	1:1.6	Surface	79.6	78.8	76.0	76.5	73.3	75.9
3	30.0	17.5	1:3.2	Surface	78.3	77.5	75.0	77.0		77.5
4	15.0	70.0	2.5:1	Surface	71.2	65.0	68.9	68.4		
5	15.0	35.0	1.3:1	Surface	75.0	75.4	75.0	75.1	73.9	
6	15.0	17.5	1:1.6	Surface	77.7	83.7	73.5	78.3		
7	7.5	70.0	5.0:1	Surface	63.7	66.3	68.4	66.1		
8	7.5	35.0	2.5:1	Surface	74.8	76.3	77.5	76.2	73.2	
9	7.5	17.5	1.3:1	Surface	76.3	79.9	75.5	77.2		
10	30.0	70.0	1.3:1	Leaf	30.3	31.8	39.1	33.7		33.1
11	30.0	35.0	1:1.6	Leaf	20.1	24.2	23.6	22.6	26.2	23.2
12	30.0	17.5	1:3.2	Leaf	20.4	22.2	23.9	22.2		22.8
13	15.0	70.0	2.5:1	Leaf	32.4	33.9	31.5	32.6		
14	15.0	35.0	1.3:1	Leaf	23.9	24.4	24.3	24.2	26.5	
15	15.0	17.5	1:1.6	Leaf	20.4	21.7	26.2	22.8		
16	7.5	70.0	5.0:1	Leaf	34.0	33.0	31.5	32.8		
17	7.5	35.0	2.5:1	Leaf	24.8	22.9	20.5	22.7	26.4	
18	7.5	17.5	1.3:1	Leaf	22.5	25.7	22.5	23.6		

(1) Mean value of % recovery at any one concentration of toxicant

(2) Mean values of % recovery at any one Triton X 100 concentration

LSD values (P = 0.05)	Surface extract	Leaf extract
Means	5.6	4.1
Means at given toxicant concentrations (1)	2.7	2.0
Means at given Triton X 100 concentrations (2)	2.7	2.0

formulations in Triton X 100. These data parallel those obtained with Brij 96, (Table 9). Table 11 shows the recovery of flamprop-methyl three and 18 hours after application of the herbicide to wheat as aqueous solutions in Brij 96. This experiment differed from those discussed above in using more concentrated surfactant solutions: in these formulations the range of surfactant concentrations corresponded to the plateau region, (Figure 5), over which uptake of the herbicide was relatively constant. In confirmation of the conclusions reached above it was found that in the absence of any marked surfactant effect all treatments gave similar % uptake.

3.6 Partition studies

A. Between the spray drop and the leaf surface

The partition of flamprop-methyl between the aqueous formulation and the lipophilic leaf surface was investigated by monitoring the depletion of herbicide in applied droplets following topical application of the formulation. Table 13 shows the partitioning behaviour of the herbicide between aqueous solutions of Triton X 100 and wheat leaves. These data show that transfer of the major part of the applied herbicide was restricted to the latter stages of the evaporation of the drop. This effect was also found with more concentrated surfactant solutions; Figure 18 shows that, following application of a solution of flamprop-methyl in a 10,000 ppm aqueous solution of Triton X 100, 70 per cent of the original applied herbicide was recoverable in the residual drop up to a few minutes prior to the drop having dried.

B. Between the herbicide residue and the epicuticular waxes

The partition of flamprop-methyl between the residue on the leaf

Table 11 : Foliar penetration from solutions of Brij 96; effect of surfactant-toxicant ratio (Experiment 20)

Trt No.	Tox ppm	Brij ppm	Ratio molar	Extract	% Recovery flamprop-methyl after 3 hours					
					Replicates			Mean value		
									(1)	(2)
1	30.0	1600	25.4:1	Surface	53.0	70.0	71.0	65.0		65.1
2	30.0	800	12.7:1	Surface	71.0	61.8	63.3	65.0	68.3	65.2
3	30.0	400	6.4:1	Surface	77.6	67.2	56.1	67.0		61.8
4	30.0	200	3.2:1	Surface	70.0	82.1	74.8	76.0		67.5
5	15.0	1600	50.8:1	Surface	64.0	73.8	85.8	74.4		
6	15.0	800	24.4:1	Surface	67.7	69.5	72.7	68.6	67.4	
7	15.0	400	12.7:1	Surface	59.7	71.7	54.5	62.0		
8	15.0	200	6.4:1	Surface	69.8	58.7	65.2	64.6		
9	7.5	1600	101.6:1	Surface	65.5	39.5	62.9	56.0		
10	7.5	800	50.8:1	Surface	62.1	57.6	67.0	62.0	59.0	
11	7.5	400	25.4:1	Surface	51.4	57.1	61.0	56.5		
12	7.5	200	12.7:1	Surface	49.5	68.2	69.0	62.0		
					% Recovery flamprop-methyl after 24 hours					
13	30.0	1600	25.4:1	Surface	12.0	9.5	16.4	12.6		12.0
14	30.0	800	12.7:1	Surface	13.2	14.7	11.0	13.0	13.6	11.5
15	30.0	400	6.4:1	Surface	10.6	17.2	14.1	14.0		12.2
16	30.0	200	3.2:1	Surface	14.1	15.8	15.5	15.0		14.3
17	15.0	1600	50.8:1	Surface	12.9	13.2	11.8	12.6		
18	15.0	800	24.4:1	Surface	10.0	10.8	10.4	10.4	12.7	
19	15.0	400	12.7:1	Surface	9.0	11.2	13.7	11.3		
20	15.0	200	6.4:1	Surface	17.7	18.1	13.3	16.4		
21	7.5	1600	101.6:1	Surface	12.0	10.6	10.1	10.9		
22	7.5	800	50.8:1	Surface	10.8	12.0	10.7	11.2	11.2	
23	7.5	400	25.4:1	Surface	10.0	16.1	7.9	11.3		
24	7.5	200	12.7:1	Surface	15.5	11.5	7.5	11.5		

Table 11 cont/

(1) Mean values of % recovery at any one concentration of toxicant

(2) Mean values of % recovery at any one Brij concentration

LSD values (P = 0.05)	3 hr data	24 hr data
Means	14.9	4.2
Means at given toxicant concentrations (1)	6.0	1.7
Means at given Brij concentrations (2)	7.2	2.0

Table 12 : Foliar penetration from solutions of Brij 96; effect of surfactant-toxicant ratio, (Experiment 20)

Brij 96 ppm	Concentration flamprop-methyl/ppm			
	30	15	7.5	
	% Recovery flamprop-methyl after 24 hours : leaf maceration			
	(mean 3 reps)			Mean
1600	95.5	94.0	101.6	97.0
800	94.0	91.6	84.0	89.9
400	79.6	83.5	95.0	86.0
200	86.5	83.0	83.0	84.2
Mean	88.9	88.0	90.9	

Table 13 : Partitioning of flamprop-methyl between aqueous solution in Triton X 100 applied as discrete droplet and leaf surface

Triton ppm	Time/mins																
	0	10	20	30	40	50	60	70	80	85	90	95	100	105	110	125	130
	% Recovery flamprop-methyl from residual drop																
10	100	88	100	97	99	96	99	98	ND	ND	75	ND	86	ND	82	62	Dry
70	100	94	92	93	91	91	87	90	ND	ND	72	ND	58	57	Dry		
200	100	90	91	84	87	88	81	87	78	ND	ND	68	72	ND	Dry		
1000	100	ND	68	95	88	96	95	82	80	69	71	ND	63	Dry			

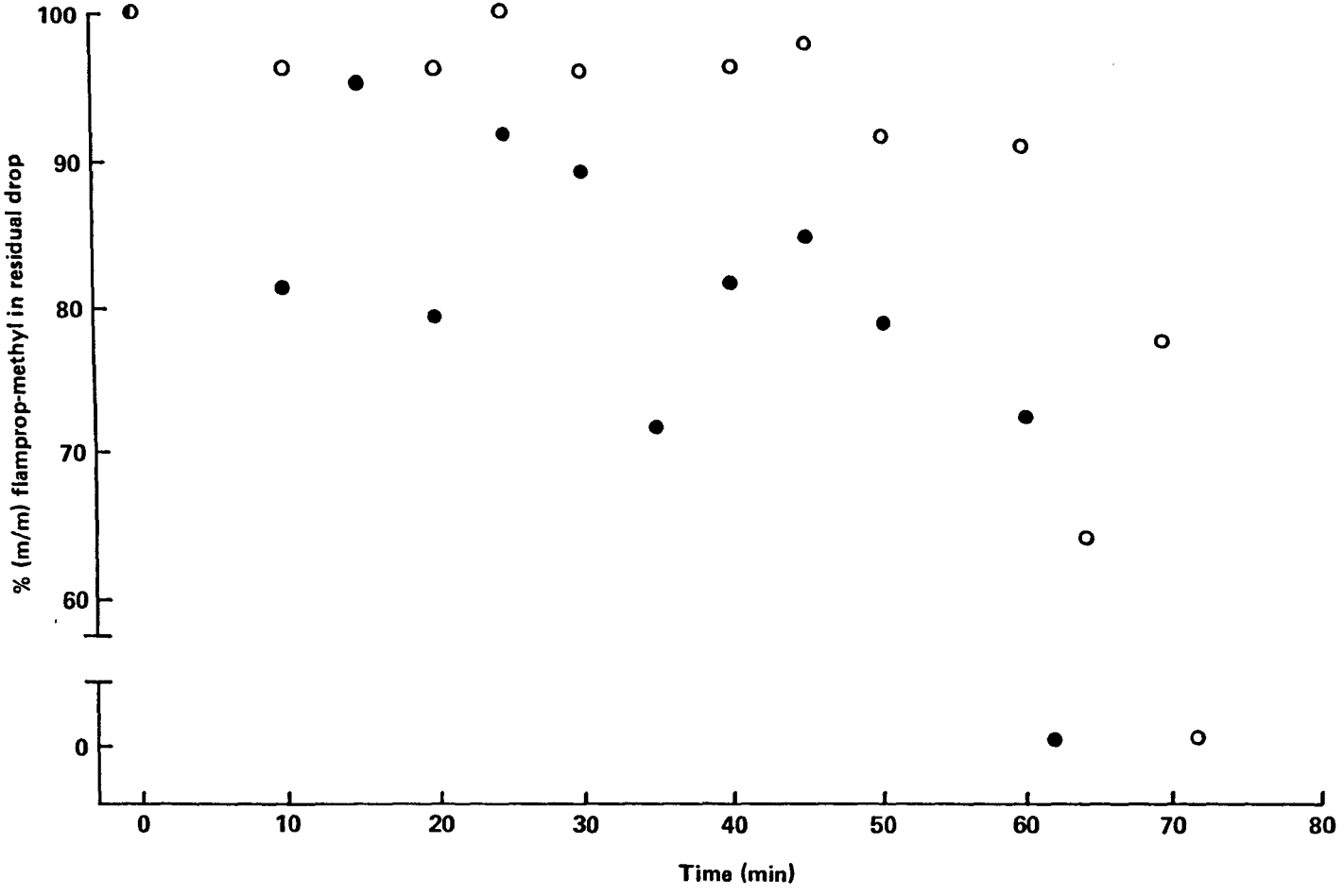


Fig 18 Partition of flamprop-methyl between topically applied drops of formulated a.i. and the leaf surface : recoveries from aqueous solutions (o) and from formulations in a 10,000 ppm solution of Triton X 100 (●)

surface and the leaf waxes was investigated with treatments for which penetration of the leaf surface had been studied previously using solvent extraction. The general trends established in these studies will be exemplified by reference to representative data.

Table 14 shows the recovery of radioactive labelled flamprop-methyl by repeated water washings of wheat treated with the herbicide formulated in Brij 96. Recovery of activity after 24 hours was largely limited to the first extract, with a second wash typically recovering less than 10 per cent of extract 1, but with small amounts of herbicide, (about 0.1 per cent), being recovered up to extract 10. This pattern of recovery broadly paralleled that found with simple aqueous solutions of the herbicide.

Leaves treated with the above formulation were also analysed to assess partition through the cuticle by extractions with a range of solvents of graded polarity. Recovery of activity, (Table 15), was associated with certain extracts, principally the first water wash, (extract 1), and immersion in hexane-acetone solutions, (extracts 6 and 7). Beyond extract 5 all solutions were to varying extents contaminated with chlorophyll: recovery in these solutions could in part or in total be contributed to by extraction from the symplast.

Table 17 shows the recoveries of flamprop-methyl achieved by water washing and extraction of the washed leaf by immersion in hexane-acetone 24 hours after application of the herbicide formulated in solutions of Triton X 100 or Brij 76. At zero time the total herbicide applied in these formulations was recoverable in a water wash. At a Triton X 100 concentration of 200 ppm the two extracts recovered a total of 63.6 per cent of the applied herbicide after 24 hours, divided

Table 14 : Foliar penetration from a solution of Brij 96 (0.01% m/v); recovery of ^{14}C-flamprop-methyl by repeated water washings

Extract	Activity recovered after 24 hours/cpm								
	Wheat					Wild oat			
	Replicates					Replicates			
1	10427 (33)[1]	1819 (7)	5749 (20)	2770 (9)	4887 (16)	5748 (20)	7497 (25)	5546 (18)	3889 (13)
2	427	541	517	393	421	214	292	281	286
3	317	214	381	307	353	250	218	175	198
4	232	650	302	192	217	172	141	130	165
5	155	231	212	193	171	120	132	118	138
6	156	194				96	118	91	
7	166	146				87	96	89	
8	128	126				102	92	79	
9	143	140				134	96	89	
10	117	130				117	95	93	

(1) Recovery in extract 1 as a % (m/m) of original application

Table 15 - Foliar penetration from a solution of Brij 96 (0.01% m/v); recovery of flamprop-methyl by repeated solvent extraction

Solvent	Method	Extract	% Recovery flamprop-methyl after 24 hours from wheat					
			Replicates					Mean
Water	Wash	1	10.7	9.7	22.0	21.3	29.0	18.5
Water	Wash	2	1.2	2.7	1.3	1.5	1.5	1.6
Hexane-acetone 10%	Wash	3	13.3	10.3	5.3	5.0	4.7	7.7
Hexane-acetone 10%	Wash	4	3.0	4.0	2.7	2.0	1.7	2.7
Hexane-acetone 10%	Wash	5	2.3	2.9	2.2	2.3	2.0	2.3
Hexane-acetone 10%	Wash	3-5	30.5	29.6	33.5	32.1	38.9	
Hexane-acetone 10%	Immersion	6	56.0	26.0	38.9	27.6	15.6	32.8
Hexane-acetone 30%	Immersion	7	11.0	18.0	12.7	21.0	27.7	18.1
Hexane-acetone 50%	Immersion	8	2.0	2.7	1.8	3.0	4.3	2.7
Hexane-acetone 70%	Immersion	9	1.0	1.0	0.5	1.7	2.0	1.2
Hexane-acetone 90%	Immersion	10	0.4	1.0	1.0	0.8	0.5	0.7
		.Total	100.9	78.3	88.4	86.2	89.0	

Table 16: Foliar penetration from a solution of Brij 96 (0.01% m/v); recovery of flamprop-methyl by repeated solvent extractions

Solvent	Method	Extract	% Recovery flamprop-methyl from wild oat after 24 hours					
			Replicates					Mean
Water	Wash	1	5.3	13.6	6.8	4.6	3.8	6.8
Water	Wash	2	0.8	1.0	1.6	0.9	1.0	1.0
Hexane-acetone 10%	Wash	3	4.1	2.8	5.8	3.8	5.1	4.3
Hexane-acetone 10%	Wash	4	5.7	4.1	6.9	4.6	5.1	5.3
Hexane-acetone 10%	Wash	5	5.8	5.1	8.1	4.0	5.9	5.8
Hexane-acetone 10%	Wash	3-5	21.7	26.6	29.2	17.9	21.0	
Hexane-acetone 10%	Immersion	6	34.5	33.9	32.1	33.2	29.2	32.6
Hexane-acetone 30%	Immersion	7	9.3	13.9	13.4	15.3	18.1	14.0
Hexane-acetone 50%	Immersion	8	3.3	4.7	3.4	4.7	4.4	4.1
Hexane-acetone 70%	Immersion	9	3.6	4.0	2.9	4.0	4.2	3.7
Hexane-acetone 90%	Immersion	10	0.9	1.0	0.7	0.9	0.6	0.8
		Total	73.3	84.1	81.7	76.0	77.6	

Table 17: Foliar penetration of flamprop-methyl into wheat and wild oat following application in aqueous surfactant.

Surfactant	Conc. ppm	Sp	Extract	% Recovery flamprop-methyl after 24 hours					
				Replicates					Mean
Triton X 100	200	WH	Water	31.5	25.7	33.0	21.7		27.9
Triton X 100	200	WH	Surface	38.8	28.8	34.1	40.7		35.6
Triton X 100	200	WH	Sum	70.3	54.5	67.1	62.4		63.6
Triton X 100	200	OA	Water	84.6	81.0	82.2	84.3	88.6	84.1
Triton X 100	200	OA	Surface	14.2	10.0	9.6	10.0	9.0	9.9
Triton X 100	200	OA	Sum	98.8	91.0	91.8	94.3	97.6	96.7
Triton X 100	1000	WH	Water	52.5	47.5	37.3	67.5	62.5	53.5
Triton X 100	1000	WH	Surface	3.9	4.9	5.5	3.1	6.6	4.8
Triton X 100	1000	WH	Sum	56.4	52.4	42.8	70.6	69.1	58.3
Triton X 100	1000	OA	Water	76.5	82.4	70.6	69.5	73.1	74.6
Triton X 100	1000	OA	Surface	3.1	2.2	4.9	6.9	3.7	4.0
Triton X 100	1000	OA	Sum	79.6	84.6	75.5	76.4	76.8	78.6
Triton X 100	10000	WH	Water	86.1	80.8	85.1	86.5		84.6
Triton X 100	10000	WH	Surface	1.6	4.3	1.5	1.9		2.3
Triton X 100	10000	WH	Sum	87.7	85.1	86.6	88.4		87.0
Triton X 100	10000	OA	Water	84.5	84.2	89.3	91.0	89.3	87.7
Triton X 100	10000	OA	Surface	3.5	4.4	2.3	2.8	1.3	2.9
Triton X 100	10000	OA	Sum	88.0	88.6	91.6	93.8	90.6	90.5
Brij 76	100	WH	Water	51.3	26.0	42.0	34.7	41.5	39.1
Brij 76	100	WH	Surface	16.4	17.3	23.8	20.0	14.1	18.3
Brij 76	100	WH	Sum	67.7	43.3	65.8	54.7	55.6	57.4
Brij 76	100	OA	Water	47.9	27.4	39.7	32.0	44.3	38.3
Brij 76	100	OA	Surface	14.2	18.5	21.4	17.5	16.9	17.7
Brij 76	100	OA	Sum	62.1	45.9	61.1	49.5	61.2	56.0

Continued over

Table 17 : continued

Surfactant	Conc. ppm	Sp	Extract	% Recovery flamprop-methyl after 24 hours					
				Recplicates					Mean
Brij 76	1000	WH	Water	32.1	33.6	34.0	33.7	30.5	32.8
Brij 76	1000	WH	Surface	29.6	24.3	27.8	22.6	24.5	25.7
Brij 76	1000	WH	Sum	61.7	57.9	61.8	56.3	55.0	58.6
Brij 76	1000	OA	Water	35.0	33.6	46.8	30.6		36.5
Brij 76	1000	OA	Surface	28.4	27.0	17.2	24.5		24.3
Brij 76	1000	OA	Sum	63.4	60.6	64.0	55.1		60.8
Brij 76	10000	WH	Water	42.5	47.5	54.3	38.6	41.2	44.8
Brij 76	10000	WH	Surface	37.8	33.9	35.6	35.0	31.2	34.7
Brij 76	10000	WH	Sum	80.3	81.4	89.9	73.6	72.4	79.5
Brij 76	10000	OA	Water	40.7	53.5	40.0	39.0	28.6	40.4
Brij 76	10000	OA	Surface	31.6	21.2	36.3	34.4	42.0	33.1
Brij 76	10000	OA	Sum	72.3	74.7	76.3	73.4	70.6	73.5

LSD values (P = 0.05)

	Water Extract	Surface Extract
For 5 obs vs 5 obs	8.9	4.6
For 4 obs vs 5 obs	9.4	4.9
For 4 obs vs 4 obs	9.9	5.2

between the water wash and the organic extract. At higher surfactant concentrations the recovery was largely confined to the water wash of wheat; combined recoveries indicated a slight improvement in uptake as the surfactant concentration was increased between 200 and 1000 ppm and a reduction in uptake on further increasing the concentration to 10,000 ppm. The trend to increased recovery in the water wash as the concentration of the surfactant in the formulation was increased was not shown by all treatments, and residues of formulations based on the Triton X-compounds 15, 35 and 45 were about equally divided between the two extracts at all concentrations. Brij 76 formulations also failed to show the depletion of recovery in the wax extract shown by Triton X 100, (Table 17), although still showing reduced uptake at higher concentrations. The pattern of redistribution shown by Triton X 100 was however shown by formulations based on the Triton X-compounds 102, 165, 305, 405 and 705.

