# Mechanisms of cuticular uptake of xenobiotics into living plants

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# ABBREVIATIONS

| | |
|---|---|
| 2,4-D | 2,4-dichlorophenoxyacetic acid |
| A | the area across which diffusion occurs ($m^2$) (Spread area of the droplet $mm^2$) |
| AI | active ingredient |
| c | concentration (mol $m^{-3}$) |
| C | concentration (nmol $\mu l^{-1}$) |
| $C_i$ | the AI concentration in the cuticle |
| $C_0$ | the AI concentration on the leaf surface |
| $C_{12}EO_3$ | triethylene glycol monododecyl ether |
| $C_{12}EO_6$ | hexaethylene glycol monododecyl ether |
| $C_{12}EO_{10}$ | decaethylene glycol monododecyl ether |
| CL | cuticular layer or secondary cuticle |
| CLSM | confocal laser scanning microscopy |
| CM | cuticular membrane |
| CMC | critical micelle concentration |
| CP | cuticle proper or primary cuticle |
| $\partial c/\partial x$ | (mol $m^{-4}$) the gradient of the concentration along the x-axis dimension. |
| D | diffusion coefficient ($m^2$ $s^{-1}$) |
| DOG | 2-deoxy-D-glucose |
| EC | emulsifiable concentrate |
| ECL | external cuticular layer |
| EO | ethylene oxide |
| EW | epicuticular wax |
| EWC | epicuticular wax crystals |
| EWF | epicuticular wax film |
| *f* | the amount penetrated at a given time (nmol) |
| ID | initial dose (nmol $mm^{-2}$) |
| J | flux per unit area (mol $m^{-2}$ $s^{-1}$) |
| K | the integral constant |
| $K_0$ | partition coefficient between the formulation residue on the leaf surface and the cuticle |
| $K_i$ | partition coefficient between the cuticle and the aqueous phase of the epidermal cell wall |
| *l* | tortuosity |
| M | the amount of solute (mol) |
| MM | molecular mass |
| mp | melting point |
| MR | molar refractivity |
| NP8 | nonylphenol with mean molar ethylene oxide content of eight |
| P | partition coefficient |
| PCW | primary cell wall |

## ABBREVIATIONS

| | |
|---|---|
| Pu | the unit partition ratio of the pesticide ($mm^{-2}$) |
| q | the penetration rate factor ($s^{-1}$) |
| RH | relative humidity |
| S | water solubility |
| SC | soluble concentrate |
| SCL | soluble cuticular lipids |
| SCW | secondary cell wall |
| TSE7.5 | Silwet L-77® (a trisiloxane ethoxylate with mean EO of 7.5) |
| t | time (s) |
| U | the total amount of penetration (i.e. the maximum uptake) (nmol) |
| V | the volume of the droplet applied (µl) |
| x | distance (m) |

# INTRODUCTION

There is a worldwide drive to reduce the quantities used and improve the field effectiveness of agrichemicals. This can be achieved by improving overall spray formulation efficacy, which is determined by the complex interactions of spray: deposition, retention to and uptake into the plant, as well as translocation to the site of biological activity within the plant. Such interactions are being addressed by developing models, based on fundamental processes. A review of the factors involved in spray formulation efficacy and their current stages of model development has been presented (Zabkiewicz, 2000, 2003). Models exist for deposition and retention, but uptake of agrichemicals into living plant foliage is complex and not yet well understood.

Adjuvants have proven beneficial in increasing the efficacy of agrichemicals, through their ability to improve the performance and consistency of the basic pesticide product. An adjuvant has been defined as "a formulant designed to enhance the activity or other properties of a pesticide mixture" (Holland, 1996). There are, broadly speaking, two routes by which adjuvants can do this. First is the minimization of off-target deposition and second, the maximization of the herbicidal effect once it is placed on the target (Reeves, 1989). The major contributors to off-target deposition are drift, in-flight volatilization, droplet shatter, bounce or runoff, washoff, and removal by wind. These losses result in pesticides never reaching the target or achieving only transitory deposit. There are two basic methods of maximising the effect of the pesticide once it is on the target. The first is to improve coverage by the spray solution, which can be accomplished by lowering the surface tension of the spray with surfactant materials. The second is by improving the penetration or uptake into the target.

Green and Hazen (1998) stated that "we often do not know how adjuvants enhance pesticide activity. We are studying interesting phenomena, but lack a comprehensive theory to predict performance or rationally select adjuvants". They concluded, "a more thorough mechanistic understanding of adjuvants will permit the development of new products with markedly superior properties. Despite some successes, we have not identified any set of universal physicochemical properties to enhance biological activity across pesticides, pests, and environmental conditions. Adjuvants need to be matched with specific applications". Coret and Chamel (1995) stated "while the mode of action of surfactants on retention of the droplets on plant surfaces can be explained from their effects on the surface tension of the

spray solution, that of surfactants on penetration of foliar-applied compounds remains largely unknown".

There have been no suitable models available to predict the uptake of agrichemicals into living plant foliage. Predictive models cannot be formulated without prior knowledge of the variables and mechanisms involved. Studies of the uptake of chemicals into plants have been largely empirical.

The objective of this PhD was to progress the understanding of the mechanisms of cuticular uptake into living plant foliage, thereby enabling uptake by important compounds such as pesticides and pollutants to be modelled. Intermediate or simple models were required that incorporated enough of the physical mechanisms of uptake to be realistic, but not so many mechanisms that the parameters were too numerous and their values impossible to determine.

There are two recognized pathways of uptake into plant foliage, stomatal infiltration and cuticular penetration. The focus of this PhD was cuticular penetration for two reasons. Firstly, the surface tensions typically provided by conventional surfactants in agrichemical sprays ($\geq$30 mN $m^{-1}$) are not low enough for stomata to be infiltrated. Secondly, stomatal infiltration occurs only in the brief period after application, while the spray deposit remains liquid, after which cuticular penetration remains the sole pathway of uptake (Stevens *et al.*, 1992).