3.7 Uptake by wild oat

The uptake by wild oat of flamprop-methyl formulated in aqueous surfactant solutions was studied in parallel with uptake by wheat. Generally little difference was found between the permeabilities of the two species, although the results could vary with formulation. Table 18 shows the recovery of flamprop-methyl from wheat and wild oat 24 hours after application of aqueous Brij 96 formulations. It can be seen from these data that uptake by the two species was similar: at the highest surfactant concentration recoveries were slightly higher from wild oat. Table 17 includes data on wild oat with herbicide formulations based on Triton X 100 and Brij 76. The uptake achieved with the latter by wheat and wild oat was similar, as was uptake at the higher Triton X 100 concentrations. Uptake from a solution in a 200 ppm Triton X 100

Table 18: Foliar penetration of flamprop-methyl into wheat and wild oat following application of aqueous Brij 96 solutions.

Surfactant conc ppm	Sp	% Recovery flamprop-methyl after 24 hours						Mean
		Surface extracts						
100	WH	45.8	28.0	35.4	34.1			37.3
100	OA	45.4	39.5	36.8	53.8	43.0	41.9	43.4
1000	WH	42.3	33.4	25.0	26.6			33.3
1000	OA	51.4	42.0	36.1	46.2	52.4	45.4	45.6
10000	WH	40.5	35.7	37.3	33.5			38.2
10000	OA	61.0	49.6	48.7	56.8	67.1	60.5	57.3

LSD value (P = 0.05)

For 4 obs vs 4 obs	7.0
For 6 obs vs 4 obs	6.4
For 6 obs vs 6 obs	5.7

solution, (Table 17), was very much greater by wheat.

3.8 Non-concerted applications

The effect on foliar uptake of application of aqueous ^{14}C-flamprop-methyl to the residue left from the prior application of a 1000 ppm solution of Brij 96 was investigated for various delays between the applications. The method used in separating the application of surfactant and herbicide in time was so designed that the period of herbicide uptake coincided for each treatment; this was achieved by staggering applications of the surfactant solution. Foliar uptake was compared over 24 hours between treatments in which application was as a complete formulation (concerted application) and treatments in which a delay of between 24 hours and 22 days was introduced between the two applications. Table 19 shows the recovery of flamprop-methyl from these treatments, effected by a water wash and immersion of the washed leaf in organic solvent. Mean values of these data were plotted graphically as a function of the time lag, (Figure 19).

The recovery of flamprop-methyl in the water wash was at a minimum when the time lag was about six days and was greatest when the time lag was in excess of eight days. The organic extract showed little variation over the time frame of this experiment (Figure 19) so that variation in total recovery was largely attributable to variation in the water wash extract. Foliar uptake was seen to be better than that from a simple aqueous solution with all treatments and to vary between about 80 per cent to 60 per cent, with greatest uptake after a time lag of about six days. The increased variability between replicates when the time lag exceeded 18 days was attributed to the complicating factor of phytotoxicity. Discernible surfactant phytotoxicity was restricted to

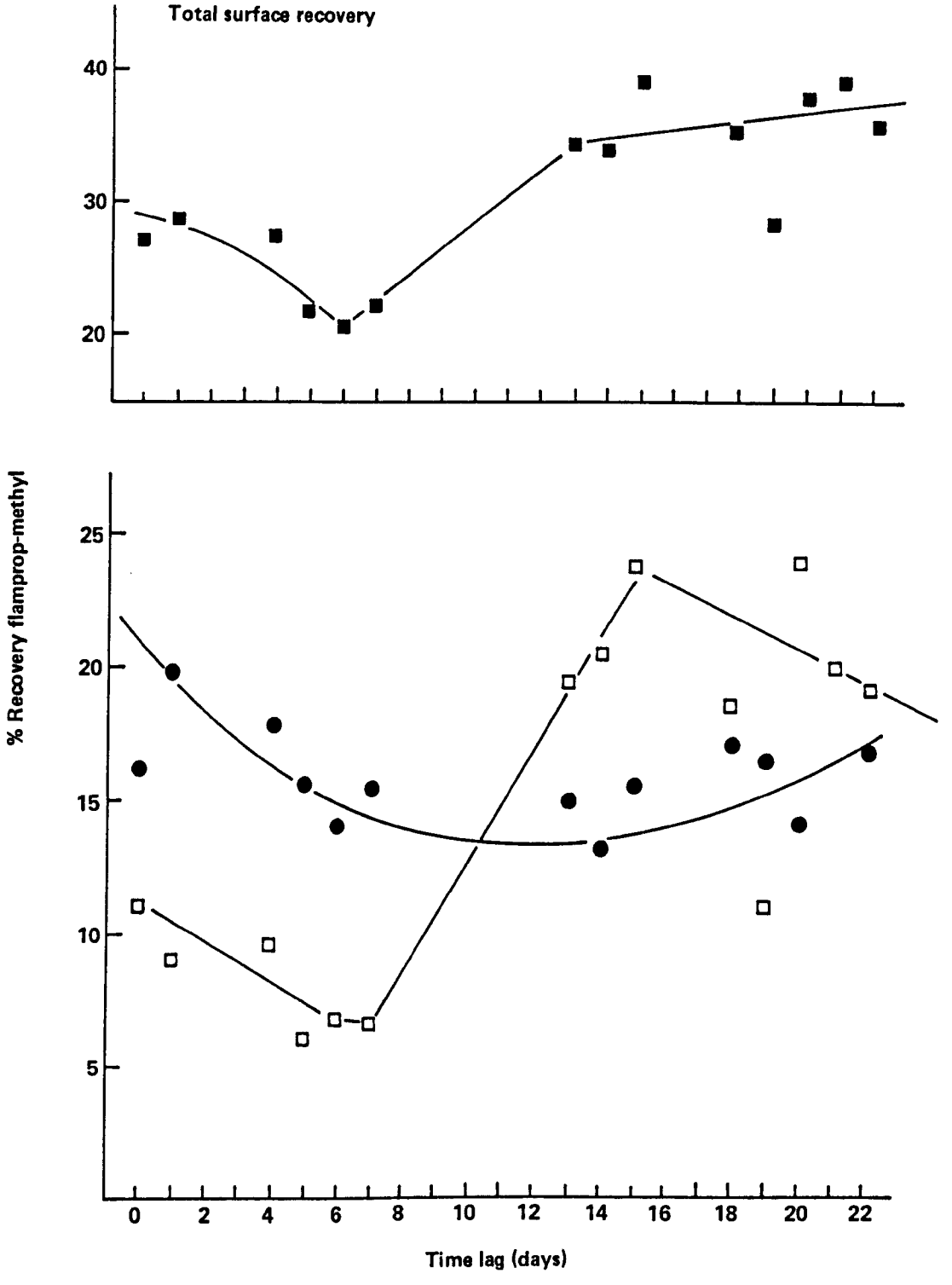


Fig 19 Non-concerted Applications

Recovery of flamprop-methyl in water wash (□—□), hexane/acetone extract (●—●) and combined water wash and hexane/acetone extract (■—■) of leaves 24 hours after application of an aqueous solution of the herbicide to the residue left from the prior application of a 1000 ppm solution of Brij 96

leaves which had been subjected to the surfactant for at least 18 days. When phytotoxicity was present, observed as extensive necrosis associated with the area of application, the recovery of flamprop-methyl in the water wash was markedly depleted, (Table 19).

This experiment was repeated with 100 ppm solutions of either Brij 96 or Triton X - 15 over a time lag of up to seven days; as before uptake was assessed 24 hours after application of the herbicide, (Tables 20 and 21). It was found that, relative to a concerted application, a marked reduction in foliar uptake accompanied the introduction of a time lag, but that uptake was then independent of the duration of the delay in application. A third type of response was found when Triton X - 45 was used in these studies at 100 ppm. In this instance (Figure 20) the initial time lag caused a smaller reduction in uptake than that found with Triton X - 15 and the reduction in uptake was increased as the time lag increased up to about three days. The distribution of the total recovery between the water wash extract and the organic extract suggested that partition into the epicuticular waxes was also reduced by the introduction of a time lag.

The effect on foliar uptake of introducing a time lag of up to seven days between application of 10,000 ppm solutions of either Brij 76 or Triton X - 305 and application of flamprop-methyl was investigated in a similar experiment to those described above. The extent of uptake was monitored by two water washes and a organic extract of the washed leaf, (Tables 22 and 23). These data suggest that the mechanism(s) by which uptake was inhibited with concentrated surfactant solutions was different for the two surfactants. With Triton X - 305, (Table 22), recoveries were largely restricted to the first water wash: uptake was markedly reduced by a 24 hour time lag and was then independent of the

Table 19 : Percentage recovery of flamprop-methyl from leaves treated separately (non-concerted application) with a 1000 ppm solution of Brij 96 and the herbicide.

Time lag[1] days	% Recovery after 24 hours in a water wash[3]					Means
	Replicates					
0[2]	12.2	8.7	8.9	14.6		11.1
1	8.5	8.2	9.9			8.9
4	8.7	4.8	14.8			9.4
5	9.5	4.7	4.2			6.1
6	7.3	5.6	7.3	6.6		6.7
7	5.3	4.1	6.7	6.2	10.7	6.6
13	16.9	17.0	24.1	19.2		19.3
14	19.0	22.8	19.8			20.5
15	20.8	23.7	23.4	27.1		23.8
18	19.3	17.8	16.8	20.2		18.5
19	10.9*	12.2*	8.0*	17.0		12.0
20	23.0	25.0	10.3*			24.0
21	11.9*	23.0	17.4			20.0
22	22.3	17.9	15.6	21.8	18.6	19.2
	% Recovery after 24 hours in hexane/acetone[3][4]					
	Replicates					
0[2]	10.3	15.0	23.6	15.6		16.1
1	13.4	22.5	23.3			19.7
4	13.3	18.5	21.9			17.9
5	16.4	17.1	13.2	15.3		15.5
6	12.1	12.7	17.7	13.7		14.0
7	16.6	23.0	12.5	10.6	14.2	15.4
13	14.8	14.0	14.7	16.4		15.0
14	17.5	9.7	12.3			13.2

Continued over

Table 19 : Continued

Time lag(1) days	% Recovery after 24 hours in a water wash(3)					Means
	Replicates					
15	14.9	12.1	22.8	12.3		15.5
18	17.7	13.4	20.5	16.2		17.0
19	16.7*	16.0*	16.1*	17.2		16.5
20	15.9	11.4	15.0*			14.1
21	20.1*	16.5	20.1			18.9
22	19.5	14.6	22.1	15.6	11.9	16.7

(1) Time lag between application of surfactant and herbicide

(2) Concerted application

(3) Recovery was made 24 hours after application of the herbicide

(4) Extractions of water washed leaves

* Leaves exhibiting severe necrosis

LSD Values (P = 0.05)	Water wash	hexane/acetone data
For 4 obs vs 4 obs	4.8	4.9
For 4 obs vs 3 obs	5.2	5.3
For 4 obs vs 5 obs	4.6	4.7
For 3 obs vs 3 obs	5.6	5.7
For 3 obs vs 5 obs	5.0	5.1
For 5 obs vs 5 obs	4.3	4.4

Table 20: Percentage receover of flamprop-methyl from leaves treated separately (non-concerted application) a 100 ppm solution of Brij 96 and the herbicide

Time lag[1] days	% Recovery after 24 hours in a water wash[3]			Means
	Replicates			
0[2]	9.4	11.0	10.5	10.3
1	49.0	40.0	38.0	42.0
2	49.0	46.7	60.0	51.9
3	40.0	46.7	29.0	38.6
4	46.7	51.0	51.0	50.0
7	44.4	44.5	55.6	48.2
	% Recovery after 24 hours in hexane/acetone[3][4]			
	Replicates			
1	9.0	7.8	7.8	8.2
2	10.0	13.3	8.4	10.6
3	15.6	13.3	13.3	14.0
4	13.9	12.2	13.3	13.1
7	13.3	10.4	10.0	11.2

(1) - (4) retain their meanings from Table 19

LSD values (P = 0.05): Water wash, 11.8; hexane/acetone, 2.7.

Table 21 : Percentage recovery of flamprop-methyl from leaves treated separately (non-concerted application) with a 100 ppm solution of Triton X 15 and the herbicide

Time lag(1) days	% Recovery after 24 hours(3) Replicates(4)			Mean
0(2)	38.4	42.7	49.3	43.5
1	80.0	82.8	88.6	83.8
2	62.8	77.1	80.0	73.3
3	85.7	82.8	65.7	78.0
4	62.8	64.3	82.8	69.9
7	77.1	85.7	82.8	81.9

(1) (3) retain their meanings from Table 19

(4) surface extracts, combining water wash and hexane/ acetone extracts

LSD value (P = 0.05) 14.5

Table 22 : Percentage recovery of flamprop-methyl from leaves treated separately (non-concerted application) with a 10,000 ppm solution of Triton X 305

Time lag[1] days	% Recovery[2] first water wash			Means
	Replicates			
1	85.6	93.3	93.3	90.7
2	97.8			97.8
3	77.8	90.0	91.1	86.3
4	93.3	91.1	86.7	90.4
7	93.3	80.0	82.2	85.0
	% Recovery - second water wash[3]			
1	0.6	0.8	1.0	0.8
2	0.6	0.6		0.6
3	0.8	1.3	1.4	1.1
4	0.8	0.7	0.6	0.7
7	0.7	0.7	1.0	0.8
	% Recovery - hexane/acetone[4]			
1	2.9	1.8	3.2	2.6
2	1.2	0.7		0.9
3	1.3	1.8	1.1	1.4
4	1.8	1.1	1.2	1.4
7	3.8	2.1	1.0	2.5

(1)-(4) retain their meanings from Table 19

LSD values (P = 0.05): first water wash, 13.4;
second water wash, 0.4;
hexane/acetone extract, 1.5

Table 23: Percentage recovery of flamprop-methyl from leaves treated separately (non-concerted application) with a 10,000 ppm solution of Brij 76 and the herbicide

Time lag[1] days	% Recovery[2] first water wash Replicates			Means
1	48.9	66.7	40.0	51.9
2	46.7	44.4	M	45.6
3	9.5	7.3	5.3	7.4
4	24.4	17.8	9.3	18.5
7	15.6	21.8	31.1	22.8
	% Recovery - second water wash[3]			
1	22.2	14.4	15.5	17.4
2	4.7	16.4	M	10.6
3	1.8	2.2	1.3	1.8
4	2.9	1.3	1.3	1.8
7	2.4	2.0	4.7	3.0
	% Recovery - hexane/acetone[4]			
1	6.7	5.8	14.4	9.0
2	5.3	11.1	M	8.2
3	11.1	17.2	22.2	16.8
4	10.0	8.7	7.8	8.8
7	6.9	11.5	8.4	8.9

(1) Time lag between application of surfactant and herbicide

(2) Recovery was made 24 hours after application of herbicide

(3) Repeat of first wash

(4) Extractions of water washed leaves

LSD values (P = 0.05): first water wash, 16.8;
second water wash, 7.6;
hexane/acetone extract 6.7

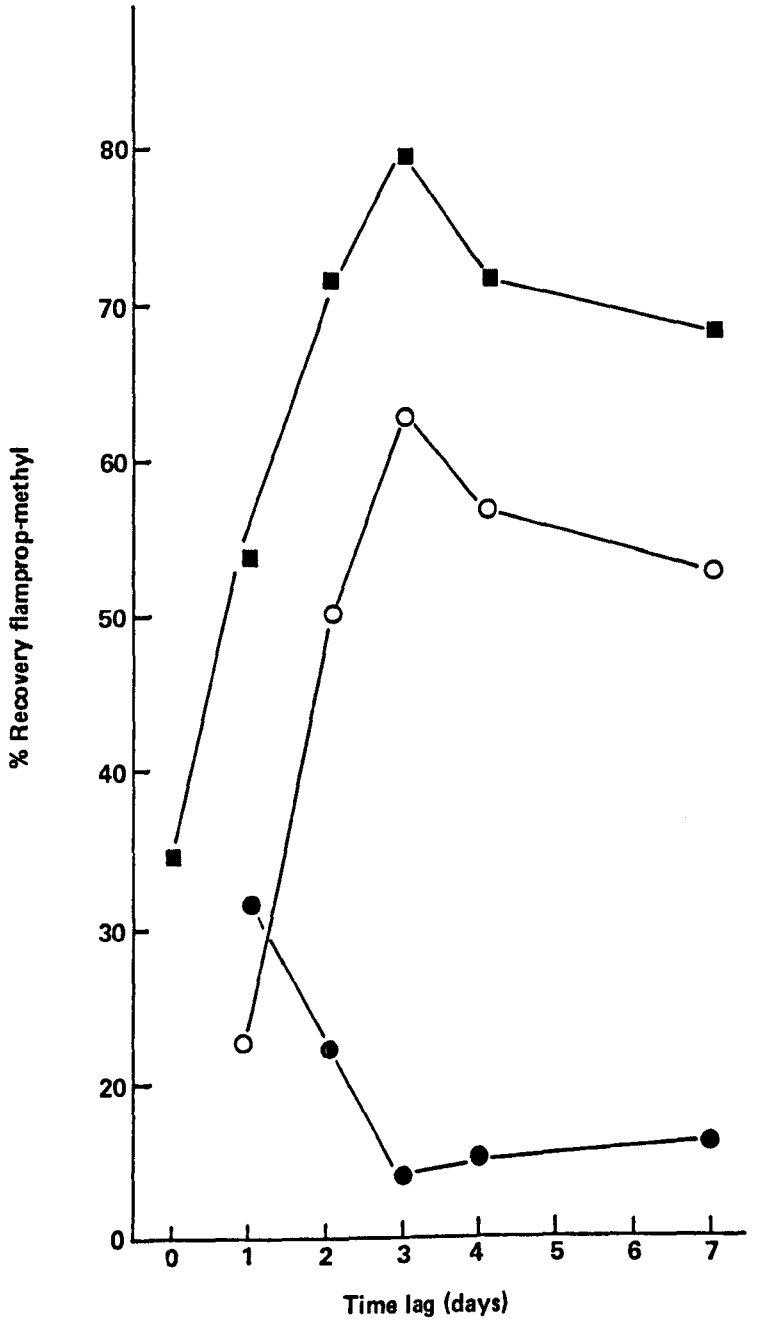


Fig 20 Non-concerted Applications

Recovery of flamprop-methyl in water wash (O—O), hexane/acetone extract (●—●) and combined water wash and hexane acetone extract (■—■) of leaves 24 hours after application of an aqueous solution of the herbicide to the residue left from the prior application of a 100 ppm solution of Triton X 45

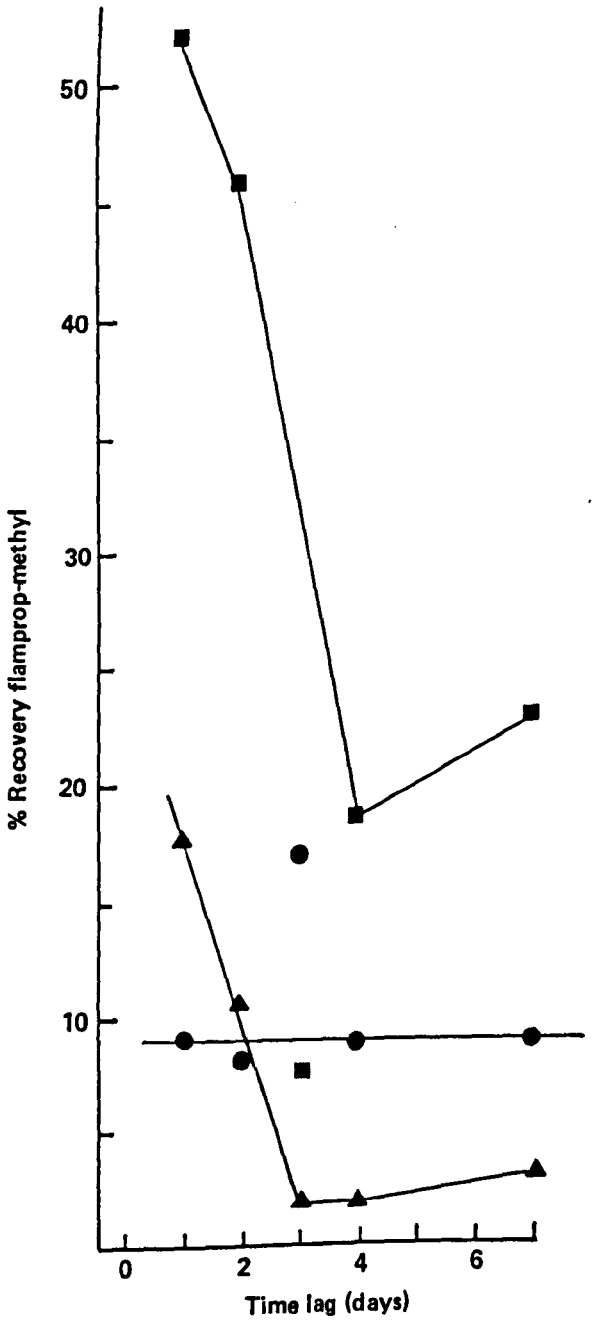


Fig 21 Non-concerted Applications

Recovery of flamprop-methyl in water wash (■—■), second water wash (▲—▲) and hexane/acetone extract (●—●) of leaves 24 hours after application of an aqueous solution of the herbicide to the residue left from the prior application of 10,000 ppm solution of Brij 76

duration of the time lag. With Brij 76 recoveries were distributed between the extracts: Figure 21 shows that as the time lag was increased then uptake was improved, (as shown by the lower recoveries in the water washes).

3.9 Transport

The distribution of radioactivity in plants treated with ^{14}C-flamprop-methyl was investigated 10 days after the application of the herbicide in solutions of Brij 96. Analyses were based on the averaged response of ten replicates for each treatment. Tables 24-26 show the recovery of flamprop-methyl in various extracts of wheat; these data are compared with those found in the absence of a surface active agent, (Table 27). All experiments were performed using comparable plants over a common time frame and are directly comparable. It would appear that the surfactant had little or no effect on translocation of flamprop-methyl out of the treated leaf; the small increase in the level of activity exported was in proportion to the increase in the mass of herbicide moved into the treated leaf. A relatively marked lowering of the activity exported to the lower (first) leaf was found when the herbicide was applied in surfactant formulations.

The redistribution of activity between acropetal, mid and basipetal segments of the treated leaf following application of ^{14}C-flamprop-methyl in a 1% (m/v) Brij 96 solution was studied over a 10 day period. Table 28 shows the absence of appreciable levels of activity in segments basipetal to the area of application.

Table 24 : Distribution of activity in plants after ten days following application of flamprop-methyl formulated in a 100 ppm solution of Brij 96

Extracts	Treated leaf	First leaf	Upper leaves	Stem	Total
	% Recovery[1]				
Surface[2][3]	13.4	0	0	0	13.4
Macerate[3]	64.3	0.2	4.6	4.0	73.1
Combustion[4]	9.3	0.1	0.6	0.9	10.9
Total	87.0	0.3	5.2	4.9	97.4

(1) Each value represents the average of ten plants
(2) Combined water wash and hexane/acetone extract
(3) Attributed to ester from TLC/GLC
(4) Attributed to acid conjugates

Table 25 : Distribution of activity in plants after ten days following application of flamprop-methyl formulated in a 1000 ppm solution of Brij 96

Extracts	Treated leaf	First leaf	Upper leaves	Stem	Total
	% Recovery(1)				
Surface(2)(3)	11.8	0	0	0	11.8
Macerate(3)	59.9	0.1	2.7	3.6	66.3
Combustion(4)	5.9	0.1	0.5	0.7	7.2
Total	77.6	0.2	3.2	4.3	85.3

(1)-(4) retain their meanings from Table 24

Table 26 : Distribution of activity in plants after ten days following application of flamprop-methyl formulated in a 10,000 ppm solution of Brij 96

Extracts	Treated leaf	First leaf	Upper leaves	Stem	Total
	% Recovery(1)				
Surface(2)(3)	15.0	0	0	0	15.0
Macerate(3)	63.9	0.2	2.4	2.5	68.9
Combustion(4)	4.2	0.2	0.5	0.6	5.6
Total	83.1	0.4	2.9	3.1	89.5

(1)-(4) retain their meanings from Table 24

Table27 : Comparison of data from Tables 24-26

Extracts	Surfactant concentration/ppm			
	100	1000	10,000	NIL
	% Recovery			
Surface	13.4	11.8	15.0	42.0
Macerate	73.1	66.3	68.9	43.8
Combustion	10.9	7.2	5.6	7.0
Total	97.4	85.3	89.5	92.8

Table 28 : Redistribution of activity in leaves treated with ^{14}C-flamprop-methyl formulations(1)

Time days	Leaf segment(2)					Total
	A	B	C	D	E	
	% Recovery of applied activity(3)					
1	5.0	3.0	92.0	0.5	0.5	99.0
4	1.0	7.0	90.0	0.5	0.5	99.0
7	15.0	7.0	80.0	0.5	0.5	103.0
10	20.0	11.0	57.0	0.5	0.5	89.0

(1) wheat was treated with flamprop-methyl formulated in a 1% (m/v) solution of Brij 96.

(2) treated leaves were divided into two acropetal segments (A,B) a mid segment (C) and two basipetal segments, (D,E). Application was to segment C.

(3) Activity was recovered by maceration in acetone. Each value was based on the mean of 10 replicates.

4. DISCUSSION AND CONCLUSIONS

Polyoxyethylene non-ionic surface active agents markedly enhance the foliar uptake by cereals of flamprop-methyl. The extent to which uptake is enhanced is largely dependent on the concentration of the formulation with respect to the surfactant, appearing to be independent of toxicant concentration within the limits of the present study, and is less dependent on hydrophobe or hydrophile structure, (especially in dilute surfactant solutions).

4.1 Effect of surfactant concentration

The herbicidal activity of foliar applied herbicide-surfactant formulations has been shown[5] to depend on the amount of surfactant applied and on the concentration of the surfactant in the formulation. Maximum herbicidal activity and toxicant uptake appear to be influenced by the surfactant concentration.[23]

Most prior work[1,45] has shown the influence of surface active agent on the expression of activity, (and penetration of toxicant), to be proportional to surfactant concentration over a relatively wide range. In an investigation of the effect of 34 ethoxylated non-ionic surfactants on the activity of various herbicides on corn and soyabean Jansen[4] found the biological activity of the formulations to increase as the surfactant concentration increased between 100 and 10,000 ppm. Exceptions to the proportionality of herbicidal activity and/or penetration enhancement and surfactant concentration have been identified. Evans and Eckert,[16] investigating various paraquat - surfactant combinations on Bromus tectorum, obtained maximum activity at surfactant concentrations of 600 and 1200 ppm; higher concentrations decreased the

observed activity. Sharma,[47] investigating asulam-octoxynol formulations on wild oats and flax, obtained maximum penetration enhancement at 1300 ppm and found a significant reduction in asulam uptake as the surfactant concentration was increased to 10,000 ppm. Studies with aminotriazole-polyoxyethylene 20 sorbitan monolaurate have also demonstrated a reduction in penetration enhancement as the surfactant concentration was increased above the optimum.[8,22]

Kirkwood et al.,[48] have shown that the surfactant concentration at which herbicidal activity is maximised can be highly specific to the system under consideration: optimum surfactant (tergitol) concentrations were determined at 500 ppm with MCPA and 5000 ppm with MCPB.

In addition to the above studies in which a well defined optimum concentration of the surfactant was established, some studies have reported progressively increasing toxicant uptake as the surfactant concentration was increased up to a point, beyond which no additional effects were noted. Babiker and Duncan[49] found that increasing the concentration of Tween 20 above 1000 ppm enhanced the uptake of asulam to only a negligible extent relative to that already effected. Cook and Duncan[50] found no significant change in the uptake of aminotriazole as the surfactant concentration was varied between 2000 and 10,000 ppm. Other formulations have been shown to exhibit no additional effects above surfactant concentrations of between 1000 and 5000 ppm.[12,51]

Surfactants are generally used in herbicidal formulations at a final concentration between 10 and 1000 ppm,[43] although more concentrated solutions have been used;[45] concentrations of up to 50,000 ppm have been reported[46] as most effective for the phenoxy compounds. The concentrations of the surfactant solutions used in the

present study were typical of levels in commercial formulations and also of the concentrations investigated in previous laboratory studies.

The discussion of the effect of surfactant concentration on the uptake of flamproplmethyl is facilitated by dividing the concentration range studied into three decades, i.e. between 10 and 100 ppm, between 100 and 1000 ppm and between 1000 and 10,000 ppm. The first decade was characterised by a rapid improvement in the rate of uptake of flamprop-methyl with increasing surfactant concentration, as evidenced by Figures 1;8 inclusive and by the data included in Tables 1 and 2 at a surfactant concentration of 100 ppm. The similarities between treatments with different surfactants at 100 ppm were noted above; with the exception of Brij 98 (Table 3) and, to a lesser extent, Brij 35, (Table 2), the Brij and Triton X surfactants showed, within either series, similar abilities at 100 ppm to promote transcuticular movement of flamprop-methyl.

Between surfactant concentrations of 100 and 1000 ppm little or no change in the penetration enhacement established over the first decade was found. The marked reduction in penetration enhancement found with Triton X 305, (Table 1: treatment numbers 24 and 25, and Figure 3), was not typical of the general response of the Triton X compounds which tended to a slight improvement in herbicide uptake over this decade (Table 1). The reduction in penetration enhancement found with Brij 35 was however shown with Brij 76, (Table 2), and Brij 98, (Table 3).

The penetration enhancement of flamprop-methyl affected by Triton X and Brij compounds was not increased by further increasing the surfactant concentration above 1000 ppm, and was, to varying extents, decreased with treatments involving surfactants having a mean polyoxyethylene content

greater than ten moles per mole of hydrophobe. The reduced penetration enhancement from these treatments was seen to depend on the oxyetheylene content of the surfactant and is discussed below.

It can be seen that the uptake of flamprop-methyl following application in solutions of non-ionic surfactants was proportional to the concentration of surfactant in the formulation over only a relatively limited range of concentrations. Penetration enhancement was maximised by the Triton X compounds at, with one exception, concentrations between about 1000 and 5000 ppm, (Table 1). Formulations based on Triton X - 305 were exceptional in showing a maximum of penetration enhancement at surfactant concentrations between 200 and 600 ppm. Formulations based on Brij surfactants showed little significant change in penetration enhancement above 100 ppm, (Table 2). The existence of a plateau region over which surfactant concentration could be increased without effecting uptake was found with the surfactants having low oxyethylene content: the consitent potentiation of foliar uptake by these compounds was apparent from the data in Tables 1 and 2. The surfactant concentration beyond which no additional effects were noted varied from about 100 ppm with Brij 92, (Figure 4), and Brij 30, (Figure 6), to 1000 ppm with Triton X - 45 and Triton X - 114 (Table 2). This analysis suggests that the variety of responses previously identified in the literature can be rationalised in terms of a common response dependent on surfactant concentration and surfactant structure.

4.2 Uptake from concentrated surfactant solutions

The effect of surfactant concentration on the penetration enhancement of flamprop-methyl depends on the oxyethylene content of

the surfactant and to a lesser extent on the nature of the hydrophobe. Suitable selection of these characteristics allows penetration enhancement to be furthered, unaffected or reduced as the surfactant concentration is increased. The mechanism(s) responsible for this variety of responses do not appear to have been identified previously.

It has been suggested[28] that surfactant concentrations above some threshold level may hinder penetration enhancement because of micelle formation. The penetration of the cuticle by the surface active agent may[52] be detrimental to herbicide uptake in impending translocation, and hence transcuticular movement; physiological effects of the surfactant resulting in the disorganization of pathways have also been suggested as an explanation of the existence of a optimum surfactant concentration.

The work of St John and Hilton[53] on the interaction of herbicides with lipids has been discussed in the context of the mechanisms operating in foliar uptake. In that discussion it was noted that the formation of a lipid layer on the leaf surface could prevent the expression of biological activity of lipid soluble toxicants by acting as a reservoir for the compound, external to the leaf surface. We have shown that the foliar uptake by cereals of Triton X polyoxyethylene surfactants is related to the mean oxyethylene content of the surfactants such that relatively rapid adsorption is found for the compounds with a low oxyethylene content. When the mean oxyethylene content exceeded 10 moles per mole of hydrophobe, application of 100 µg of the surfactant resulted in a surface residue of between 50 µg and 100 µg persisting over up to 48 hours after treatment. This observation suggests a mechanism for the detrimental effect of concentrated surfactant solutions on penetration enhancement; by forming a persistent layer on the

leaf surface the residue of surfactant might inhibit herbicide uptake, acting as a resistance to uptake in series to the resistance offered by the modified external leaf structure. It would be expected that the reduction in penetration enhancement in concentrated surfactant solutions would be proportional to the magnitude of the surfactant residue and that in the absence of a surfactant residue penetration enhancement would not be hindered.

Figure 22 shows the foliar uptake of flamprop-methyl, following the application of the herbicide in 10,000 ppm aqueous Triton X solutions, (data from Table 1), as a function of the surface residue of the surfactant. It can be seen that an excellent correlation exists between the surfactants that were rapidly absorbed by the leaf and the surfactants that did not inhibit penetration enhancement; the consistent potentiation of the foliar uptake of flamprop-methyl achieved with formulations based on the Triton X - compounds 15, 35, 45 and 114 was well correlated with the total adsorption of these surfactants within 24 hours. The relationship between the magnitude of the surfactant residue and the reduction in penetration enhancement was less clear when the deposit persisted for longer periods. The magnitude of the surfactant residue increased as the mean oxyethylene content of the surfactant varied between 10 and 70 moles, whereas herbicide uptake showed a significant minimum at a mean oxyethylene content of 16 moles. St. John and Hilton[53] have shown that the ability of a surface deposit to reduce uptake will be influenced by the chemical nature of the deposit; in the present context two points appear worthy of note: i), when evaluated in molar terms the surface residue of surfactant decreases as the mean oxyethylene content varied between 10 and 70 moles,

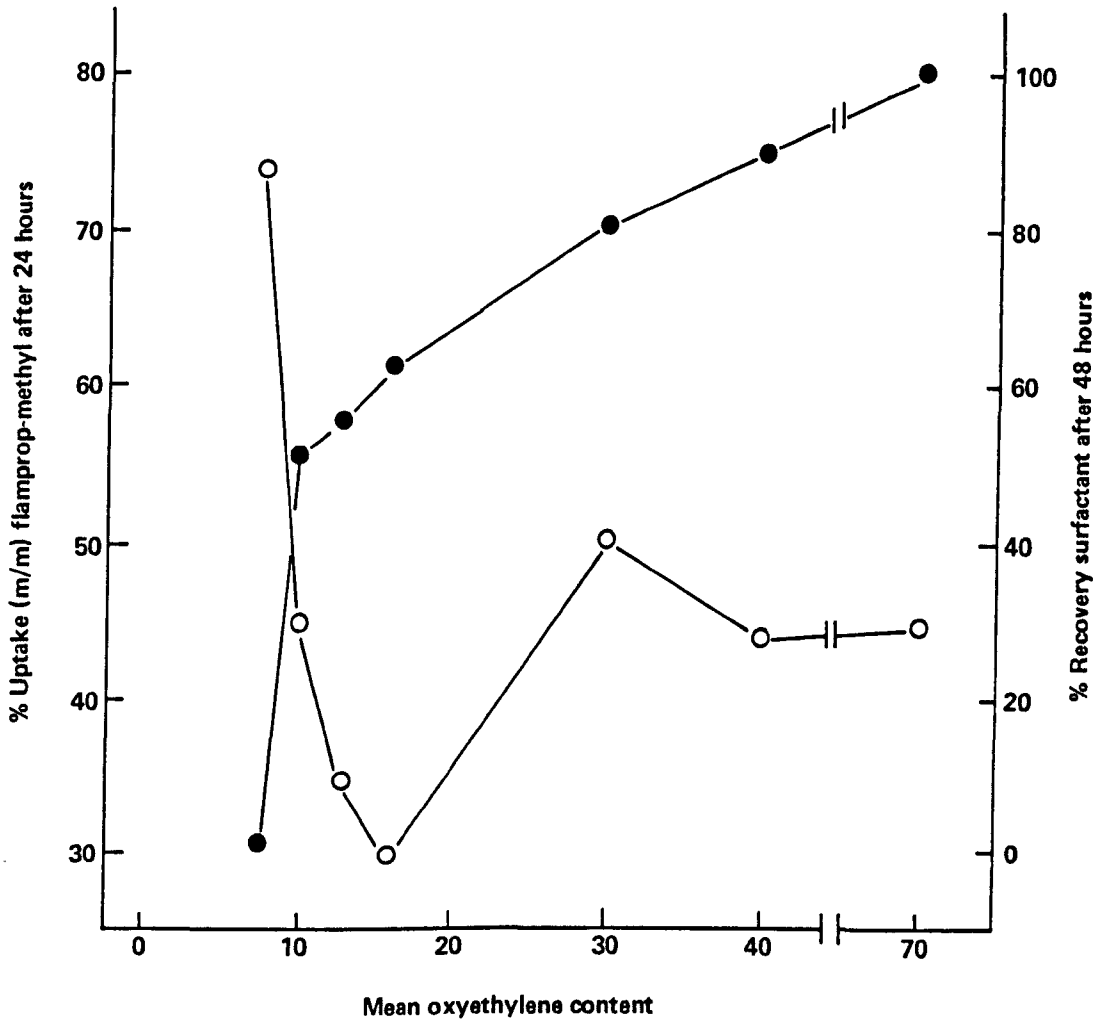


Fig 22 Foliar uptake of flamprop-methyl 24 hours after treatment with the herbicide formulated in 10,000 ppm aqueous Triton X solutions (O—O) and recovery of surfactant from the leaf surface 48 hours after the application of 10,000 ppm aqueous Triton X solutions (●—●) as a function of the mean oxyethylene content of the surfactant

and

ii), the lipophilicity of the surfactant residue decreases as the oxyethylene content of the surfactant increases.