There is general agreement that uptake through the leaf cuticle is a diffusion process (Price, 1982) and therefore this PhD considered diffusion as the predominant mechanism for uptake of formulations into plant foliage. The majority of studies available in the literature have considered uptake into whole plants in terms of percentage values. To enable a true and mechanistically more relevant comparison among xenobiotics, moles were considered a more appropriate unit of measure to use in the PhD studies than percent or mass. Although concentration has been used in models developed using isolated cuticles, when the initial solution deposit (droplet) rapidly becomes a deposit residue due to solvent evaporation, the initial droplet concentration becomes irrelevant. Hence, it was considered more appropriate to consider the dose (amount per unit area) either applied (Zabkiewicz and Forster, 2001) or over time on the leaf surface. For the current studies the dose applied was derived from a measurement of the spread area and molar amount of xenobiotic applied per droplet to give moles per unit area of xenobiotic applied.

Due to the large differences in behaviour between lipophilic and hydrophilic compounds observed in the literature, compounds ranging in lipophilicity were studied. As surfactants of different EO (ethylene oxide) chain length have been shown to have different

effects on different active ingredients, a range of surfactant EO chain lengths were studied, as well as an organosilicone surfactant with quite different spreading properties. In an attempt to explain differences in uptake among species, a range of species were investigated.

**Scope of Work**

This thesis consists of a literature review, four series of studies arranged into four independent chapters, followed by a generalised discussion. A published conference presentation and a published poster presentation are given in Appendix I and II respectively.

The objective of initial studies (Chapter 3) was to determine whether the uptake of model xenobiotics differing in lipophilicity and in the presence of a range of surfactants could be described by a novel but simple relationship involving the initial dose of xenobiotic applied. The objective of the studies outlined in Chapter 4 was to verify the relationship developed in Chapter 3, using three pesticides, applied as commercial and model formulations in the presence of a wide range of surfactants, into different plants. In a new approach further studies (Chapter 5) used this relationship to establish the relative importance of species, active ingredient (AI), AI concentration and surfactant to uptake. Chapters 3-5 considered uptake at only one time interval (24 hours). The objective of the research outlined in Chapter 6 was to study uptake at different time intervals over 24 hours and determine whether a logistic-kinetic penetration model developed using isolated plant cuticles could be applied to whole plant uptake. Extensions of the studies outlined in Chapter 3 were presented at an International conference (Appendix I and II). These papers set out to illustrate how the equations developed in Chapter 1 could be applied to estimate uptake per unit area, total uptake or percent uptake (Appendix I), and attempted to relate anomalies in the mass uptake relationship found at high concentrations to the presence of precipitates (Appendix II).

# LITERATURE REVIEW

## The Plant Cuticle

All primary aerial surfaces of vascular plants are covered by a thin, superficial film, the cuticle, which is composed of soluble and polymeric lipids (Jeffree, 1996). The most important function of the cuticle is the protection of land-living plants from desiccation (Schönherr, 1982) but it is also a barrier to the uptake of foliar-applied chemicals. The thickness of plant cuticles varies from less than 0.5 μm to more than 15 μm. The epicuticular waxes (EW), which form a waxy bloom on the surfaces of many plants, are conspicuous and readily accessible for investigation, and their properties, structure and chemistry have consequently been much studied (Baker, 1982; Jeffree, 1986; Riederer and Markstädter, 1996). Epicuticular waxes come in many diverse forms (platelets, granules, ribbons, rodlets etc.)(Chamel, 1986). The mechanism by which the EW is delivered to the plant surface remains unconfirmed (Jeffree, 1996, 2006). Atomic force microscopy has recently shown (Koch *et al.*, 2004) that regeneration of epicuticular lipids on living plant surfaces is a highly dynamic and comparatively swift process, but there was no visible evidence of the source of the wax. The structure of EW crystallites is predominantly a process of self-assembly, with the crystal morphology determined by dominant chemical constituents (Jeffree, 1974, 1986; Jeffree *et al.*, 1975, 1976; Holloway *et al.*, 1976; Lister and Thair, 1981; Jetter and Reiderer, 1994, 1995; Jetter *et al.*, 1996). The existence of an amorphous wax layer underlying the EW crystallites was proposed by Jeffree *et al.* (1975, 1976). However, the current consensus of opinion is that the film may be crystalline, even though it does not appear to be so in SEM images (Jeffree, 2006). Although the epicuticular waxes primarily govern the wettability of the plant surface (Holloway, 1970), they do not function as the primary permeability barrier, which is now believed to be governed by cuticular waxes embedded in cutin (Riederer, 1991; Schönherr and Mérida, 1981; Schreiber *et al.*, 1996a). The diffusion of non-electrolytes is thought to take place in an amorphous methylene group environment formed by cutin and the non-crystalline wax fraction (Schönherr, 1982; Reiderer and Schreiber, 1995; Baur *et al.*, 1997). There is evidence that wax embedded in the cuticle is chemically distinct from EW (Martin, 1960), and recent developments in methods of isolating the EW without contaminating the extract with intracuticular wax (Jetter *et al.*, 2000) have strengthened this view (Jeffree, 2006). Plant cuticular waxes are composed of varying amounts of mainly long-

chain alkanes, 1-alkanols, aldehydes, alkanoic acids, alkyl esters and additional classes of aliphatic substances (Merk *et al*, 1998). Cyclic constituents like triterpenoids and sterols may also be present (Tulloch, 1976; Holloway, 1984; Bianchi, 1995; Riederer and Markstädter, 1996). The structure of the plant cuticle is so heterogeneous that there is no typical cuticle (Holloway, 1982). However, an example schematic of a cuticle is given in Figure 2.1. This model cuticle is composed of the following layers: (a) the epicuticular wax crystals [EWC], (b) the epicuticular wax film [EWF], (c) the cuticle proper [CP] or primary cuticle composed of cutin and cutan, or various mixtures of them, as well as waxes, forming an electron-dense lamellae, (d) the cuticular layer [CL] or secondary cuticle, with an inner reticulate region, frequently forming the bulk of the cuticle when fully developed and which may or may not contain cellulose, (e) the primary cell wall [PCW] and (f) the secondary cell wall [SCW]. Holloway (1982) identified six structural types of cuticle: (1) lamellate CP, reticulate CL, (2) faintly lamellate CP, gradually merging with reticulate CL, (3) all regions reticulate, (4) all regions lamellate, (6) mainly amorphous. Holloway (1982) and Jeffree (1996) allocated species to cuticle types. Recently Jeffree (2006) proposed a seventh cuticle type having an amorphous CP with chaotically lamellate external cuticular layer (ECL) penetrated by intracuticular wax microfibrils.