Thus the complex form of the uptake curve (Figure 22) can arise as a consequence of interaction between these conflicting trends. In general the form of this curve would be expected to be dependent on the character of the herbicide and the surfactant and on the tendency for the surfactant to be absorbed by the species under consideration. Although no significant differences between wheat and wild oat were found in the penetration enhancement of flamprop-methyl by concentrated surfactant solutions, (Tables 17 and 18), the effect of species might be of importance if the comparison was between more dissimilar species.

If the reduction in penetration enhancement with concentrated surfactant solutions occurred as a consequence of a persistant surface deposit of the surfactant, then factors which served to reduce this residue should result in increased uptake. Earlier studies have shown the progressive depletion of the surface residue of Triton X - compounds, following foliar application of aqueous surfactant solutions, over a period of seven days. Reduction in penetration enhancement would be expected to diminish with time as a result of the progressive depletion of the surfactant residue. This postulate was investigated by introducing a time lag between application of the surfactant and flamprop-methyl to allow prior redistribution of the surfactant residue. The interpretation of these results was complicated by the adverse effect of a time lag on the performance of the treatments at or near optimum surfactant concentrations. Thus the reduction in penetration enhancement, relative to concerted treatments, found to occur when a 24 hour time lag was

used to separate application of 100 ppm solutions of Triton X - 15, (Table 21), Triton X - 45, (Figure 20), and Brij 96 (Table 20) suggested that the prior redistribution of surfactant could diminish the mechanism by which uptake was potentiated. The results obtained with treatments based on 10,000 ppm Triton X - 305 solutions showed a marked reduction in the potentiation of uptake relative to concerted application which was independent of the duration of the time lag. When this experiment was performed with Brij 76 uptake was not adversely effected by a 24 hour time lag and was seen to improve as the time lag increased up to seven days (Figure 21).

4.3 Effect of surfactant structure

In general it has been shown[45] that one ionogenic class of surface active agents is not greatly superior over another as far as biological enhancement of herbicides is concerned but great variation within individual classes has been demonstrated. Studies[23] on structure - activity relationships between herbicides and homologous series of surfactants have indicated that definite relationships exist between the herbicide and surfactant structure for maximum herbicide penetration. Structure variation in anionic and cationic surface active compounds has been discussed by Jansen.[54] For the ethoxylated non-ionic surfactants structural variation can be considered in terms of the length of the hydrophobic and hydrophilic molecular chains, the nature of the hydrophobe and the type of linkage involved, viz., ether or ester.

Jansen,[4,29] in an investigation of the relationship of the structure of ethoxylated, (ether type), non-ionic surfactants to the herbicidal activity of water soluble herbicides (Amitrole, Dalapon,

2,4-D, and DNBP) found that the variability in effectiveness at a surfactant concentration of 10,000 ppm was associated with differences in the chemical structure of both the hydrophilic and hydrophobic moieties. Much of this variability was related to the differences in the hydrophiles, viz., the mean oxyethylene contents, but was influenced by the hydrophobic moiety and also varied with the plant species and herbicide. Specific intermolecular relationships between the herbicide and surfactant had been suggested by previous workers, e.g., by Freed and Montgomery[11,40] investigating Amitrole - surfactant combinations, and by Hughes and Freed[13] working with IAA; in these studies the degree of interaction was shown to be markedly influenced by the chemical nature of the surfactant used. The work by Jansen extended the scope of the interaction to include the plant species, and emphasized the specificity of the herbicide - surfactant - plant interaction. Smith[55] evaluated structure - activity relationships for Amitrole, Dalapon and Paraquat - surfactant combinations; maximum activity was influenced by the oxyethylene content but this relationship was seen to depend on the concentration of the surface active agent. In support of the conclusion reached by Jansen, variations in the hydrophobe were found to be less important in influencing the phytotoxicity of the herbicide. Studies[5] using herbicide with low water solubilities have also demonstrated a similar relationship between the activity of the herbicide and the structure of surfactants incorporated into the spray solution.

a. hydrophobe effects

Variation in the chain length of the hydrophobe had little effect on the uptake of flamprop-methyl within the surfactant types included

in the present study. Figure 14 shows the uptake of flamprop-methyl over seven days following application of formulations in 100 ppm aqueous solutions of alkylphenol ethoxylates. It can be seen that, at a constant oxyethylene content, variation in the alkyl group between C8 and C12 had no significant effect on uptake. With the alkyl ethoxylates uptake was in some instances seen to be influenced by hydrophobe nature at relatively high concentrations. Table 2 shows that the Brij compounds 96, 56, and 76 effected similar penetration enhancement at 100 ppm but differed in the extent to which this was maintained at higher concentrations. At surfactant concentrations of 10,000 ppm penetration enhancement was maintained with Brij 96 (92% uptake), was reduced with Brij 56, (73.5%), and was very much lessened with Brij 76, (32%). This difference was somewhat surprising in view of the close structural similarity of these surfactants; Brij 96 and Brij 76 are based on the C18 hydrocarbon which is saturated in Brij 76 and is derived predominantly from octadec-9-en-1-ol with Brij 96. Uptake from formulations based on the unsaturated hydrophobe was not generally different from other treatments when comparison was made at different but common oxyethylene contents. Thus, Brij 92 and Brij 30 (derived from dodecan-1-ol) showed no difference in enhancing the foliar uptake of flamprop-methyl over the range of surfactant concentrations studied, as did Brij 98 and Brij 35.

Although the Brij compounds tended to enhance herbicide uptake to a greater extent than was generally achieved with the Triton X compounds the difference between alkyl and alkylaryl hydrophobes was less than that found with other herbicide - surfactant treatments; in an investigation of DNBP - surfactant treatments on corn and soybean Jansen[29] found that surfactants derived from monohydric alcohols enhanced biological activity but that alkylphenol ethoxylates had no effect.

b. hydrophile effects

Previous studies[23] of biological effect following foliar application of herbicides formulated with polyoxyethylene non-ionic surface active agents have shown a maxima in toxicity enhancement with increasing mean oxyethylene content, beyond which the effect of the surfactant was diminished. Valkenburg and Yapel Jr.,[7] have suggested that the existence of an optimum oxyethylene content may be interpreted in terms of an optimum hydrophile - lipophile balance (HLB). The HLB system of surfactant characterisation is related to the solubility of the surfactant in that at high HLB values the compounds are very water soluble and at low HLB values lipid solubility is maximised. Because HLB is related to solubilities it can also be equated with partition coefficients. A high HLB can be equated with a large water-lipid partition co-efficient. Valkenburg and Yapel Jr. suggest that an optimum HLB implies an optimum partition coefficient for activity enhancement.

Behrens[56] and Hull[45] have suggested that for each herbicide - formulation - species combination there is an optimum HLB requirement for the surfactant in the formulation and that this is unchanged if different surfactant types are used, although the maximum level of efficiency achieved may vary. Atlas Chemical Industries have reported a HLB between 9 and 10 as giving the greatest increase in the activity of Dalapon, DNBP and Diuron. This generalization is not supported by the results obtained by Jansen;[29] herbicide activity and surfactant toxicity were seen to be more influenced by chemical type than by the HLB. The results obtained with flamprop-methyl formulations also suggested the absence of a strong correlation between penetration enhancement and HLB, e.g. formulations based on Brij 96 and Brij 76

were markedly dissimilar in performance at all concentrations above 100 ppm (Table 2) although these surfactants have a common HLB of 12.4. Penetration enhancement by Brij 92 and Brij 30 was identical at all concentrations (Table 2); the HLB values of these surfactants are 4.9 and 9.7 respectively.

Returning to the effect of oxyethylene content per se, it was apparent that with formulations based on the Brij surfactants penetration enhancement was reduced when the oxyethylene content was about 20 moles. This effect can be seen in comparisons between surfactants having a common hydrophobe, i.e. between the Brij compounds 92, 96 and 98, and between 30 and 35 (Tables 2 and 3). Unlike the reduction in the penetration enhancement effected by certain Triton X compounds at high surfactant concentrations, the reduced performance of Brij 35 and Brij 98 was seen at all concentrations up to 10,000 ppm. The effect was concentration dependent with Brij 35 however and in this was comparable to that seen with Triton X adjuvants.

Figure 23 shows the uptake by wheat of flamprop-methyl over 24 hours following application of the herbicide in aqueous Triton X solutions with surfactant concentrations of 100 and 1000 ppm. At 100 ppm little effect of oxyethylene content is discernible; based on mean values the optimum oxyethylene content would appear to be between 10 and 30 moles. At 1000 ppm it was apparent that over 24 hours herbicide uptake was potentiated by surfactants having an oxyethylene content at or below 10 moles.

Figure 24 shows the uptake by wheat of flamprop-methyl over 24 hours following application in 5000 ppm aqueous Triton X solutions. It can be seen that penetration enhancement was apparently markedly dependent on

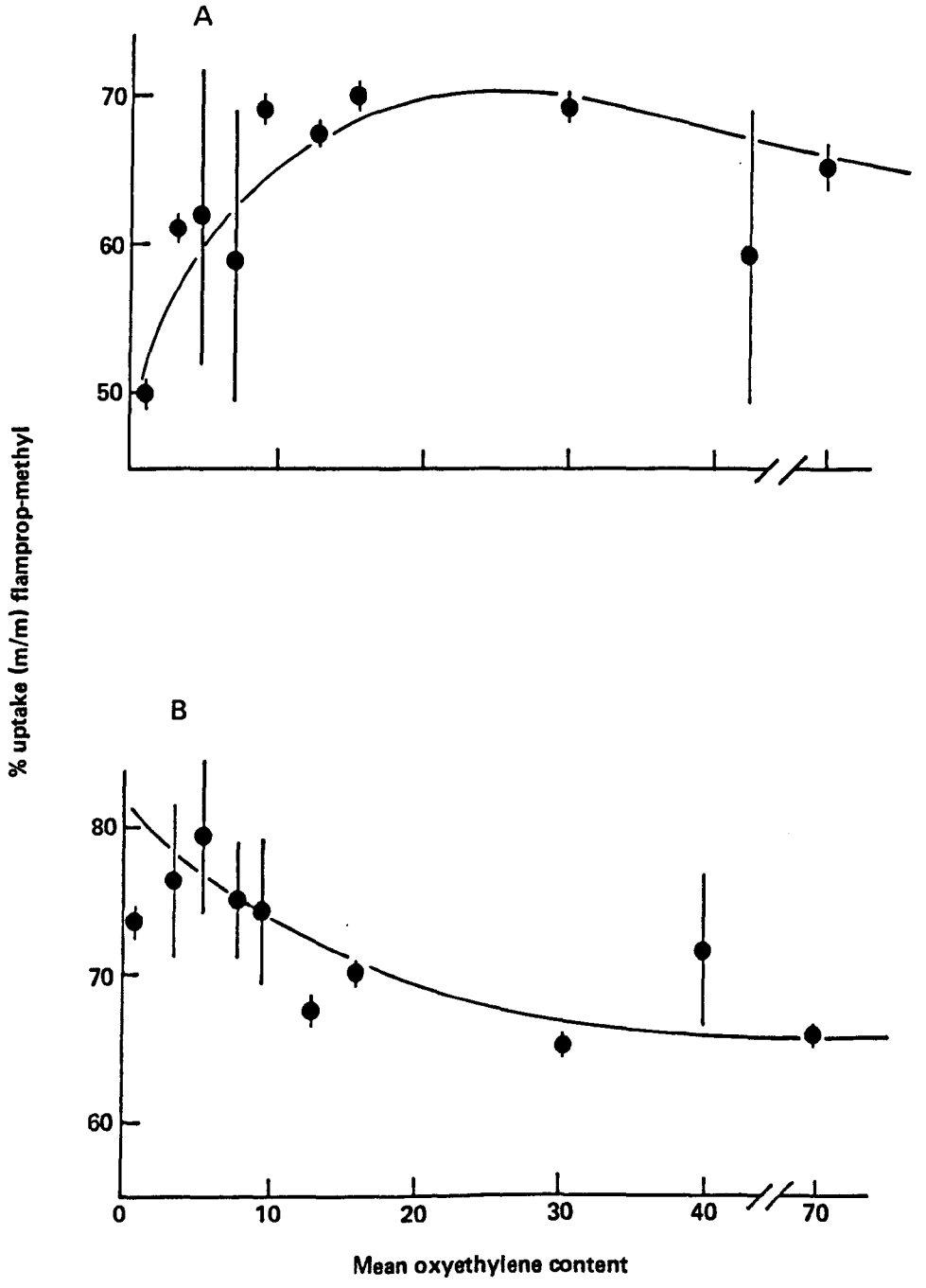


Fig 23 Foliar uptake by wheat of flamprop-methyl 24 hours after application of the herbicide formulated in (A) 100 ppm and (B) 1000 ppm aqueous Triton X solutions as a function of the mean oxyethylene content of the surfactant. Vertical bars show variation about mean values (4 reps.)

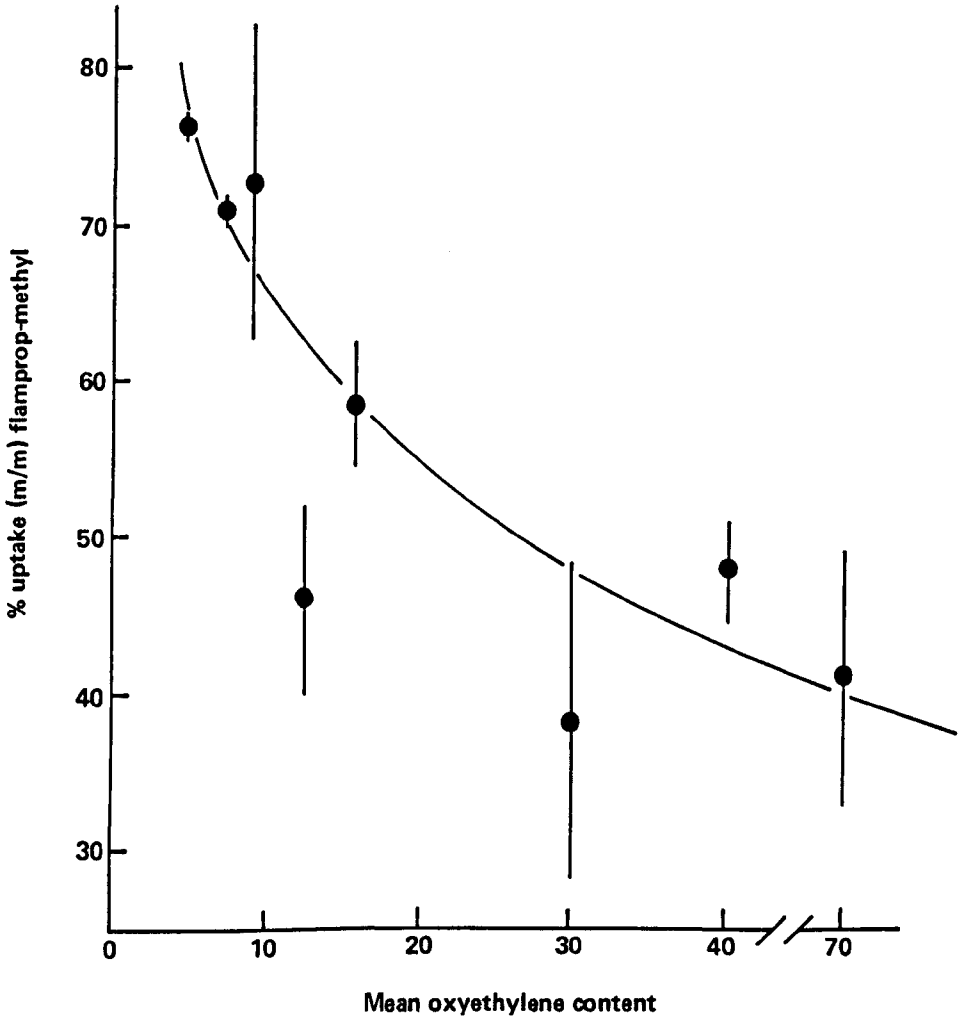


Fig 24 Foliar uptake by wheat of flamprop-methyl 24 hours after application of the herbicide formulated in 5000 ppm aqueous Triton X solutions as a function of the mean oxyethylene content of the surfactant. Vertical bars show variation about mean values (4 reps.)

oxyethylene content and was maximised at or below 10 moles. A similar result was also obtained at a surfactant concentration of 10,000 ppm (Figure 25). These results parallel those reported in many of the studies alluded to earlier, both in terms of optimum oxyethylene content (5-20 moles) and in the tendency for this optimum to move to lower oxyethylene contents as the concentration of the surfactant was increased. This dependency on concentration was also apparent when uptake was compared between formulations at equimolar surfactant concentrations (so eliminating any differential effect from the hydrophobe): Figure 26 shows that as the molarity of the surfactant solutions was increased then (i), the variation in herbicide uptake with oxyethylene content was more marked and (ii), the optimum oxyethylene content was reduced from about 16 moles (at $0.4.10^{-3}$ moles.dm^{-3}) to about 5 moles (at 3.10^{-3} moles.dm^{-3}).