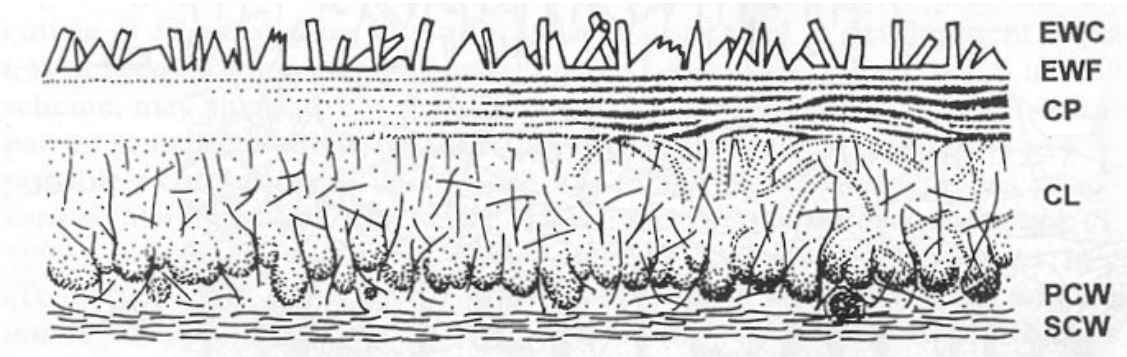



**Figure 2.1**. An example schematic of a plant cuticle composed of the following layers: (a) the epicuticular wax crystals [EWC], (b) the epicuticular wax film [EWF], (c) the cuticle proper [CP] or primary cuticle, (d) the cuticular layer [CL] or secondary cuticle, (e) the primary cell wall [PCW] and (f) the secondary cell wall [SCW]. From Jeffree (1996).

### *Pathways of uptake through plant cuticles*

*Stomatal infiltration*

Claims had been made that stomata were portals for the entry, by mass flow, of spray solutions into leaves (Sands and Bachelard, 1973). Schönherr and Bukovac (1972) indicated that pore infiltration was unlikely to occur when surface tension exceeded 30 mN $m^{-1}$. This was supported by subsequent work (Greene and Bukovac, 1974; Neumann and Prinz, 1974). Surface tensions typically provided by conventional surfactants in agrichemical sprays (≥ 30 mN $m^{-1}$) are not low enough for stomata to be infiltrated. A novel class of surfactant, organosilicone surfactants, can reduce aqueous surface tensions to 20 to 25 mN $m^{-1}$, which can induce stomatal infiltration. Stevens *et al.* (1991) quantified the contribution of the stomatal pathway by determining uptake at intervals as short as 10 min after application. It was found that the high level of uptake was reduced when stomatal aperture was manipulated using illumination, or complete closure of the stomata caused by leaf excision and wilting. Stomatal infiltration is readily observed using transmitted light, which makes waterlogged intercellular air spaces of the leaf visible (Stevens *et al.*, 1992). Buick *et al.* (1992) used fluorescent dye and UV microscopy to identify stomatal infiltration, while direct visual observation by confocal laser scanning microscopy (CLSM) has only recently been reported (Gaskin *et al.*, 1998; see Fig. 2.2). Stomatal infiltration can occur only in the brief period after application, while the spray deposits remain liquid. Thereafter, cuticular penetration remains as the sole pathway of uptake (Stevens *et al.*, 1992).

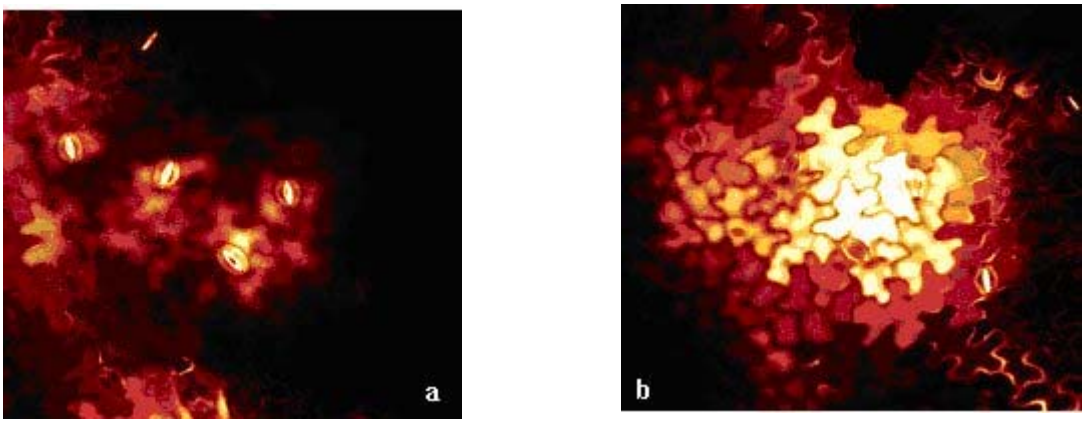



**Figure 2.2**. Confocal laser scanning microscopy (CLSM) images of bean foliage after treatment with organosilicone solution (0.5%): (a) near the adaxial surface of bean leaf with solution visible in stomatal pores and adjacent cells and (b) 20-25 μm below adaxial surface of bean leaf. From Gaskin *et al.*, 1998.

*Cuticular penetration*

Molecules penetrating plant cuticles have often been speculated to follow two different paths of diffusion. Stock and Holloway (1993) state that from existing information on the fine structure of plant cuticles (Holloway, 1982), it is possible to surmise on the whereabouts of any putative polar or apolar pathways. The apolar route, presumably, would be through the predominantly amorphous and polymeric cutin matrix with its associated embedded waxes. However a polar structural route through the cuticle could be provided via the network of polysaccharide fibrils (Stock and Holloway, 1993; Jeffree, 1996). Jeffree (2006) points out that although the reticulum (of polysaccharide microfibrils) demonstrably traverses the entire CL in *Agave* (Wattendorf and Holloway, 1980), and also in *Picea abies* (Tenberge, 1989, 1992) it stops short of the ECL in *Clivia*, which has notably low permeability to water (Schmidt *et al.*, 1981; Schmidt and Schönherr, 1982). Nevertheless, agrochemicals might be able to penetrate cuticles by more than one route, the relative amounts varying according to their physicochemical properties and the modifying influences of any added surfactants (Stock and Holloway, 1993).

Small, polar molecules such as water have been surmised to penetrate the cuticle via polar pores (Franke, 1967; Sekse, 1995). Wax microchannels were reported in freeze-etched cuticles of *Trifolium repens* (Hall, 1967), and Miller (1986) reported the existence of discrete, natural cuticular pores concomitant with anticlinally-oriented transcuticular canals in leaves of more than 50 species, but his observations were made with the light microscope. Electron microscopy has consistently failed to reveal transcuticular pores in the sense of open transcuticular channels via which wax, or other materials, might pass freely. Jeffree (2006) comments on the relative sizes of wax crystals and the cuticle and concludes that firstly the lengths (height) of the wax crystallites is typically greater than the thickness of the CP, and in some instances thicker than the entire CM, and secondly no trace of any pores of dimensions corresponding with the crystallites is ever visible. He points out that pores large enough to act as paths for wax extrusion could not hide undetected.

However, there is substantial experimental evidence that the plant cuticle behaves toward the penetrating molecules like a homogeneous membrane (Schönherr and Riederer, 1989; Kerstiens, 1996; Schreiber and Riederer, 1996). Schreiber and Riederer (1996) concluded that the fact that transport of small and polar water molecules was highly correlated with the D (diffusion coefficient) of lipophilic and comparatively large octadecanoic acid molecules indicated that there is only one diffusional path across the cuticular wax barrier

which must be taken by all molecules traversing the cuticle, irrespective of their size and their polarity. Niederl *et al*. (1998) provided further substantial evidence for the conclusions drawn above concerning the homogeneous structure of plant cuticles as transport barriers.

Nevertheless, although the concept of cuticular transport, via polar pores, has not been generally accepted, there is a growing body of evidence in the literature that advocates their existence (Schönherr, 2000; Schreiber *et al.*, 2001; Schönherr and Schreiber, 2004; Schlegel *et al.*, 2005; Schreiber, 2005, 2006). The evidence proposed for polar paths of transport is that although diffusion of charged substances like organic ionic compounds (e.g. glyphosate) and inorganic ions (e.g. $K^+$ and $Ca^{2+}$) across the lipophilic transport barrier of cuticles should not be possible (e.g. Briggs and Bromilow, 1994) it is well documented that they can penetrate both isolated astomatous cuticles as well as intact stomatous leaf cuticles (Schreiber, 2006). Schreiber (2006) outlines further evidence for a separate route compared to lipophilic molecules including the lack of effect of temperature, cuticular wax extraction, and plasticisers that enhanced lipophilic molecules, on the uptake of organic ionic compounds and inorganic ions. In addition, the dependence of cuticular penetration of lipophilic molecules on the size of molecules is much stronger (Buchholz *et al.*, 1998) compared to that of inorganic ions (Schönherr and Schreiber, 2004). Schreiber (2006) hypothesised that for water, being an uncharged polar molecule, both pathways of transport are probably accessible. However, the existence of polar pores for transport through the cuticle is still a controversial issue.

**Diffusion**

There is general agreement that uptake through the leaf cuticle is a diffusion process (Price, 1982). Diffusion is the net movement of molecules from an area of high concentration to an area of lower concentration until both concentrations are equal. In gases, diffusion processes are fast (10 cm $min^{-1}$), whereas they are much slower in liquids (0.05 cm $min^{-1}$) and solids (0.00001 cm $min^{-1}$) (Cussler, 1997).

The first mathematical treatment of diffusion was established by Fick (1855) who developed a law for diffusion in one dimension:

$$J = -Aj = -AD\,\partial c/\partial x \qquad (2.1)$$

Where J is the flux (mol $m^{-2}$ $s^{-1}$), j the flux per unit area (mol $s^{-1}$), A the area across which diffusion occurs ($m^{-2}$), D the diffusion coefficient ($m^2$ $s^{-1}$), c the concentration (mol $m^{-3}$), x the

distance (m) and $\partial c/\partial x$ (mol $m^{-4}$) the gradient of the concentration along the x axis. This equation is known as Fick's first law. In the case of diffusion without convection and a unitary area, eqn 2.1 can be written as:

$$J = -D\,\partial c/\partial x \qquad (2.2)$$

A simple way of considering the flux per unit area is the amount of a solute that diffuses through a unit area per unit of time, i.e.

$$J = M/At \qquad (2.3)$$

where M is the amount of solute (mol) , A the area across which diffusion occurs ($m^2$) and t is time (s). Uptake (U; mol $m^{-2}$) over a specific time (t) can be determined in terms of mol uptake per unit area, i.e.:

$$U = Jt = M/A = Dt\,\partial c/\partial x \qquad (2.4)$$

Fick's law has been modified for plant cuticles (Price, 1982; Schönherr and Baur, 1994, 1996) into

$$J = D(K_0C_0 - K_iC_i) / lx \qquad (2.5)$$

The term in the brackets is termed the "driving force" for diffusion. $K_0$ is the partition coefficient between the formulation residue on the leaf surface and the cuticle; $K_i$ is the partition coefficient between the cuticle and the aqueous phase of the epidermal cell wall; $C_0$ is the AI concentration on the leaf surface, while $C_i$ is the AI concentration in the cuticle (see Fig. 2.3). The effective cuticular thickness ($lx$) can be greater than the apparent or measured thickness ($x$). This is due to the fact that diffusion of xenobiotics is thought to take place only in the amorphous regions (Riederer and Schreiber, 1995). The crystalline regions are not accessible to the diffusing molecules, acting as impermeable barriers which increase the tortuosity and thus the length of the diffusion path (Buchholz *et al.*, 1998; Baur *et al.*, 1999).