4.4 Effect of surface tension, interfacial tension and the wetting properties of the surfactant

Perhaps the best known property of surface active agents is their effect on the surface and interfacial tensions of solvents.[3] The magnitude of this effect is intimately related to the structure and concentration of the surfactant, and has been used to explain the relationship between structure/concentration and penetration enhancement in terms of improved contact, greater effective area and stomatal infiltration. The general concensus of published opinion however, suggests that the capacity of these compounds to lower surface tension, and their ability to increase wetability as determined by contact angle measurements, cannot fully explain surfactant potentiation of foliar uptake save in exceptional cases.[1,4,12,15,23,29,54] The maximum

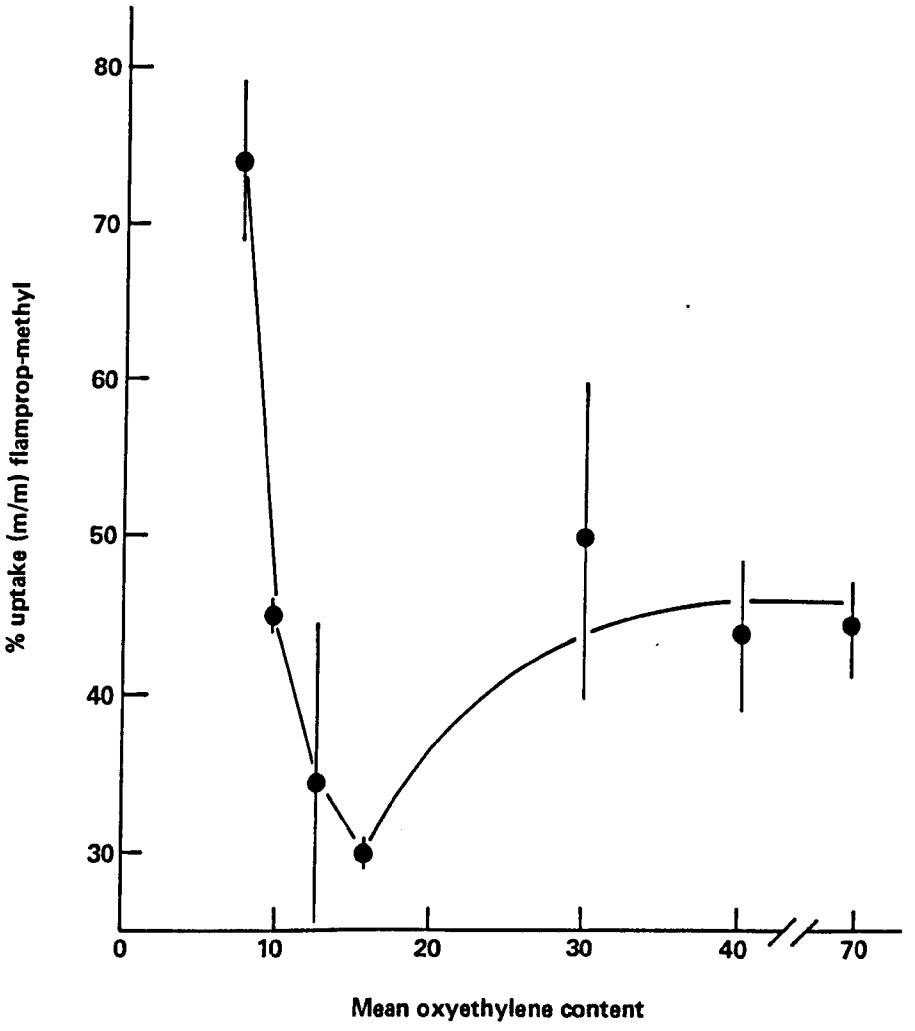


Fig 25 Foliar uptake by wheat of flamprop-methyl 24 hours after application of the herbicide formulated in 10,000 ppm aqueous Triton X solutions as a function of the mean oxyethylene content of the surfactant. Vertical bars show variation about mean values (4 reps.)

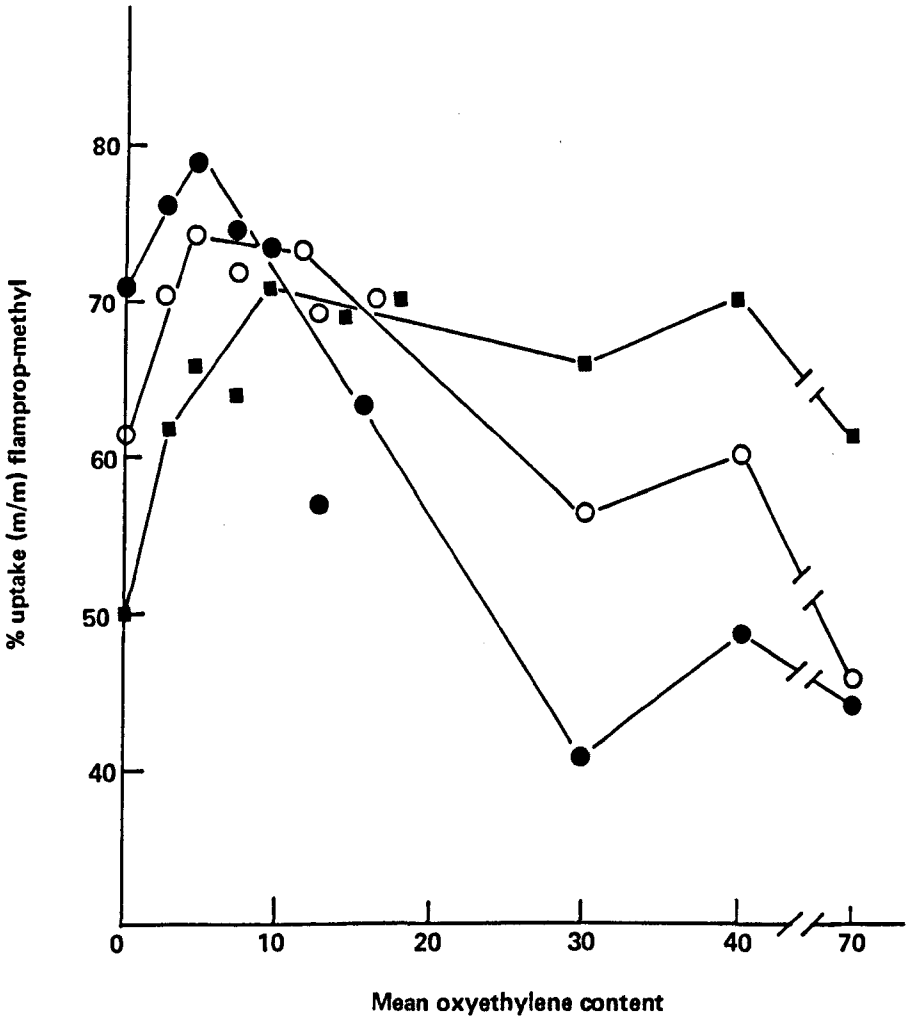


Fig 26 Foliar uptake by wheat of flamprop-methyl 24 hours after application of the herbicide formulated in 0.4 x 10^{-3} mole dm^{-3} (■—■), 1 x 10^{-3} mole.dm^{-3} (○—○) and 3 x 10^{-3} mole.dm^{-3} (●—●) equimolar solutions of the Triton X surfactants as a function of the mean oxyethylene content of the surfactant.

expression of the lowering of surface tension is attained at low surfactant concentrations; though the effective concentration varies somewhat between preparations it is commonly[28] between 10 and 1000 ppm. An important consideration is that at higher concentrations the surface tension remains more or less constant. According to Jansen[14] surfactants have been found to exhibit their greatest effects on the biological activity of pesticides at concentrations greater than 1000 ppm, and one must therefore conclude that these biological effects are better correlated with other properties than with surface tension changes. It has also been reported,[11,13,15,18,40] that herbicide solutions with similar surface tensions but containing different surfactants can show marked variation in penetration enhancement.

Figures 27 and 28 show the uptake by wheat of flamprop-methyl as a function of the surface tension of the applied solution for Triton X and Brij surfactant solutions respectively. It can be seen that there is little or no correlation discernible between penetration enhancement and surface tension for either surfactant series and that solutions at similar surface tensions could promote uptake to very different extents. Similar lack of correlation was obtained when uptake was considered as a function of the surface tension at the CMC or the limiting surface tension of the surfactants.

It has been suggested[51] that the surface tension of a solution could not be expected, on theoretical grounds, to be an entirely reliable index of the ability of a solution to wet a surface as it takes no account of the nature of the surface or the solution/surface interface. From this one might expect better correlation between penetration enhancement and interfacial tension, with the proviso that

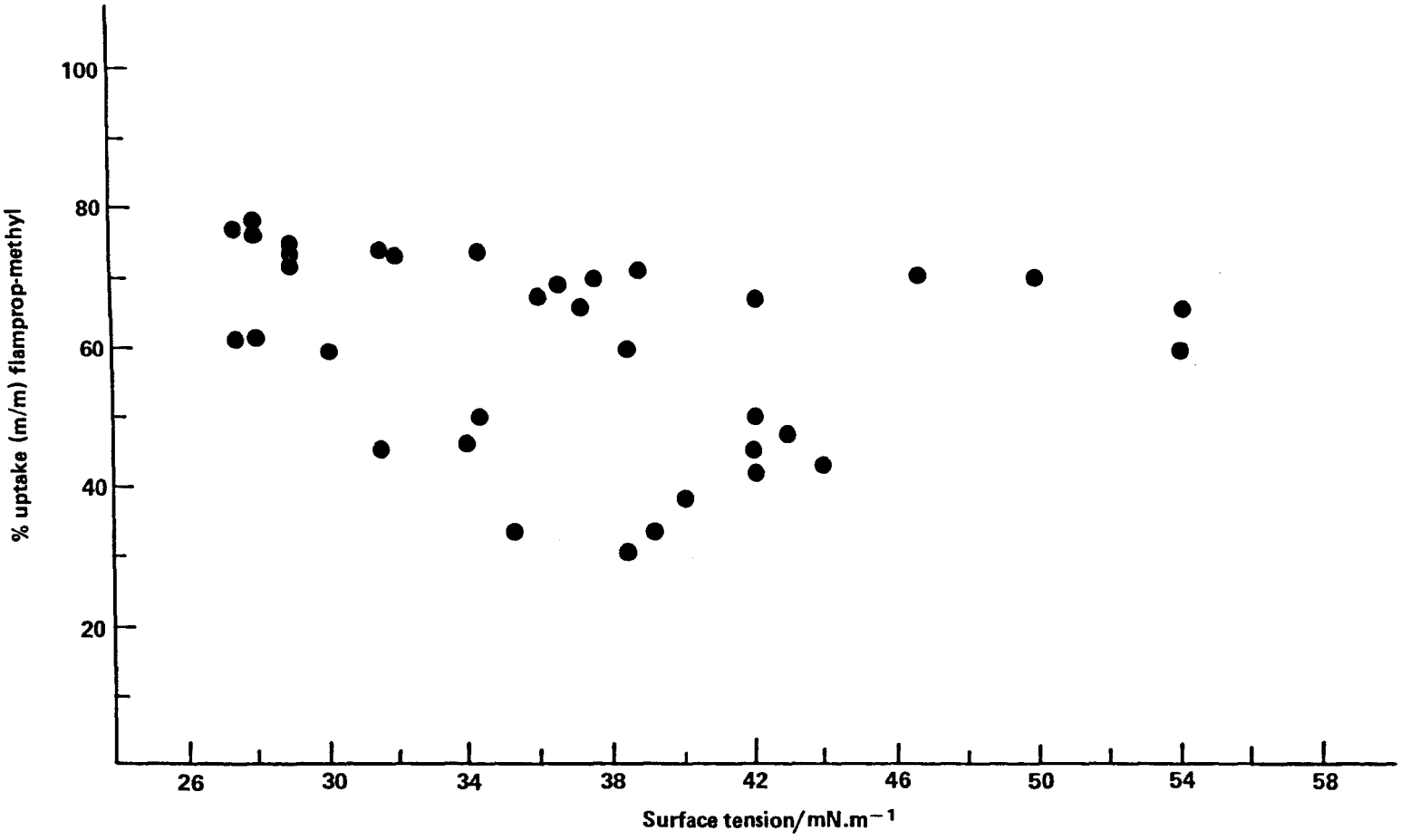


Fig 27 Foliar uptake by wheat of flamprop-methyl after application of the herbicide formulated in aqueous solutions of the Triton X surfactants as a function of the surface tension of the formulation

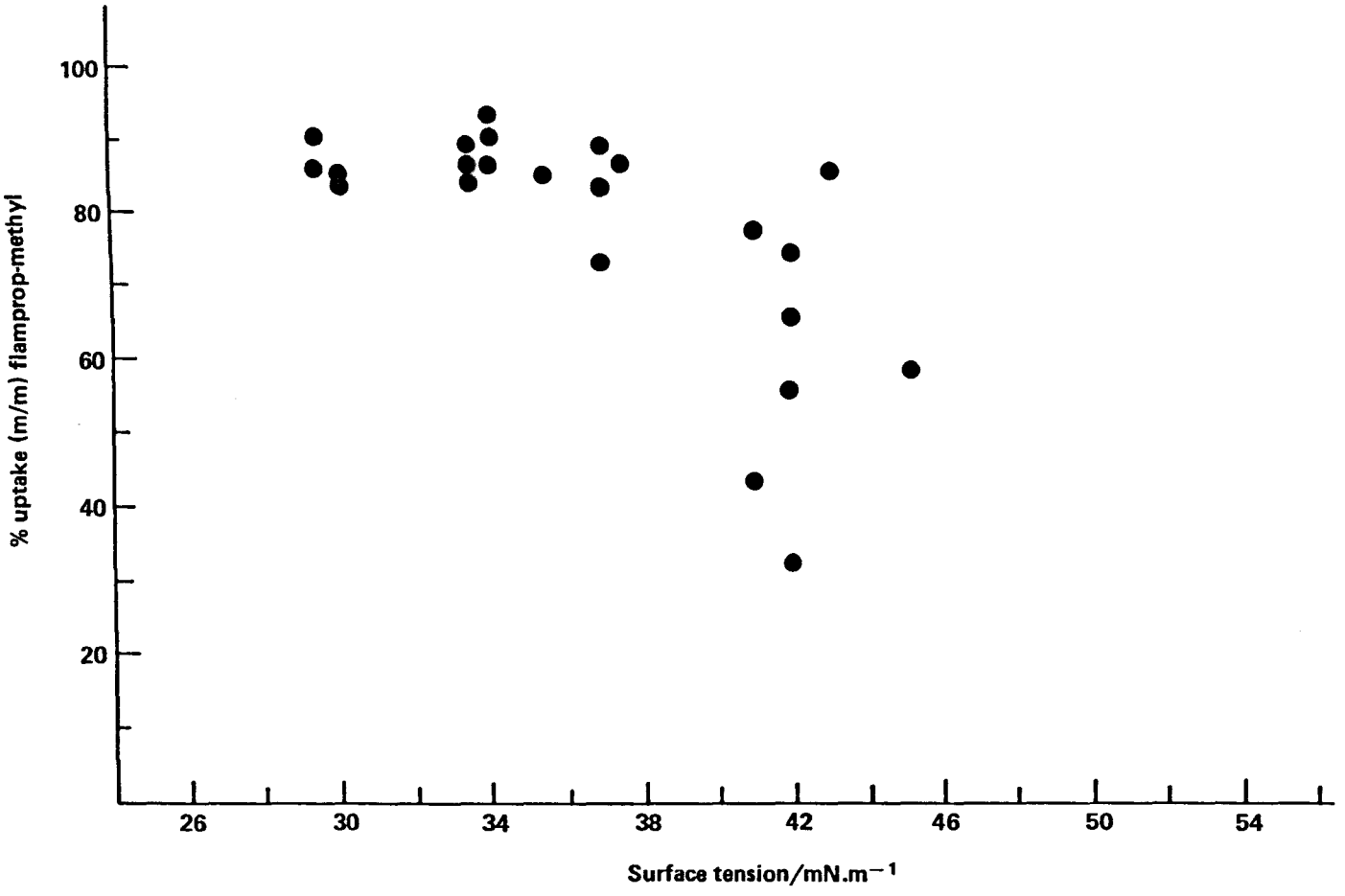


Fig 28 Foliar uptake by wheat of flamprop-methyl after application of the herbicide formulated in aqueous solutions of the Brij surfactants as a function of the surface tension of the formulation

The experimentally determined value of this property is known to be highly dependent on whether the surfactant is initially dissolved in the aqueous or oil phase, on the time allowed for the establishment of equilibrium between the two phases, and on the choice of the oil phase. The wetting properties of surfactant solutions can also be considered in terms of such parameters as the contact angle, the spreading coefficient, or a specific test such as the Draves test. Jansen[4] considered no single physical property to prove adequate for indexing the spreading/wetting characteristics of pesticidal sprays. Both the contact angle and the spreading coefficient have been reported[13,15,57] as showing little or no correlation with toxicity enhancement, although Sands and Bachelard[51] investigating the uptake of Picloram by Eucalypt leaf discs, found uptake to be well correlated with contact angle both when different surfactant types were used and when the surfactant concentration was varied. Becher and Becher[58] have suggested that herbicidal activity can be related to both the contact angle and the surface tension by plotting the spreading pressure (π), and activity index vs. HLB. The relationship between π and the contact angle can be expressed as $\pi = \gamma_L \cos \theta$ where γ_L is the surface tension of the liquid and θ the contact angle between the liquid and a solid surface. Becher and Becher demonstrated that surfactants in their series which exhibited maximum π corresponded to those adjuvants of similar HLB which Jansen[4] et al., had shown to optimise herbicidal activity.

Figure 29 shows the uptake by wheat of flamprop-methyl as a function of the interfacial tension of the applied solution measured against toluene and the highly aliphatic Acroprime 90. The determination of

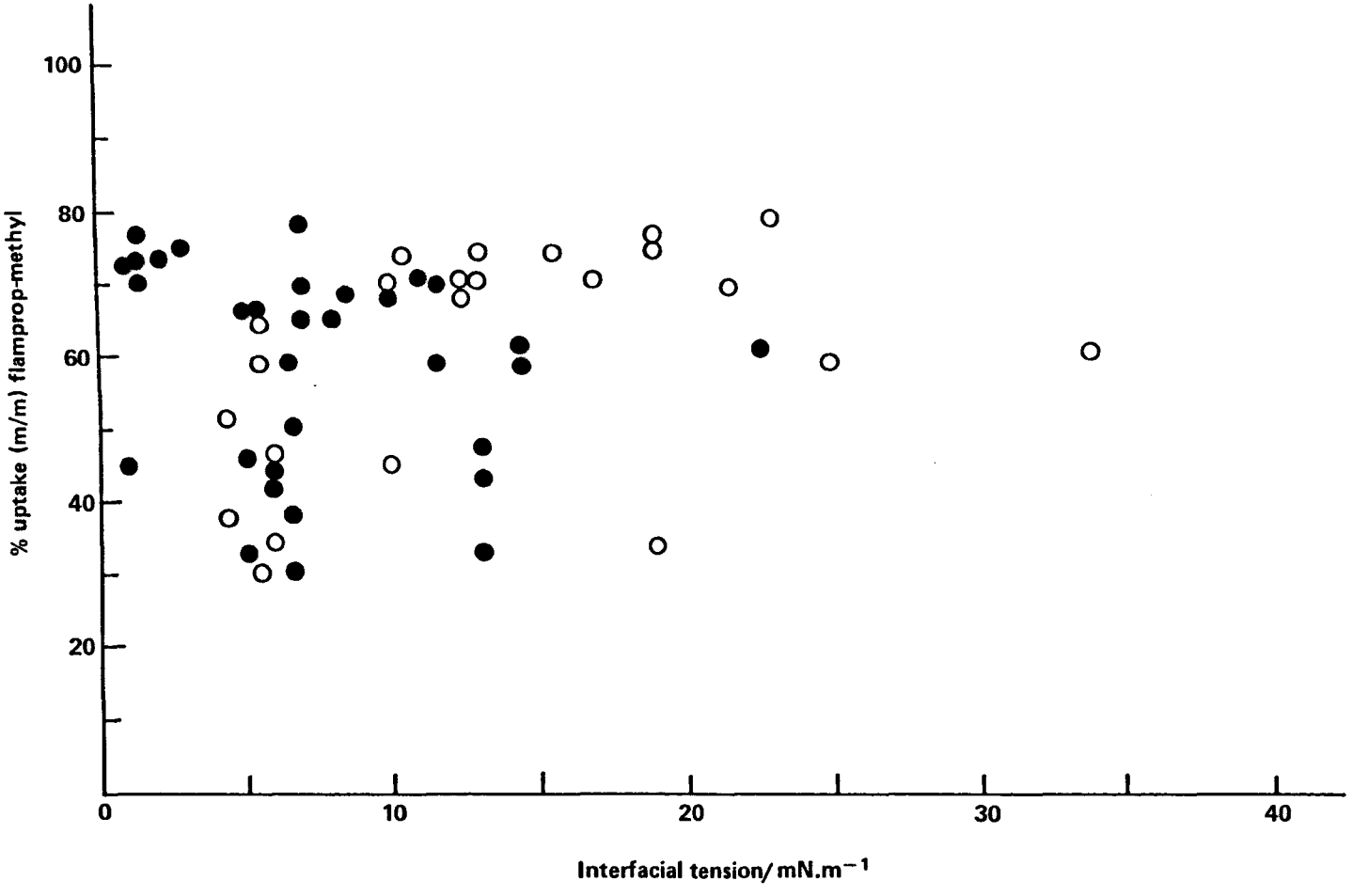


Fig 29

Foliar uptake by wheat of flamprop-methyl after application of the herbicide formulated in aqueous solutions of the Triton X surfactants as a function of the interfacial tension measured between the formulation and either Acroprime 90 (●—●) or toluene (○—○)

these parameters was considered earlier, when it was noted that the data obtained with Acroprime 90 are similar to that reported by Crook et al.,[59] using iso-octane. It can be seen that correlations were non-existent between these interfacial tensions and the penetration enhancement of flamprop-methyl.

Figure 30 shows flamprop-methyl uptake as a function of the spreading coefficient of the surfactant solutions on either toluene or Acroprime 90. Again little or no correlation is obvious from this data. Correlations between the wetting power of the surfactant solution as measured by the Draves test and penetration enhancement were also non-existent.

4.5 Uptake from short chain alcohol solutions

A series of aqueous solutions of flamprop-methyl were formulated containing various short chain (C1-C6) alcohols. The surface tension of aqueous solutions of the alcohols is shown in Figure 31: foliar uptake from these solutions was found to be greatest from a saturated aqueous solution of hexan-1-ol, which can be seen to have a surface tension about 30 $mN.m^{-1}$. This solution effected at least comparable wetting on cereal leaves to that achieved by the most surface active surfactants studied. Figure 32 shows the foliar uptake by wheat of flamprop-methyl following application of an aqueous solution saturated with hexan-1-ol. It can be seen that in the presence of the alcohol uptake was about 40% (m/m) over 24 hours; although this rate of uptake was superior to that found with aqueous herbicide solutions it was considerably less than that achieved with many of the surfactants investigated, despite the fact that many of these surfactants were considerably less efficient in wetting the leaf surface.

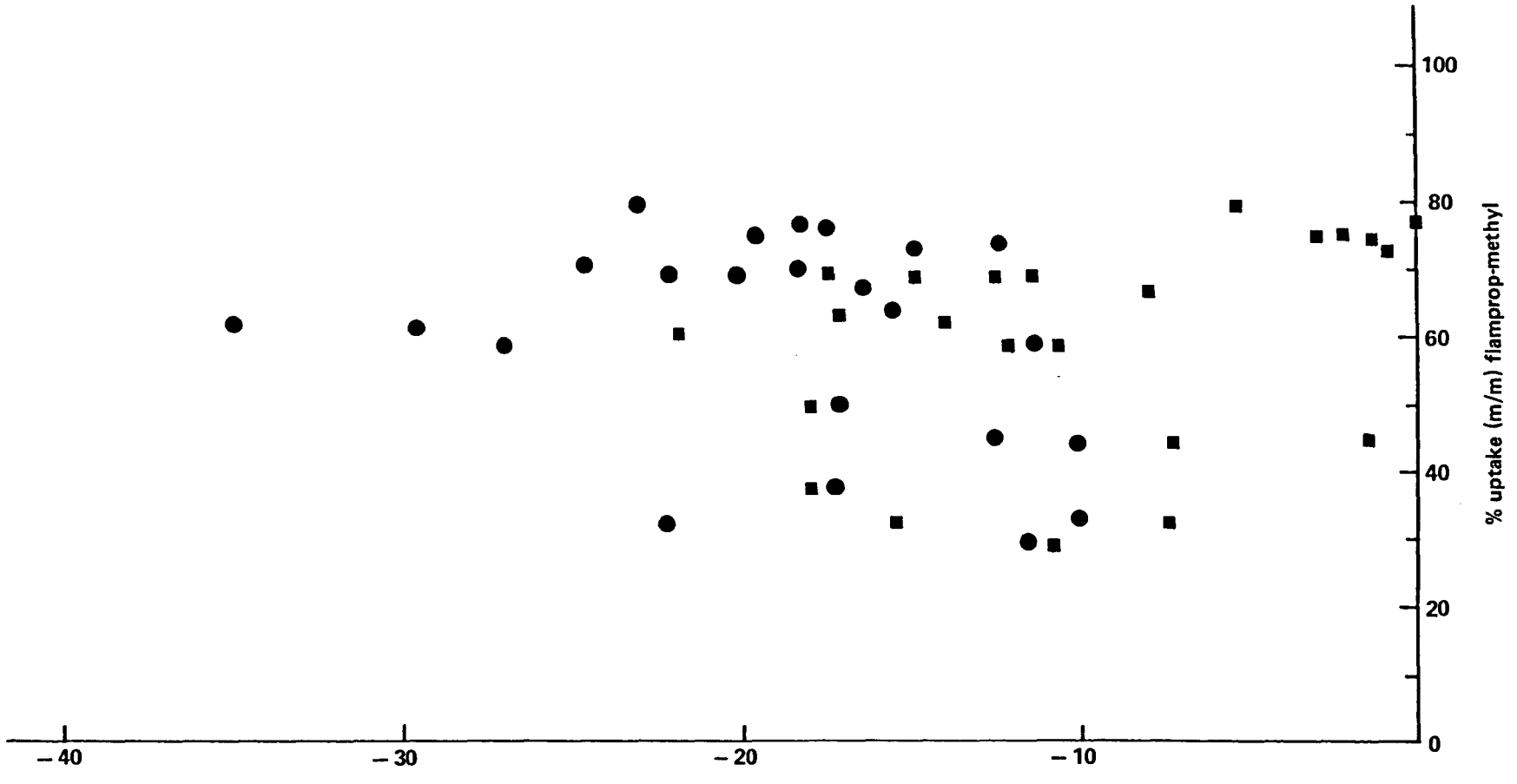


Fig 30

Foliar uptake by wheat of flamprop-methyl after application of the herbicide formulated in aqueous solutions of the Triton X surfactants as a function of the spreading coefficient between the surfactant solution and either Acroprime 90 (■—■) or toluene (●—●)

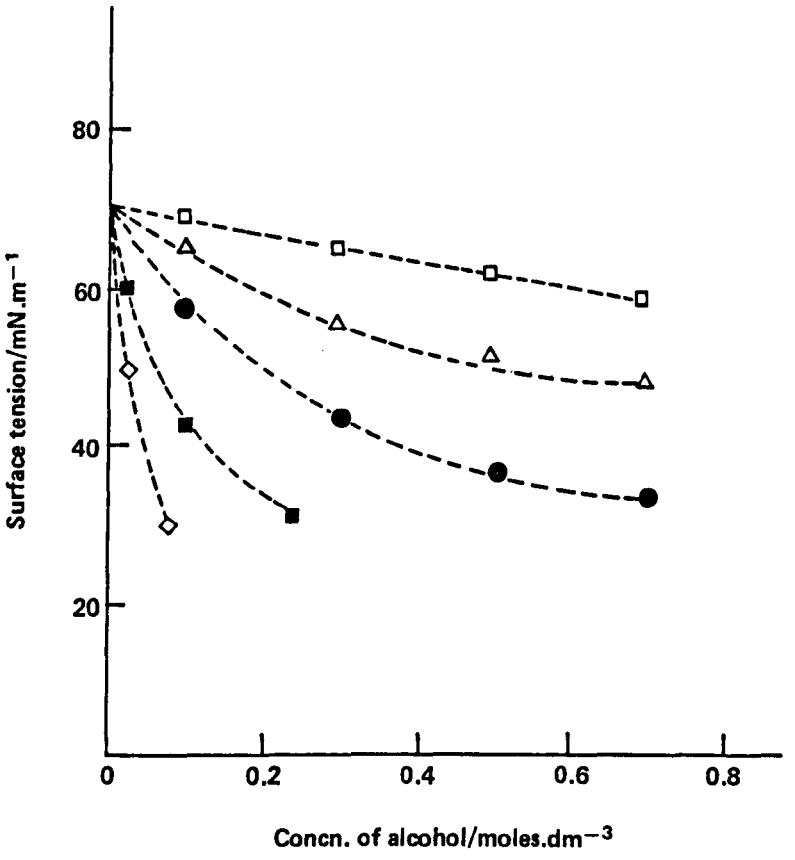


Fig 31

Surface tension (Du Nuoy) of aqueous solutions of alcohols

□ Ethanol
△ Propan–1–ol
● Butan–1–ol
■ Pentan–1–ol
◇ Hexan–1–ol

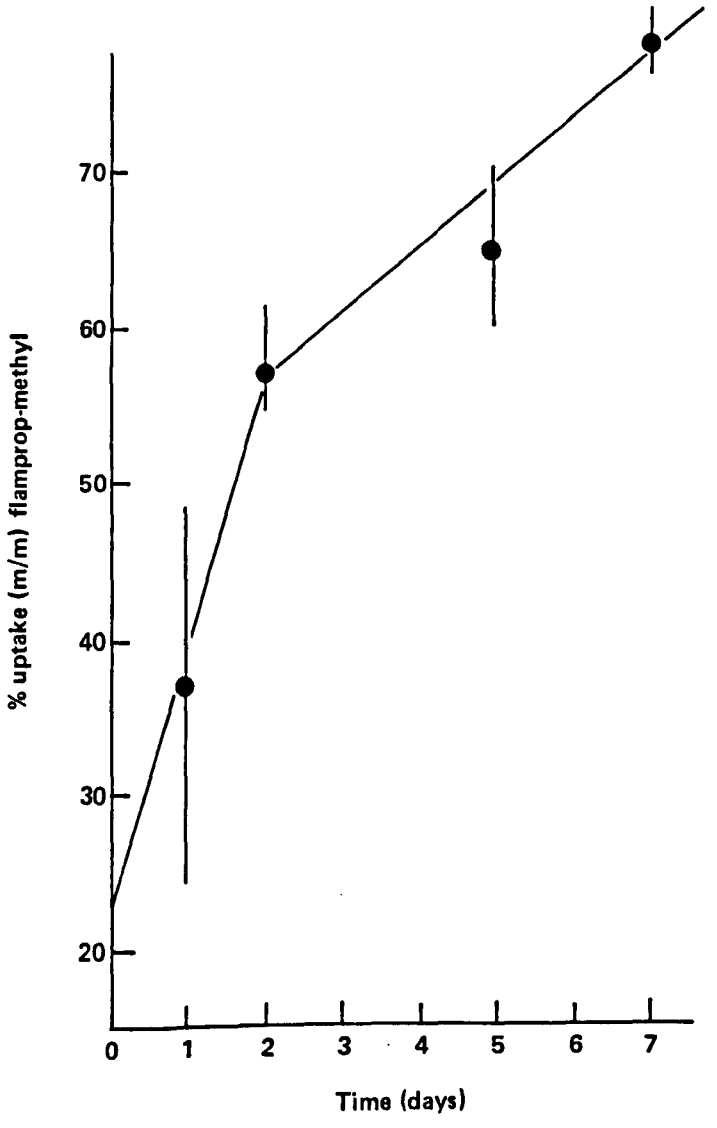


Fig 32 Foliar uptake by wheat of flamprop-methyl after application of the herbicide formulated in a saturated aqueous solution of hexan-1-ol.
Vertical bars show variation about mean values (5 reps.)

4.6 Non concerted applications and leaf wetting

In the recovery of the drop deposit from treated leaves it was observed that the treated area of leaf was altered in its wettability so that subsequent application of distilled water to that area resulted in a degree of wetting comparable to that achieved by the original treatment. This phenomenon was also observed when a time lag was introduced between the application of the surfactant solution and the herbicide solution, the latter spreading over the area wetted by the former. As a consequence, comparison of concerted versus separated application was at a constant level of wetting/spreading for any one treatment. The marked difference in effectiveness between the application methods, noted earlier, reflects the small contribution of improved contact to the enhancement of the foliar uptake of flamprop-methyl by surfactants. This result, and the absence of any correlation between surface properties and penetration enhancement, suggests that the potentiation of the foliar uptake of flamprop-methyl was attributable to interactions subtler and more specific than mere surface activity and increased wetting.

4.7 Stomatal penetration

Stomata have been implicated[60] in the process of foliar penetration in two roles, firstly, that the guard and accessory cells per se may be preferred sites of entry,[61-63] and secondly that under certain conditions mass movement of the spray solution through the stomatal pore into the substomatal chamber can occur,[64-68] The penetration of liquids into the intercellular air space of the leaves through open stomata has received considerable attention. Franke,[69] has suggested that, if it occurs, such mass movement of the spray solution does not constitute penetration since the internal cuticle remains as a lipoidal barrier which

must be transgressed. It has however been argued,[67] that once within the leaf spaces substances can be absorbed over an extended period and that the thinner inner cuticle will be more readily penetrated.

There is a general concensus[65,66,70] that pure water does not enter into the intercellular space through open stomata, unless external pressure is applied.[68] Reports concerned with the effect of surfactants on promoting stomatal penetration by aqueous solutions are contradictory; while penetration has been reported[1,51,66,67,71-73] other investigators[65,74-76] have concluded that mass movement does not readily occur. When stomatal penetration has been reported the mechanism would appear to be complex,[60] and the surface tension of the aqueous solution would seem not to be the sole determining factor.

Schonherr and Bukovac [68] have shown from theoretical considerations that spontaneous penetration of the stomatal pore will be limited to those solutions forming a zero contact angle with the wall of the pore. Experimental data obtained gave good qualitative agreement with theory; spontaneous penetration of stomatal and substomatal chambers was seen to be dependent on the liquids surface tension being less than the critical surface tension of the leaf surface, that liquid surface tension above which all liquids show finite contact angles on the surface.[77] These studies suggest that solutions with surface tensions below 30 $mN.m^{-1}$ can penetrate stomata at ordinary pressures. This result was supported by data obtained with organosilicone based surfactants[78] which are capable of lowering the surface tension to about 20 $mN.m^{-1}$. From this, it is suggested that a substantial contribution to penetration enhancement from stomatal penetration would be manifested as an abrupt improvement in uptake at and below some well defined surface tension. Above this surface tension little penetration enhancement would

be found since the surface tension would be too high to permit mass movement through stomata, while at and below the necessary surface tension penetration would be spontaneous.

The type of response predicted above was not evident in the data obtained with flamprop-methyl/surfactant formulations. That the correlation between penetration enhancement and surface tension implicit in the above reasoning was not present in this data is apparent from Figures 27 and 28. It is also apparent that penetration enhancement was increased linearly with increasing surfactant content up to maximum uptake, (Figures 1-8), and that the data did not conform to the pattern expected of an abrupt increase in uptake at some surfactant concentration corresponding to spontaneous stomatal penetration. This result can be interpreted in two ways:

(i) that the surface tension of the formulations was too high to permit spontaneous stomatal infiltration. Shafrin and Zisman[79] have reported the critical surface tension of pure CH_3 surfaces as 25 $mN.m^{-1}$; if the critical surface tension of the leaf surface approached this value then it would be below the limiting surface tension of all of the formulations.

(ii) that stomatal penetration had occurred but that this had no effect on the observed rate of uptake of flamprop-methyl.

The present studies did not distinguish between these interpretations.

4.8 Compartmental analysis of uptake data

It was shown earlier that the permeation of the cuticle can be discussed in terms of resistances acting on separate stages of the process of transcuticular movement. The relative importance of such

resistances should vary with the nature of the solute and the type of plant. It was also noted that most relevant published work has assumed the main resistance to foliar uptake to be associated with movement across the epicuticular waxes. Resistances due to binding or adsorption sites must be of only minor interest when the solute molecule has zero charge status and low polarity but diffusion in a solid, semi-crystalline phase is still a relatively slow process in comparison to movement through less well ordered phases and to partition between phases.

If one assumes that the lowering of diffusional resistance by a surfactant is effected by a surfactant/lipid interaction at some site remote from the site of application, then the magnitude of the effect might reasonably be expected to depend on the nature of the surfactant and the amount present at that site. The availability of the surfactant can be investigated using the compartmental model of foliar uptake introduced earlier and reproduced as Figure 33.

With the simplifying assumptions that diffusion follows Ficks first law and that the concentration at the inner interface, $C_{2,3}$, is zero, then the concentration gradient across the lipid layer will depend on the concentration at the interface $C_{1,2}$. Under these conditions the total amount of diffusant within compartment 2 will depend on $C_{1,2}$ and will increase with increasing $C_{1,2}$. Factors influencing $C_{1,2}$ have been discussed above: in particular it was suggested that $C_{1,2}$ might be independent of the amount applied to compartment 1, and of $K_{1,2}$, because of the limitations imposed by the saturation concentration of the solute in the monolayer δx. For a polyoxyethylene surfactant molecule spatial restrictions dictate that the interfacial monolayer

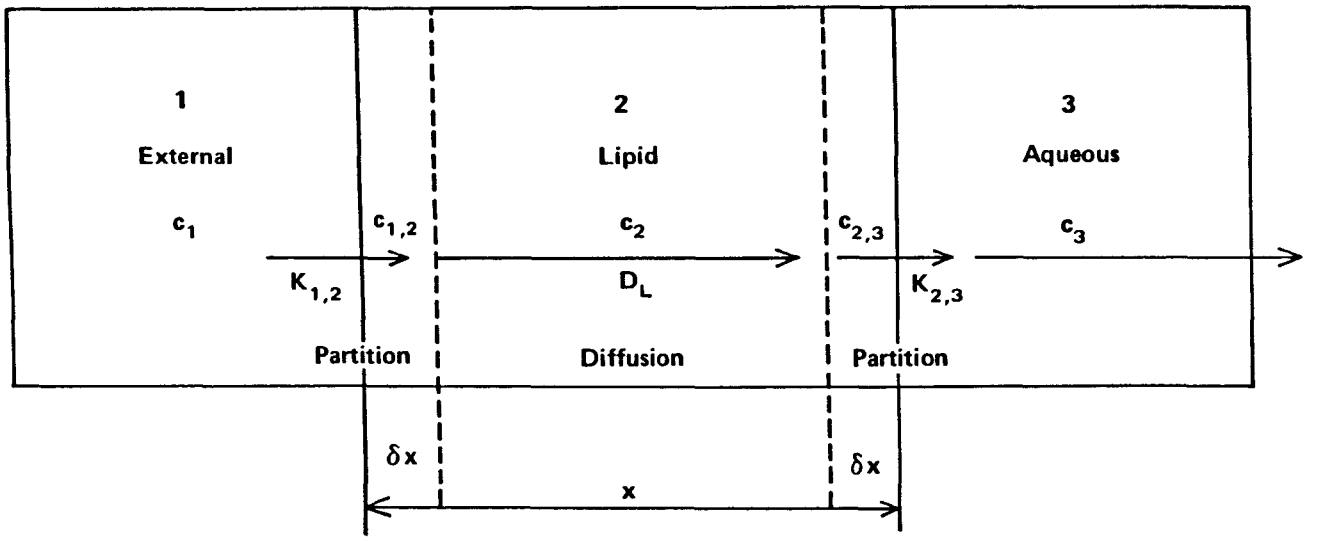


Fig 33

A compartmental model of foliar uptake, reproduced from Chapter 1.

must contain only very small amounts of material relative to the amounts commonly applied by topical placement of droplets. It may be concluded that at any one time the total amount of surfactant in compartment 2, and the amount at a site within this compartment, can be independent of the amount applied and independent of the partition coefficient unless the application is of low volume of dilute solution or the solute is very hydrophilic.