Isolated cuticle studies have confirmed the factors involved in cuticular diffusion (Schönherr and Baur, 1994, 1996) and recently an excellent overview has been written on the different models that have been used to study cuticular penetration (Watanabe, 2002).

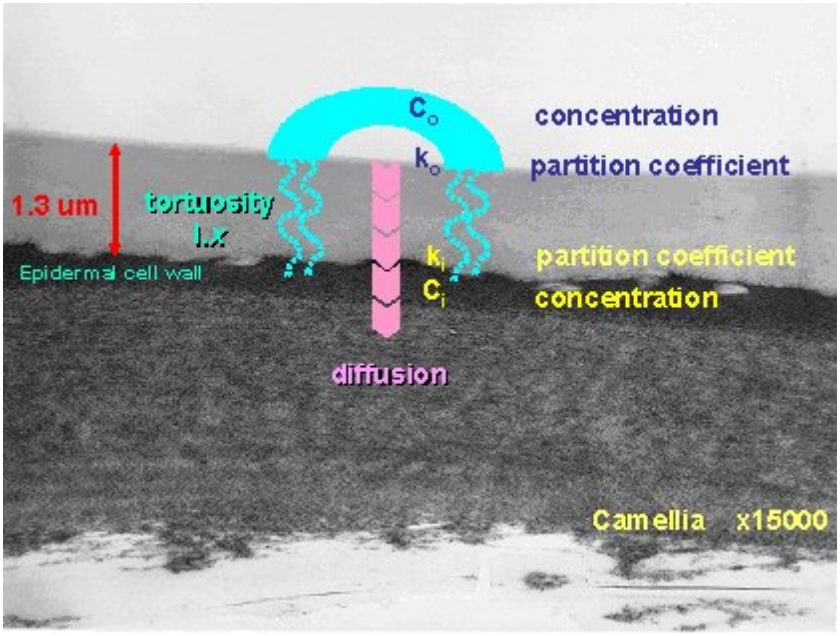



**Figure 2.3**. Schematic illustrating the factors involved in the diffusion of a compound across a plant cuticle (in this case, a camellia leaf cross section). From Zabkiewicz, 2003.

Although the variables in eqn 2.5 have been determined for isolated cuticles, it would be extremely difficult to derive them all using whole plant systems. It is easy to determine the amount of AI in the donor and receiver solution, on each side of an isolated cuticle (Schönherr and Baur, 1994), and therefore the amount within the isolated cuticle (or even determine the amount within the cuticle directly). However, quantifying the amount of active ingredient within just the cuticle in a whole plant system is not currently possible. It is clear that the mass or mol applied per unit area is important (Liu *et al.*, 1996).

Fick's first law of diffusion as modified for plant cuticles *in vitro* (eqn 2.5) may not be appropriate for *in vivo* situations where the applied quantity is a finite dose (from a droplet deposit). Watanabe (2002) found that the models dealing with non-equilibrium transcuticular penetration kinetics did not fully quantify all the kinetic parameters involved in penetration from a droplet, and he has recently developed a non-steady state, non-equilibrium model, termed "the logistic-kinetic penetration model".

Although concentration has been used in models developed using isolated cuticles, when the initial solution deposit (droplet) rapidly becomes a deposit residue due to solvent

evaporation, the initial droplet concentration becomes irrelevant. Hence, it may be more appropriate to consider the dose (amount per unit area) either applied (Zabkiewicz and Forster, 2001) or over time on the leaf surface.

**Uptake of hydrophilic versus lipophilic compounds**

If hydrophilic xenobiotics can traverse the cuticle, then it is hypothesised that there is an infinite sink due to translocation of the xenobiotic and uptake is directly related to concentration. With highly lipophilic xenobiotics, uptake is negatively correlated with concentration as the leaf (cuticle) is the sink. Hence the thicker the cuticle or heavier wax deposits, the higher the concentration that can be absorbed. Stevens (1984) used this reasoning to explain the effect of the concentration of 3 chemicals ranging in lipophilicity on the uptake into 4 plant species.

Many researchers have found that lipophilic compounds are rapidly sorbed by plant cuticles (Chamel, 1986; Schönherr and Reiderer, 1988; Chamel *et. al.*, 1991; Chaumat and Chamel, 1991; Chamel *et al.*, 1992; Santier and Chamel, 1992; Chamel and Vitton, 1996). However Baker and Chamel (1990) found that while diclofop-methyl (log P 4.6) was sorbed within the epidermis, movement of dinoseb (log P 4.1) through the epidermis was 6-fold that of atrazine (log P 2.05).

Santier and Chamel (1992) found that glyphosate was consistently weakly retained within cuticles isolated from all four species considered, confirming the low affinity of this hydrophilic herbicide for the lipid polymer.

**Effect of surfactants on the penetration of active ingredients into plant cuticles**

Many authors (Steurbaut and Dejonckheere, 1988; Steurbaut *et al*., 1989; Chamel *et al.*, 1992; Coret and Chamel, 1994) have found that low ethylene oxide (EO; EO3 - EO6) surfactants can significantly increase the transfer of lipophilic compounds across isolated plant cuticles (to a different extent among species). It has been proposed (Chamel *et al.*, 1992) that to exert their action on cuticular permeability the surfactants must be retained within the cuticle, therefore it could be assumed that cuticular sorption would be greater for the surfactant with the shorter chain length because it is more lipophilic. It has been observed that surfactants, in particular with a low degree of ethoxylation, have the potential to penetrate into the cuticle (Anderson and Girling, 1983; Stock *et al.*, 1992; Baur and Schönherr, 1997).

This is also supported by results with octylphenoxy surfactants showing that the degree of sorption for cuticles was inversely related to the degree of ethoxylation (Schafer *et al.,* 1989). Penetration of the surfactant will cause time dependent effects on both mobility and driving force, and the results indicate that distinct differences exist among the selected surfactants.

High EO (EO16 – EO40) surfactants have been found not to have a significant effect on the diffusion of lipophilic compounds across isolated cuticles (Chamel *et al.*, 1992; Coret and Chamel, 1994)

These results are in complete agreement with data reported for intact plants which showed that foliar penetration of lipophilic compounds was improved by surfactants with a short ethoxylated chain (Stevens and Bukovac, 1987; Holloway *et al.*, 1989; Stock, 1990; Holloway and Stock, 1990; Holloway *et al.*, 1992; Kirkwood *et al.*, 1992; Stock *et al.*, 1993).

It has been suggested (Coret and Chamel, 1994) that surfactants with short EO chains are not only smaller, less viscous compounds, but are generally more lipophilic. Therefore, their interaction with the hydrophobic components of the cuticle will be more intense. The authors said that this was shown in the case of the cuticular waxes by the more pronounced lowering of their melting point. Thus, better diffusion could result from an increase of fluidity or disorganisation of the cuticular waxes. Their small size may also allow them to diffuse more easily into recesses of the cutin polymer, thereby increasing its free volume which, according to Fujita (1968), should increase diffusion coefficients of solutes in the latter.

This was shown experimentally by Schreiber *et al.* (1996b) who studied the interaction of surfactants with the waxy transport barrier of cuticles using ESR-spectroscopy. It was found that a significant fraction of the spin probe was still in a rigid environment in the pure wax at 40°C, whereas in the presence of the C6E3, the spin probe was in a liquid environment, where it could rotate freely around its own axis. Thus, the different effects which are induced by the different surfactants on the mobilities of the investigated compounds are based on different degrees of fluidity of the wax environment where diffusion takes place. With those surfactants that reach higher concentrations in the wax, the wax barrier is obviously in a more fluid state at a given temperature compared with other surfactants. The authors concluded that under real conditions, foliar uptake occurs from highly concentrated residues, consisting of the formulation components and the active ingredient. Surfactant effects will become independent of the critical micelle concentration (CMC) and will be related to ratios of amounts of surfactants and wax and to surfactant mobility in waxes.

Further experimental evidence that monodisperse alcohol ethoxylates significantly increase the degree of disorder in cuticular waxes is available from NMR measurements

(Reynhardt and Riederer, 1991, 1994; Schreiber *et al.*, 1997). This is interpreted as an increase in fluidity of the amorphous fraction of the cuticular wax which in consequence leads to an increased mobility of the diffusion of the xenobiotic and therefore enhanced uptake (Reiderer, 2006).

The results of a study by Baur *et al.* (1997) showed that the amounts of surfactant sorbed in isolated cuticles decreased in the same order as their general effects on solute mobility.

Many authors (Stevens and Bukovac, 1987; Holloway *et al.*, 1989; Stock, 1990; Holloway and Stock, 1990; Gaskin and Holloway, 1992; Holloway and Edgerton, 1992; Stock *et al.*, 1993) have found that uptake of highly hydrophilic molecules (log P < -3.0) into intact foliage is enhanced best by surfactants of high EO content (EO15 – EO20), while surfactants of lower EO content can fail to improve, or even reduce uptake (Gaskin and Holloway, 1992). However, anomalies do exist with Baur (1999) concluding that there was no simple relationship between ethylene oxide content and surfactant effect on the uptake of methylglucose into isolated cuticles.

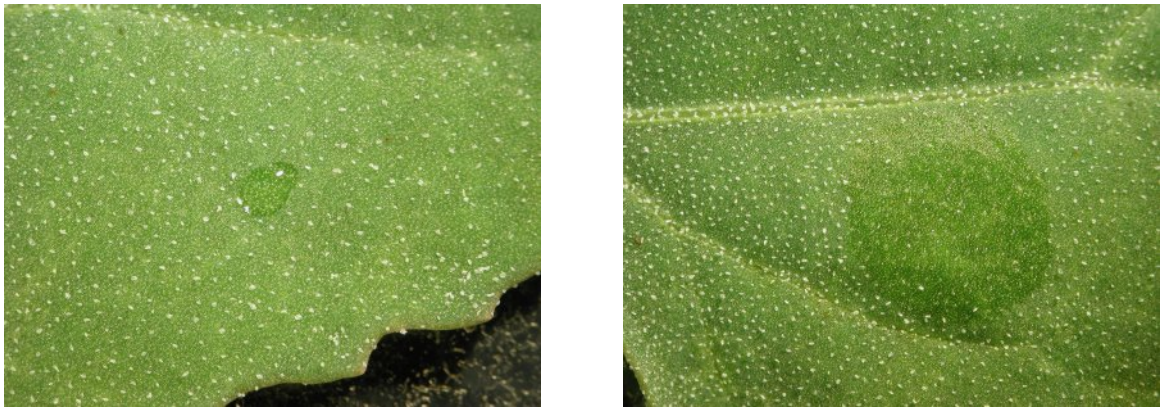
Compounds of intermediate lipophilicity have been found (Holloway *et al.*, 1989; Stock, 1990; Holloway and Stock, 1990, Stock *et al.,* 1993) to show little preference for surfactant EO content to maximise their foliage uptake.

Surfactants can prevent crystallisation of the active ingredient on the cuticle surface, thereby increasing its bioavailability, as has been reported in the case of glyphosate (MacIsaac *et al.*, 1991).