The period over which the surfactant level in compartment 2 is maintained will depend on the amount of material applied to compartment 1 and on the diffusion coefficient D_L, which in turn may depend on the molecular structure of the surfactant. If the lowering of diffusional resistance to other solutes by the surfactant is directly related to the amount of surfactant in the lipid phase and depends on the continued presence of the surfactant in that phase then :

(i) at low surfactant concentrations the necessary level of surfactant to modify cuticular resistance may not be sustained by the external phase

(ii) at optimum surfactant concentrations the lowering of the diffusional resistance of the cuticle will be maintained over a period sufficient for herbicide uptake to proceed to near completion, and

(iii) at supra-optimum surfactant concentrations changes in penetration enhancement occur as a consequence of a persistent residue of the surfactant in the external phase.

Generally the experimentally determined enhancement of uptake by surfactants appeared to show some similarities to the responses predicted from a compartmental model. The absence of any marked effect of surfactant structure on flamprop-methyl penetration, save at supra-optimum concen-

trations, and the absence of a correlation between penetration enhancement and partition coefficients were explicable in terms of the analysis presented above. The small variation in the effectiveness of surfactants with differing oxyethylene contents to promote uptake when used in dilute solutions may be due to differences in the intrinsic abilities to modify the lipid phase.

Experiments with non-concerted applications gave evidence for the importance of the time factor to the mechanism involved in the surfactant induced enhancement of uptake. The reduction in uptake relative to concerted applications when a short time lag was introduced between surfactant and herbicide applications demonstrated that the mechanism involved a transient interaction and that this was rapidly diminished. One difficulty encountered in attempts to correlate experimental data with the proposed model was that uptake from dilute solutions had been shown to be potentiated over relatively long periods. The preceding discussion has however assumed that penetration enhancement depends on the continued presence of the surfactant which cannot be sustained by dilute applications. It is noteworthy that in such cases of continued uptake, it occurred at much reduced rates after about 24 hours. Continued uptake may be explained by some residual effect of the surfactant on the epicuticular structure by which transcuticular movement can continue at increased rates: relative to unaided uptake. Such an effect would not contradict previous discussions providing the level of penetration enhancement achieved was somewhat less than that effected by the initial interaction.

Finally it should be noted that surfactant induced potentiation of uptake is here explained in terms of a surfactant/lipid interaction by which epicuticular wax structure is made more permeable to flamprop-

methyl, and that this interaction has no direct herbicide component, viz., flamprop-methyl does not actively interact in this model. Such a concept was in accord with the absence of any discernible effect on uptake of the herbicide content of the formulations and of the herbicide: surfactant ratio. Other studies have however found that the herbicide: surfactant ratio appears to influence transcuticular movement. Babiker et al.,[80] investigating the penetration of bean leaves by amitrole in the presence of Tween 20, found that (i) amitrole penetration decreased with increasing amitrole concentration at a fixed concentration of Tween 20, and (ii) that when the ratio of herbicide:surfactant was kept about constant (1:2) similar penetration data were obtained irrespective of amitrole concentration. Amitrole differs from flamprop-methyl in its greater aqueous solubility and in being mobile in both apoplast and symplast. Other differences exist between these data, including the plant used. More significantly, the amount of solute applied differs markedly between these studies: amitrole uptake was investigated following application of between 30 and 720 µg per leaf whereas the highest dose of flamprop-methyl applied in these experiments was 0.3 µg. The dosage used in experiments with amitrole is arguably more typical of that used by other workers. The mechanism suggested for the surfactant induced enhancement of foliar uptake can be considered as a type of facilitated diffusion. Facilitated diffusion is generally concentration dependant and can be saturated at high concentrations.[81] Thus it may be that the type of effect described in this study is only amenable to study when relatively low dosage application is made to the leaf.

REFERENCES

Chapter 1

1. Sargent, J.A. Ann. Rev. Plant Physiol. 16, 1, (1965).
2. Sargent, J.A. 8th., Brit. Weed Control Conf. Proc. p.804, (1966).
3. Overbeek, J. van. Ann. Rev. Plant Physiol. 7, 355, (1956).
4. Currier, H.B. and Dybing, C.D. Weeds 7, 195, (1959).
5. Mitchell, J.W., Smale, B.C. and Metcalf, R.L. Adv. Pest Control Research 3, 359 (1960).
6. Hoskins, W.H. Residue Reviews 1, 66, (1962).
7. Crafts, A.S. and Foy, C.L. Residue Reviews 1, 112, (1962).
8. Ebling, W. Residue Reviews 3, 35, (1963).
9. Mitchell, J.W. and Lindner, P.J. Residue Reviews 2, 51 (1963).
10. Crafts, A.S. In 'The Physiology and Biochemistry of Herbicides' pp 75-110. Academic Press: London and New York, (1964).
11. Foy, C.L. J.Agr. Fd. Chem. 12, 473, (1964).
12. Hull, H.M. In 'Abdorption and translocation of organic substances in plants' 7th, Ann. Symp. Amer. Soc. Plant Physiol. S.Sect p45, (1964).
13. Hull, H.M. Residue Reviews 31, 1, (1970).
14. Linskens, H.F., Heinen, W. and Stoffers, A.L. Residue Reviews 8, 136, (1965).
15. Martin, J.T. Ann. Rept. East Malling Research Sta. 1960 p40, (1961).
16. Audus, L.J. Agrochim. 11, 309, (1967).
17. Franke, W. Ann. Rev. Plant Physiol. 18, 281, (1967).
18. Foy, C.L., Whitworth, J.W., Muzik, T.J. and Currier, H.B. In 'Environmental and other factors in the response of plants to herbicides'. Oregon Agr. Expt. Sta. Tech. Bull. 100, 3, (1967).

19. Hammerton, J.L. Weeds 15, 330, (1967).

20. Hull, H.M., Barrier, G.E., Frans, R.E., Hilton, J.L., Knake, E.L., Moreland, D.E. and Zick, W.H. Herbicide Handbook of the Weed Society of America, Geneva. Humphery Press: New York, (1967).

21. Norris, L.A. Herbicide Veg. Management Symp. Proc. p56, (1967).

22. Price, C.E. Repts. Progr. Appl. Chem. 310, (1974).

23. Price, C.E. In 'Herbicides and Fungicides. Factors affecting their activity'. (ed. McFarlane, N.R.). The Chemical Society: Special publ. number 29. p42, (1977).

24. Kirkwood, R.C. ibid. p67.

25. Orgell, W.H. Ph.D. Thesis, Univ. of Calif., Davis, (1954).

26. Collander, R. In 'Plant Physiology, a treatise. Vol.II. Plants in relation to water and solutes'. pp.3-102, Academic Press: New York and London, (1959).

27. Orgell, W.H. Iowa Acad. Sci. Proc. 64, 189, (1957).

28. Robertson, M.M., Parham, P.H. and Bukovac, M.J. J.Agric. Fd. Chem. 19, 754, (1971).

29. Kirkwood, R.C., Dalziel, J., Matlib, A. and Somerville, L. Pestic. Sci. 3, 307, (1972).

30. Lopez, H. Ph.D. Thesis, Oregon State Univ., (1971).

31. Sargent, J.A. and Blackman, G.E. J. exp. Bot. 20, 542, (1969).

32. Kennedy, C.D. Pestic. Sci. 2, 69, (1971).

33. Kennedy, C.D. and Stewart, R.A. In 'Herbicides and Fungicides. Factors affecting their activity'. (ed. McFarlane, N.R.), The Chemical Society: Special publ. number 29, (1977).

34. Kennedy, C.D. and Harvey, J.M. Pestic. Sci 3, 715, (1972).

35. Smith, A.E. Physiologia Pl. 27, 338, (1972).

36. Smith, A.E. Weed Sci. 20, 46, (1972).

37. Bukovac, M.J., Sargent, J.A., Powell, R.G. and Blackman, G.E. J. exp. Bot. 22, 598, (1971).

38. Bukovac, M.J., Rasmussen, H.P. and Shull, V.E. Scanning Electron Microscopy III, pp.213-223, (1981).

39. Yamada, Y. Studies on foliar adsorbtion of nutrients by using radioisotopes. Ph.D Thesis, Kyoto Univ. Japan, (1962).

40. Yamada, Y., Wittwer, S.H. and Bukovac, M.J. Plant Physiol. 40 (1), 170, (1965).

41. Darlington, W.A. and Cirulis, N. Plant Physiol. Lanc. 38, 442, (1963).

42. Bukovac, M.J. and Norris, R.F. Plant Physiol. 42, S-48, (1967).

43. Norris, R.F. and Bukovac, M.J. Pestic. Sci. 3, 705, (1972).

44. Lieb, W.R. and Stein, W.D. Current topics membranes transp. 2, 1, (1971).

45. Kumins, C.A. and Kwei, T.K. In 'Diffusion in Polymers'. (eds. Crank, J. and Park, G.S.) pp.107-140, Academic Press: New York, (1968).

46. Lieb, W.R. and Stein, W.D. Nature (London) 224, 240, (1969).

47. Cohen, M.H. and Turnbull, D. J. Chem. Phys. 31, 1164, (1959).

48. Grncarevic, M. and Radler, F. Planta 75, 23 (1967).

49. Skoss, J.D. Bot. Gaz. 117, 55, (1955).

50. Possingham, J.V., Chambers, T.C., Radler, F. and Grncarevic, M. Austral. J. Biol. Sci. 20, 1149, (1967).

51. Hall, D.M. and Jones, R.L. Nature (London) 191, 95, (1961).

52. Bukovac, M.J. Amer. Soc. Hort. Sci. Proc. 87, 131, (1965).

53. Volk, R. and McAuliffe, C. Soil Sci. Soc. Amer. Proc. 18, 308, (1954).

54. Haas, K. and Schonnherr, J. Planta 146, 399, (1979).

55. Baker, E.A. Bukovac, M.J., Flore, J.A. J. Amer. Soc. Hort. Sci. 104, 611, (1979).

56. Baker, E.A. and Bukovac, M.J. Ann. appl. Biol. 67, 243, (1971).

57. Herrett, R.A. and Linck, A.J. Physiologia Pl. 14, 767, (1961).

58. Bukovac, M.J., Flore, J.A. and Baker, E.A. J. Amer. Soc. Hort. Sci. 104, 611, (1979).

59. Darlington, W.A. and Barry, J.B. J. agric. Fd. Chem. 13, 76, (1965).

60. Webster, D.H. Weeds 10, 250, (1962).

61. Kirkwood, R.C., Dalziel, J., Matlib, A. and Somerville, L. Proc. 9th., Brit. Weed Control Conf. p651, (1968).

62. Ong, B.Y., Falk, R.H. and Bayer, D.E. Plant Physiol. 51(2), 415, (1973).

63. Smith, C.N. and Nalewaza, J.D. Weed Sci. 20, 36, (1972).

64. St. John, J.B. and Hilton, J.L. Weed Sci. 21, 477, (1973).

65. Brian, R.C. Ann. appl. Biol. 59, 81, (1967).

66. Crafts, A.S. Hilgardia 26, 287, (1956).

67. Kirkwood, R.C. Proc. 11th., Brit. Weed Control Conf. p1117, (1972).

68. Robertson, M.M. and Kirkwood, R.C. Weed Res. 9, 224, (1969).

69. Crafts, A.S. Ann. Rev. Plant Physiol. 4, 253, (1953).

70. Norris, L.A. and Freed, V.H. Weed Res. 6, 203, (1966).

71. Czaza, A. TH. Planta 57, 669, (1962).

72. Scott, F.M., Hammer, K.C. and Baker, E. Science 125, 339, (1957).

73. Weaver, R.J. and De Rose, H.R. Bot. Gaz. 107, 509, (1946).

74. Adam, N.K. Disc. Faraday Soc. 3, 5, (1948).

75. Schonherr, J. and Bukovac, M.J. Plant Physiol. 49, 813, (1972).

76. Boynton, D. A. Rev. Pl. Physiol. 5, 31, (1954).

77. Wittwer, S.H. and Teubner, F.G. Ann. Rev. Plant Physiol.10, 13, (1959).

78. Goodman, R.N. and Goldberg, H.S. Phytopathology 50 , 851, (1960).

79. Kamimura, S. Dissertation Abstr. 25 (10), 5486, (1965).

80. McFarlane, J.C. and Berry, W.L. Plant Physiol 53 (5), 623, (1974).

81. Price, C.E, Boatman, S.G. and Boddy, B.J. J. exp. Bot. 26, 521, (1975).

82. Orgell, W.H. Plant Phys. 30, 78, (1955).

83. Crafts, A.S. The Chemistry and Mode of Action of Herbicides. Interscience: New York, (1961).

84. Davies, P.J., Drennan, D.S.H., Fryer, J.D. and Holly, K. Weed Research 8, 233, (1968).

85. Basler, E., Todd, G.W. and Meyer, R.E. Plant Physiol. 36, 573, (1961).

86. Pallas, J.E. Jr., and Williams, G.G. Bot. Gaz. 123, 175, (1962).

87. Yamada, Y., Jyung, W.H. Wittwer, S.H. and Bukovac, M.J. Amer. Soc. Hort. Sci. Proc. 87, 429, (1965).

88. Esau, K. Plant Anatomy, 2nd Edn., Wiley: New York, (1965).

89. Norris, R.F. and Bukovac, M.J. Amer. J. Bot. 55, 975, (1968).

90. Franke, W. Amer. J. Bot. 48, 683, (1961).

91. Franke, W. In 'Absorption and translocation of organic substances in plants', 7th., Ann. Symp. Amer. Soc. Plant Physiol. S-Sect. p95, (1964).

92. Franke, W. Pestic. Sci. 1, 164, (1970).

93. Franke, W. Residue Rev. 38, 81, (1971).

94. Schonherr, J. and Bukovac, M.J. Planta 92, 189, (1970).

95. Crafts, A.S. In 'Proc. of a Symp. on the use of Isotopes in Weed

Research'. Oct. 25-29, 1965, Vienna Internat. Atomic Energy Agency, Vienna, Austria pp.3-7, (1966).

96. Crafts, A.S. and Crisp, C.E. Phloem transport in plants. Freeman: San Franscisco, (1971).

97. Ashton, F.M. and Crafts, A.S. Mode of Action of Herbicides. John Wiley and Sons: New York, (1973).

98. Hay, J.R. In 'Herbicides. Physiology, Biochemistry and Ecology' (ed. Audus, L.J.) Vol. 1, p365, Academic Press: London and New York, (1976).

99. Currier, H.B. and Dybing, C.D. Weeds, 7, 195, (1959).

100. Foy, C.L. Weeds 10, 35, (1962).

101. Day, B.E. Plant Physiol, 27, 143, (1952).

102. Little, E.C.S. and Blackman, G.E. New Phytol. 62, 173, (1963).

103. Robertson, M.M. and Kirkwood, R.C. Weed Res. 10, 102, (1970).

104. Hay, J.R. In 'Herbicides. Physiology, Biochemistry and Ecology', (ex. Audus, L.J.), Vol. 1, pp.365-396, Academic Press: London and New York, (1976).

105. Danielli, J.F. Collston Papers 7, 1, (1954).

106. Seaman, D. Chem. and Ind. p159, (1979).

107. Jyung, W.H. and Wittwer, S.H. Am. J. Bot. 51, 437, (1964).

108. Baur, J.R. Bovey, R.W. and Benedict, C.R. Agron. J. 62, 627, (1970).

109. Luckwill, L.C. and Lloyd-Jones, C.P. J. Hort. Sci. 37, 190, (1962).

110. Holloway, P.J. and Baker, E.A. Plant Physiol. 43, 1878, (1968).

111. Martin, J.T. and Juniper, B.E. The Cuticles of Plants. Edward Arnold, (1970).

112. Crowdy, S.H. In 'Systemic Fungicides', (ed Marsh, R.W.) p92, Longman: London, (1972).

113. McCready, C.C. New Phytol. 62, 3, (1963).

114. Collander, R. Acta. Chem. Scand. 3, 717, (1949).

115. Jennings, D.H. The Absorption of solutes by Plant Cells. Oliver and Boyd: Edinburgh, (1963).

116. Coupland, D. and Peel, A.J. Planta, 103, 249, (1972).

117. Geiger, D.R. and Cataldo, D.A. Plant Physiol. 44, 45, (1969).

118. Geiger, D.R. Encycl. Plant Physiol. New Ser. 1, 395, (1975).

119. Geiger, D.R., Malone, J. and Cataldo, D.A. Amer. J. Bot. 58, 672, (1971).

120. Kuo, J., O'Brian, T.P. and Canny, M.J. Planta 121, 97, (1974).

121. Olsen, P. Planta 123, 199, (1975).

122. Shih, C.Y. and Currier, H.B. Amer. J. Bot. 56, 464, (1969).

123. Kursanov, A.L. Izv. Akad. Nauk. SSSR Ser. Biol. 1, 3, (1967).

124. Stocking, C.R., Williams, G.R. and Ongun, A. Biochem. Biophys. Res. Commun. 10, 416, (1963).

125. Hawker, J.S. Aust. J. biol. Sci. 18, 959, (1965).

126. Kriedemann, P.E. Planta 73, 175, (1967).

127. Brovchenko, M.I. Fiziol, Rast. 12, 270, (1965).

128. Barrier, G.E. and Loomis, W.E. Plant Physiol. 32, 225, (1957).

129. Sovonick, S.A., Geiger, D.R. and Fellows, R.J. Pl. Physiol. Lancaster 54, 886, (1974).

130. Field, R.J. and Peel, A.J. New Phytol. 70, 997, (1971).

131. Moorby, J.J. exp. Bot. 15, 457, (1964).

132. Crisp, C.E. In 'Pesticide Chemistry. Proc. 2nd., Internat. I.U.P.A.C, Congr. Pest. Chem', (ed. Tahori, A.S.). Vol. 1, Insecticides, p211. Gordon and Breach: London, (1972).

133. Crisp, C.E. and Look, M. Adv. Pestic. Sci. Plenary Lect. Symp. Pap. Int. Congr. Pestic. Chem. 4th, 1978. 3, 430, (1979).