**Effect of dose (pesticide deposit per unit area) on uptake**

Dose (moles per unit area) applied is determined by both the concentration of the xenobiotic applied and the total spread area of the droplet. The addition of different surfactants can alter the spread area of a droplet significantly, and to different extents (eg. see Fig. 2.4). It has been stated (Holloway *et al.*, 1992; Stock and Holloway, 1993; Stock *et al.*, 1993) that there is no general correlation between the efficiency of surfactant-induced uptake and the apparent contact area of the corresponding deposit. Thus, it would appear that any spreading effects are incidental to this phenomena because a good activator surfactant may result in the formation of a deposit of the agrochemical covering only a small area, an intermediate area or even a large area of the target leaf (Stock and Holloway, 1993). However Stock and Holloway (1993) did acknowledge that uptake of lipophilic compounds is generally

favoured by the addition of nonionic surfactants with low mean molar ethylene oxide (EO) contents (5-6) which have higher surface activity and are, consequently, good spreaders. On the other hand, surfactants of higher EO contents (15-20), which have very poor spreading properties are the best activators for water-soluble compounds. Nevertheless, they said that surfactant EO content and spreading have little influence on the efficiency of uptake enhancement of compounds of intermediate polarity. However, other researchers have found the dose of AI applied to be important to uptake, particularly in the case of hydrophilic actives. Liu and Zabkiewicz (1997) found that uptake of glyphosate varied with both surfactant and glyphosate concentration, with glyphosate concentration per unit area being positively correlated with uptake.



**a** **b**

**Figure 2.4.** Example of the differences in spread area of DOG (0.075%) on a *Chenopodium album* leaf surface due to the droplets containing different surfactants. (a) contains $C_{12}EO_6$ and has a spread area of 1.2 $mm^2$ while (b) contains Silwet L-77 and has a spread area of 31.1 $mm^2$. From Forster *et al.*, 2003.

Gaskin and Holloway (1992) found that the concentration of glyphosate in formulations had a significant effect ($P < 0.001$) on absorption of the [$^{14}C$] AI by both wheat and field bean. At both sampling intervals studied, there was generally less uptake of radiolabel from formulations containing glyphosate at 0.5 g $litre^{-1}$ than from the higher concentrations, especially by field bean. There was no difference in uptake between concentrations of either 5 or 10 g $litre^{-1}$. However, the authors concluded that the study of surfactant-concentration-uptake relationships for glyphosate-mono(iso-propylammonium) provided little definitive information about the mechanisms by which particular surfactants improve uptake of the herbicide. Köcher and Kocur (1993) found that droplet spreading was paralleled by very low rates of foliar uptake of [$^{14}C$]glufosinate, but could not provide a reason for this.

## Uptake differences among plant species

A plethora of authors, in both the whole plant and isolated cuticle fields, have found significant differences in uptake among species (e.g. Chamel *et al.*, 1992; Santier and Chamel, 1996; Gouret *et al.*, 1993; Stevens, 1984; Price and Anderson, 1985; Baker, Hayes and Butler, 1992). Chamel and Vitton (1996) draw the conclusion that the structural characteristics of the cuticles are of major importance and must be given particular attention when attempting to establish models to predict the transfer of xenobiotics through plant cuticles. Price and Anderson (1985) concluded that the difference between the uptake patterns for the various species studied were large, and meant that it could not be assumed that high uptake of one formulation into a particular species would lead to similar behaviour in another.

## Previous attempts to model uptake *in vivo*

Organic compounds with a wide range of physicochemical properties are used as agrochemicals. Many studies have been performed in an attempt to relate the uptake of agrochemicals to the physicochemical properties of the active ingredients (AI's) and formulants.

An attempt to correlate AI physical properties with uptake into whole plants was undertaken by Stevens (1984). The study measured the uptake of glucose and 15 agrochemicals (dissolved in aqueous acetone) into four species (maize, rape, strawberry and sugar beet) and attempted to correlate the properties of the AI to uptake. However this study found that there was no simple relationship.

Baker *et al.* (1992) studied the uptake of 26 chemicals (herbicides, fungicides, growth regulators, insecticides and model compounds) formulated with and without Ethylan TU (NP8) surfactant into the same four species. Logarithms of physicochemical properties (partition coefficient [P], water solubility [S], and molecular weight [MW], together with deposit area and melting point [m.p.] values) were used to investigate relationships with uptake. Regressions using the entire group of 26 chemicals showed poor correlation between uptake and individual variables. Polynomials in log P, log S, log MW and m.p. were used to test for complex relationships. A quadratic polynomial in log P, log S and m.p. accounted for slightly more (77%) of the variability between means for total uptake than a cubic polynomial in log P and log S (75%). However, the authors concluded that, although both regressions

were significant, the 'lack of fit' indicated that other factors needed to be included to account fully for the variability in rates of uptake.

Stock (1990) developed a mathematical model to describe surfactant-induced uptake activation of neutral, model organic compounds. The uptake of methylglucose, phenylurea, cyanazine, WL110547 and permethrin could be combined in a complex matrix to relate log P, surfactant EO (ethylene oxide) content, surfactant concentration and molar refractivity (MR, a corrected form of molar volume), to uptake into either bean ($R^2 = 0.85$) or wheat ($R^2 = 0.79$). The surfactants used were all aliphatic alcohol ethoxylates. Stock felt that due to the empirical approach used, such equations would be of limited value in predicting actual uptake values on the basis of log P, MR and surfactant EO content. The inclusion of additional compounds of differing molecular properties would significantly influence the equations, necessitating re-analysis. In addition, the model was derived from uptake values at only one time interval. Stock stated that this approach had already been criticised because any variations in uptake kinetics were not taken into account; kinetics can vary due to both EO content and surfactant concentration for a particular compound-species combination.

The *in vivo* studies performed in an attempt to relate the uptake of agrochemicals to the physicochemical properties of the AI's and formulants, have been relatively unsuccessful since there have been no suitable models of this form available to predict the uptake of agrochemicals into plant foliage.