134. Vernon, L.P. and Aronoff, S. Arch. Biochim. Biophys. 36, 383, (1952).

135. Yamaguchi, S. Hilgardia 36, 349, (1965).

136. Crafts, A.S. and Yamaguchi, S. Hilgardia 27, 241, (1958).

137. Chang, F.Y. and Vanden Born, W.H. Weed Sci. 16, 176, (1968).

138. Carter, H.C. and Naylor, A.W. Bot. Gaz. 122, 138, (1960).

139. Crafts, A.S. Translocation in Plants, Rinehart and Winston: New York, (1961).

140. Foy, C.L. Plant Physiol. Lancaster 36, 688, (1961).

141. Mason, G.W. Doctorate dissertation, Univ. of Calif., Davis, Calif., (1960).

142. Sagar, G.R. Proc. Br. Weed Control Conf. pp.271-278, (1960).

143. Sharma, M.P., Vanden Born, W.H. and McBeath, D.K. Weed Res. 18 (8), 169, (1978).

144. Anderson, J.L. and Thomson, W.W. Residue Rev. 47, 167, (1973).

145. Butta , J.G. and Steffens, G.L. Physiologia Pl. 24, 431, (1971).

146. Jenner, C.F. Rept. Waite. Agr. Res. Inst. S. Austral. p36, (1963).

147. Davenport, H.E. Proc. Roy. Soc. B157, 332, (1963).

148. Ashton, F.M. Weeds 6, 257, (1958).

149. O'Brien, T.J. Diss Abstr. B 28(12), 4846, (1968).

150. Freed, V.H. Res Pestic. Proc. Conf. Davis. Calif. 1964, pp.159-171, (Pub. 1965).

151. Rohrbaugh, L.M. and Rice, E.L. Bot. Gaz. 111, 85, (1949).

152. Hay, J.R. and Thimann, K.V. Plant Physiol. 31, 446, (1956).

153. Herrett, R.A. and Bagley, W.P. J. Agric. Fd. Chem. 12, 17, (1964).

154. Eliasson, L. Physiologia Pl. 18, 506, (1965).

155. Pallas, J.E. and Crafts, A.S. Science, N.Y. 125, 192, (1956).

156. Levi, E. Science, N.Y. 137, 343, (1962).

157. Slade, P. and Bell, E.G. Weed Res. 6, 267, (1966).

158. Bridges, R.C. and Farrington, J.A. Pestic. Sci. 5, 365, (1974).

159. Jeffcoat, B. and Harries, W.N. Pestic. Sci. 4, 891, (1973).

160. Jeffcoat, B., Harries, W.N. and Thomas, D.B. Pestic. Sci 8, 1, (1977).

161. Jeffcoat, B. and Harries, W.N. Pestic. Sci 6, 283, (1975).

162. Grayson, B.T. and Stokes, S. Pestic. Sci 9, 595, (1978).

163. Roberts, T.R. Pestic. Sci. 8, 463, (1977).

164. Benyon, K.I., Roberts, T.R. and Wright, A.N. Pestic. Sci. 6, 429, (1974).

165. Benyon, K.I., Roberts, T.R., Stoydin, G and Wright, A.N. Pestic. Sci. 6, 443, (1974).

166. Dutton, A.J., Roberts, T.R. and Wright, A.N. Chemosophere 5 (3), 195, (1976).

167. Jooste J. VD. W and Moreland, D.E. Phytochem. 2, 263, (1963).

168. Schwartz, H.M., Biedron, D.I., von Holdt, M.M. and Rehm, S. Phytochem. 3, 189, (1964).

169. Hill, B.D. and Stobbe, E.H. Weed Res. 18 (4), 223, (1978).

170. Hill, B.D., Stobbe, E.H. and Jones, B.L. Weed Res. 18, 149, (1978).

171. Dal Nogare, S. and Juvet, R.S. Gas Liquid Chromatography: Theory and Practice, Wiley - Interscience: New York, (1962).

172. Turner, J.C. Sample Preparation for Liquid Scintillation Counting, Review 6, The Radiochemical Centre, Amersham, England, (1967).

173. Muzik, T.J. In 'Herbicides. Physiology, Biochemistry, Ecology', (ed. Audus, L.J.), Academic Press: London (1976).

174. Prasad, R. and Blackman, G.E. J. exp. Bot. 16, 86, (1965).

Chapter 2

1. Glassman, H.N. Bact. Revs. 12, 105, (1949).
2. Osipow, L.W. Surface Chemistry. Theory and Industrial Applications. Reinhold Publ. Co.: New York, (1962).
3. Colwell, C.E. and Rixon, W.E. Am. Dyestuff Reptr. 50, No. 18, 39, (1961).
4. Davies, J.T. and Rideal, E.K. Interfacial Phenomena, (2nd edn.), Academic Press, (1963).
5. Sisley, J.P., (translated and revised by Wood, P.J.), Encyclopedia of Surface Active Agents. Chemical Publ. Co. Inc: New York, (1952).
6. Adamson, A.W. The Physical Chemistry of Surfaces. Wiley - Interscience: New York, (1960).
7. Schick, M.J. Non-Ionic Surfactants. Marcel Dekker Inc: New York, (1967).
8. Sherman, P. Emulsion Science. Academic Press: London and New York, (1968).
9. Schonfeld, D. Surface Active Ethylene Oxide Adducts. Pergamon Press: London, (1969).
10. Shaw, D.J. Introduction to Colloid and Surface Chemistry. Butterworths: London, (1970).
11. De Boer, J.H. The Dynamical Character of Adsorption, (2nd. edn.) Oxford Univ. Press, (1968).
12. Kanellopoulos, A.G. Chem and Ind. p951, (1974).
13. Griffen, W.C. J. Soc. Cosmetic Chemists 1, 311, (1949).
14. Becher, P. In 'Non-Ionic Surfactants', (ed. Schick, M.J.) p606, Marcel Dekker Inc: New York, (1967).
15. Enyeart, C.R. ibid, p44.

16. Shachat, N. and Greenwald, W.L. ibid., p8.

17. Bruson, H.A, and Stein, O., (to Rohm and Haas Co.) US. 2, 143, 759 (1939).

18. Flory, P. J. Am. Chem. Soc. 62, 1561 (1940).

19. Kelly, J. and Greenwald, H.L. J. Phys. Chem. 62, 1096, (1958).

20. Fineman, M.N., Brown, G.L. and Myers, R.J. J. Phys, Chem. 56, 963, (1952).

21. Konishi, K. and Yamaguchi, S. Anal. Chem. 38, 1755, (1966).

22. Nadeau, H.G., Oaks, D., Nichols, W.A. and Carr, L.P. Anal. Chem. 36, 1914 (1964).

23. Gibbons, R.A. Nature 200, 665, (1963).

24. Satkowski, W.B., Huang, S.K. and Liss, R.L. In 'Non-Ionic Surfactants', (ed. Schick, M.J.) p86, Marcel Dekker Inc: New York, (1967).

25. Nadeau, H.G. and Siggia, S. ibid, p868.

26. Greff, R.A. and Flanagan, P.W. J. Am. Oil Chem. Soc. 40, 118, (1963).

27. Crowe, M.O.L. Anal. Chem. 13, 845, (1941).

28. Skelly, N.E. and Crummett, W.B. Anachem. Conf. Wayne State Univ. Detroit, Mich., (1964).

29. Reilly, R. and Rae, R. Physicochemical Methods, Vol. 1, Methven: London, (1943).

30. Freud, B.B. and Freud, H.Z. J. Am. Chem. Soc. 52, 1772, (1930).

31. Boyd, G.E. and Livingston, H.K. J. Am. Chem. Soc. 64, 2383, (1942).

32. AATCC 17-1952.

33. Greenwald, H.L. and Brown, G.L. J. Phys. Chem. 58, 825, (1954).

34. Rohm and Haas, Triton Surface Active Agents, (1967).

35. Crook, E.H., Fordyce, D.B. and Trebbi, G.F. J. Phys. Chem. 67, 1987, (1963).

36. Hsiao, L., Dunning, H.W. and Lorenz, P.B. J. Phys. Chem 60, 657, (1950).

37. Crook, E.H., Fordyce, D.B. and Trebbi, G.F. J. Colloid Sci. 20, 191, (1965).

Chapter 3

1. St. John, J.B. and Hilton, J.L. Weed Sci 21, 477, (1973).
2. Crafts, A.S. and Yamaguchi, S. Manual 35, Calif. Agr. Expt. Sta. Extension Service, (1964).
3. Smith, L.W. and Foy, C.L. Weeds 15, 67, (1967).
4. Smith, L.W. and Foy, C.L. J. Agr. Food Chem. 14, 117, (1966).
5. Norris, L.A. and Freed, V.H. W. Weed Cont. Conf. Research Progress Rept. p85, (1963).
6. Foy, C.L. and Smith, L.W. Proc. Western Weed Control Conf. p88, (1973).
7. Price, C.E. In 'Herbicides and Fungicides. Factors affecting their activity'. (ed. McFarlane, N.R.), p67, The Chemical Society Special Publ. number 29, (1977).
8. Hull, H.K. Proc. Western Weed Control Conf. p87 (1973).
9. Nadeau, H.G. and Siggia, S. In 'Non-Ionic Surfactants', (ed. Schick, M.J.), Marcel Dekker Inc: New York, (1967).
10. Ginn, M.E., Church, C.L. and Harris, J.C. Anal. Chem. 33, 145, (1961).
11. Skelly, N.E. and Crummett, W.B. Anachem. Conf. Wayne State Univ. Detroit, Mich., (1964).
12. Kelly, J. and Greenwald, H.L. J. Phys. Chem. 62, 1096, (1958).
13. Nadeau, H.G., Oaks, D. Nichols, W.A. and Carr, L.P. Anal. Chem. 36, 1914, (1964).
14. Gildenberg, L. and Trowbridge, J.R. J. Am. Oil Chem. Soc. 42, 70, (1965).
15. Hamilton, R.J. and Sewall, P.A.
16. Huber, J.F.K., Kolder, F.F.M. and Miller, J.M. Anal. Chem. 44, 105, (1972).

17. Carey, M.A. and Persinger, H.E. J. Chromatog. Sci. 10, 587, (1972).

18. Krejici, M., Rounda, M. and Vaurouch, V. J. Chromatogr. 91, 549, (1974).

19. Allen, F.C. and Rice, R.I. J. Chromatogr. 110, 151, (1975).

20. Turner, L.P., McCullough, D. and Jackewitz, A. J. Am. Oil Chem. Soc. 53, 691, (1976).

21. Calzolari, C., Fauretto, L. and Stancher, B. J. Chromatogr. 47, 209, (1970).

22. Cassidy, R.M. and Niro, C.M. J. Chromatogr. 126, 787, (1976).

23. Cassidy, R.M. J. Liq. Chromatogr. 1 (2), 241, (1978).

24. Pedley, J.B. Shell Research Limited, Sittingbourne Research Centre. Private communication.

25. Kelly, J. and Greenward, H.L. J. Phys. Chem. 62, 1096, (1958).

26. Melander, W.R., Chen, B.K. and Horvath, C. J. Chromatogr. 185 (1), 99, (1979).

27. Lieb, W.R. and Stein, W.D. Current topics Membranes Transp. 2, 1, (1971).

28. Skelland, In 'Diffusion in Polymers', (eds Crank, J. and Parks, G.S.), Academic Press: New York, (1970).

29. Crook, E.H., Fordyce, D.B. and Trebbi, G.F. J. Colloid Sci. 20, 191, (1965).

30. Becher, P. In 'Non-Ionic Surfactants', (Ed. Schick, M.J.), Marcel Dekker Inc: New York and London, (1967).

31. Jost, W. 'Diffusion. In Solids, Liquids, Gases', (3rd edn.). Academic Press: New York and London, (1960).

Chapter 4

1. Foy, C.L. J. Agr. Fd. Chem. 12, 473, (1964).
2. Sharma, M.P. and Vanden Born, W.H. Weed Sci. 18, 57, (1970).
3. Holly, K. and Turner, D.J. Adv. Pestic. Sci. Plenary Lect. Symp. Pap. Int. Congr. Pestic. Chem. 4th., 1978. 3, 726, (Pub. 1979).
4. Jansen, L.L., Gentner, W.A. and Shaw, W.C. Weeds 9, 381, (1961).
5. McWhorter, C.G. Weeds 11, 83, (1963).
6. Yapel, Jr., A.F. Advan. Chem. Ser. 114, 183, (1972).
7. Van Valkenburg, W. and Yapel, Jr., A.F. Advan. Chem. Ser. 114, 252, (1972).
8. Babiker, A.G.T. and Duncan, H.J. Weed Research 15 (2), 123, (1975).
9. Muller, L.E., Carr, P.H. and Loomis, W.H. Am. J. Bot. 41, 593, (1954).
10. Weintraub, R.L., Yeatman, J.B., Brown, J.W., Throne, J.A. Skoss, J.B. and Conover, J.R. Proc. 8th N.E. Weed Cont. Conf. p5, (1954).
11. Freed, V.H. and Montgomery, M. Weeds 6, 386, (1958).
12. Currier, H.B. and Dybing, C.D. Weeds 7, 195, (1959).
13. Hughes, R.E. and Freed, V.H. Weeds 9, 54, (1961).
14. Staniforth, D.W. and Loomis, W.E. Science 109, 628, (1949).
15. Foy, C.L. and Smith, L.W. Weeds 13, 15, (1965).
16. Evans, R.A. and Eckert Jr., R.E. Weeds 13, 150, (1965).
17. Corns, W.G. and Dai, T. Can. J. Plant Sci. 47, 711, (1967).
18. Smith, L.W. and Foy, C.L. Weeds 15, 67, (1967).
19. Darlington, W.A. and Barry, J.B. J. Agri. Fd. Chem. 13, 76, (1965).
20. Robertson, M.M., Parham, P.H. and Bukovac, M.J. J. Agric. Fd. Chem. 19, 754, (1971).

21. Chow, P.N.P. Proc. Plant Growth Reg. Working Group, Hot Springs, Arkansas, 4th Ann. Meeting 310-313, (1977).

22. Cook, G.T., Abdel, G.T. Babiker, A.G.T. and Duncan, H.J. Pestic. Sci. 8, 137, (1977).

23. Foy, C.L. and Smith, L.W. In 'Pesticidal Formulations Research. Physical and Colloidal Chemical Aspects', Adv. Chem. Series 86, 55, (1969).

24. Bland, P.D. and Brian, R.C. Pestic. Sci. 6, 419, (1975).

25. Temple, R.E. and Hilton, H.W. Weeds 11 (4), 297, (1963).

26. Holly, K. In 'The Physiology and Biochemistry of Herbicides', p423, Academic Press: New York, (1964).

27. Seaman, D. Chem. and Ind. p159, (1979).

28. Parr, J.F. and Norman, A.G. Bot. Gaz. 126 (2), 86, (1965).

29. Jansen, L.L. Weeds 12, 251, (1964).

30. Furmidge, C.G.L. J. Sci. Fd. Agric. 10, 267, (1959).

31. St. John, J.B., Bartels, P.G. and Hilton, J.L. Weed Sci. 22, 233, (1974).

32. Towne, C.A., Bartlets, P.G. and Hilton, J.L. Weed Sci. 26 (2), 182, (1978).

33. Healey, P.L., Ernst, R. and Ardith, J. New Phytol. 70, 477, (1971).

34. Holly, K. Ann. appl. Biol. 44, 195, (1956).

35. Rademacher, B. Proc. 2nd Br. Weed Control Conf. pp.401-405, (1954).

36. Dewey, O.R., Gregory, P. and Pfeiffer, R.K. Proc. 3rd Br. Weed Control Conf. pp.313-326, (1956).

37. Hall, D.M. and Jones, R.L. Nature (London) 191, 95, (1961).

38. Aberg, E. In 'The Physiology and Biochemistry of Herbicies' pp.401-422. Academic Press:London and New York, (1964).

39. Turner, D.J. Pestic, Sci. 3, 323, (1972).

40. Freed, V.H. and Montgomery, M. Weeds 6, 386, (1957).

41. Freed, V.H. and Witt, J.M. In 'Pesticide Formulation Research', (ed. Gould, R.F.), Adv in Chem Series 86, 70, (1968).

42. Eyring, H., Lumry, R. and Woodbury, J.W. Record Chem. Progress 10, 100, (1949).

43. Price, C.E. Repts. Progr. Appl. Chem. p310, (1974).

44. Lawrence, A.S.C. J. Soc. Cosmet. Chem. 22 (8), 505, (1971).

45. Hull, H.M. Residue Reviews 31, 1, (1970).

46. Kanellopoulos, A.G. Chem. and Ind. 23, 951, (1974).

47. Sharma, M.P., Vandon Born, W.H. and McBeath, D.K. Weed Res 18 (3), 169, (1978).

48. Kirkwood, R.C., Dalziel, J., Matlib, A. and Somerville, L. Pestic. Sci. 3, 307, (1972).

49. Babiker, A.G.T. and Duncan, H.J. Weed Res. 14 (6), 375, (1974).

50. Cook, G.T. and Duncan, H.J. Pestic. Sci. 9 (6), 535, (1978).

51. Sands, R. and Bachelard, E.P. New Phytol. 72, 69, (1973).

52. Lindner, P.L. Emulsions Technol 1, 179, (1974).

53. St. John, J.B. and Hilton, J.L. Weed Sci. 21, 477, (1973).

54. Jansen, L.L. Weeds 13, 117, (1965).

55. Smith, L.W., Foy, C.L. and Bayer, D.E. Weed Res 6, 233, (1966).

56. Behrens, R.W. Weeds 12, 255, (1964).

57. Dorscher, K.P. and Buchholtz, K.P. Agron. J. 48, 59, (1956).

58. Becher, P. and Becher, D. Adv. in Chem. Series Number 86, 15, (1969).

59. Crook, E.H., Fordyce, D.B. and Trebbi, G.F. J. Phys. Chem. 67, 1987, (1963).

60. Greene, D.W. and Bukovac, M.J. Amer. J. Bot. 61, 100, (1974).

61. Weber, F. Protoplasma 10, 608, (1930).

62. Franke, W. In 'Adbsorption and translocation of organic substances in plants', 7th., Ann. Symp. Amer. Soc. Plant Physiol. S- Sect. p95, (1964).

63. Neumann, S.T. and Jacob, F. Naturwiss, 55, 89, (1968).

64. Ebeling, W. Hilgardia 12, 665, (1939).

65. Turrell, F.M. Bot. Gaz. 108, 476, (1947).

66. Dybing, C.D. and Currier, H.B. Plant Physiol. 36, 169, (1961).

67. Currier, H.B., Pickering, E.R. and Foy, C.L. Weeds 12, 301, (1964).

68. Schonherr, J. and Bukovac, M.J. Plant Physiol. 49, 813, (1972).

69. Franke, W. Amer. J. Bot. 48, 683, (1961).

70. Adam, N.K. Disc. Faraday Soc. 3, 5, (1948).

71. Cook, J.A. and Boynton, D. Proc. Amer. Soc. Hort. Sci. 59, 82, (1952).

72. Skoss, J.D. Bot. Gaz. 117, 55, (1955).

73. Eddings, J.L. and Brown, A.L. Plant Physiol. 42, 15, (1967).

74. Weaver, R.J. and DeRose, H.R. Bot. Gaz. 107, 509, (1946).

75. Sargent, J.A. and Blackman, G.E. J. exp. Bot. 13, 348, (1962).

76. Jyung, W.H., Wittwer, S.H. and Bukovac, M.J. Proc. Am. Soc. Hort. Sci. 86, 361, (1965).

77. Bernett, M.K. and Zisman, W.A. J. Phys. Chem. 63, 1241, (1959).

78. Neumann, P.M. and Prinz, P. J. Sci. Pd. Agric. 25, 221, (1974).

79. Shafrin, E.G. and Zisman, W.A. J. Phys. Chem. 64, 519, (1960).

80. Babiker, A.G.T., Cook, G.T. and Duncan, H.J. In 'Herbicides and Fungicides. Factors affecting their activity' (ed. McFarlane, N.R.)

The Chem. Soc. Special Publ No. 29, p93, (1977).

81. Robertson, M.M. and Kirkwood, R.C. Weed Res. 9, 224, (1969).