Although Fick's law of diffusion has been modified for plants (Price, 1982), this approach has historically not been used to study cuticular uptake into whole plants. Recently (Satchivi *et al.*, 2000a, 2000b) a dynamic non-linear simulation model has been developed for whole plant transport and allocation of foliar-applied xenobiotics. This model includes an equation describing the cuticular sorption process based on models developed by Schönherr and co-workers, with the addition of parameters describing surfactant effects and taking relative humidity into account. The difficulty with this model is the number of factors required, including many isolated cuticle factors (e.g. xenobiotic diffusion coefficient, wax/water partition coefficient, cuticle/water partition coefficient, thickness of the limiting skin, xenobiotic concentration in the formulation residue and in the cuticular membrane, and the critical micelle concentration of the surfactant). The validation of this model (Satchivi *et al.*, 2000b) did not distinguish among plant species, and a single cuticle thickness was used for all plant species. At present, a physical measurement cannot establish the "thickness" of the limiting skin, as this is not simply the thickness of the cuticle, or the wax layer, but the length of the diffusion path through the limiting skin (Riederer and Schreiber, 1995).

A method of modelling foliar uptake of pesticides using finite difference techniques has also been presented recently (Lamb *et al.*, 2001). Again numerous inputs are required for the model, such as diffusion coefficient, partition coefficient between droplet and cuticle and between cuticle and plant, cuticle thickness, droplet volume and diameter, and the duration of the experiment. Species differences were taken into account in this model in the form of cuticle thickness, and prediction versus actual uptake appeared good. However, this time dependent diffusion model is in one spatial dimension (the depth of the leaf) and does not consider droplet spread effects.

In conclusion, a very limited number of extremely detailed uptake models do exist (Satchivi *et al.*, 2000a, 2000b, 2001) but by their very nature they have a large number of parameters that tend to be plant and active ingredient specific that may be difficult, if not impossible to determine in most cases. The results from these types of models are often quite good but care must be taken that they are not used outside of their limitations (Trapp, 2004). What are lacking are intermediate models that have enough of the physical mechanisms incorporated into them to be realistic but not so many that the parameters are too numerous and their values impossible to determine.

# Mechanisms of cuticular uptake of xenobiotics into living plants: 1. Influence of xenobiotic dose on the uptake of three model compounds applied in the absence and presence of surfactants into *Chenopodium album*, *Hedera helix* and *Stephanotis floribunda* leaves.

## INTRODUCTION

Organic compounds with a wide range of physicochemical properties are used as agrochemicals. Many studies have attempted to relate the uptake of agrochemicals to the physicochemical properties of the active ingredients (AI's) and formulants.

One attempt to correlate AI physical properties with uptake into whole plants was undertaken by Stevens (1984). The study measured the uptake of glucose and 15 agrochemicals (dissolved in aqueous acetone) into four species (maize, rape, strawberry and sugar beet) and attempted to correlate the properties of the AI to uptake. However this study found that there was no simple relationship.

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Stock (1990) developed a mathematical model to describe surfactant-induced uptake activation of neutral, model organic compounds. The uptake of methylglucose, phenylurea, cyanazine, WL110547 and permethrin could be combined in a complex matrix to relate log *P*, surfactant EO (ethylene oxide) content, surfactant concentration and molar refractivity (*MR*), to uptake into either bean ($R^2$ = 0.85) or wheat ($R^2$ = 0.79). The surfactants used were all aliphatic alcohol ethoxylates. Stock felt that, due to the empirical approach used, such equations would be of limited value in predicting actual uptake values on the basis of log *P*, *MR* and surfactant EO content. The inclusion of additional compounds of differing molecular properties would significantly influence the equations, necessitating re-analysis. In addition, the model was derived from uptake values at only one time interval. Stock stated that this approach had already been criticised because any variations in uptake kinetics were not taken into account; kinetics can vary according to both EO content and surfactant concentration for a particular compound-species combination.

The *in vivo* studies performed in an attempt to relate the uptake of agrochemicals to the physicochemical properties of the AI and formulants have been relatively unsuccessful as there are currently no suitable models available to predict the uptake of agrochemicals into plant foliage.

There is general agreement that uptake through the leaf cuticle is a diffusion process (Price, 1982). The physical diffusion of substances through membranes or skins is described by Fick's law:

$$J = D\ \partial c/\partial x \qquad (3.1)$$

where J is the flux per unit area (mol $m^{-2}$ $s^{-1}$), D the diffusion coefficient ($m^2$ $s^{-1}$), c the concentration (mol $m^{-3}$), x the distance (m) and $\partial c/\partial x$ (mol $m^{-4}$) the gradient of the concentration along the x-axis dimension. A simple way of considering the flux per unit area is the amount of a solute that diffuses through a unit area per unit of time, ie

$$J = M/At \qquad (3.2)$$

where M is the amount of solute (mol), A the area across which diffusion occurs ($m^2$) and t is time (s). Uptake (U; mol $m^{-2}$) over a specific time (t) can be determined in terms of mol uptake per unit area, ie

$$U = Jt = M/A = Dt\ \partial c/\partial x \qquad (3.3)$$

Fick's law has been modified for plant cuticles (Price, 1982; Schönherr and Baur, 1994, 1996) into

$$J = D(K_0C_0 - K_iC_i) / lx \qquad (3.4)$$

The term in the brackets is termed the "driving force" for diffusion. $K_0$ is the partition coefficient between the formulation residue on the leaf surface and the cuticle; $K_i$ is the partition coefficient between the cuticle and the aqueous phase of the epidermal cell wall; $C_0$ is the AI concentration on the leaf surface, while $C_i$ is the AI concentration in the cuticle. The effective cuticular thickness ($lx$) can be greater than the apparent or measured thickness ($x$).

Isolated cuticle studies have confirmed the factors involved (Schönherr and Baur, 1994, 1996) and recently an excellent overview has been written on the different models that have been used to study cuticular penetration (Watanabe, 2002).

Although the variables in eqn (3.4) have been determined for isolated cuticles, it would be extremely difficult to derive them all using whole plant systems. It is easy to determine the amount of AI in the donor and receiver solutions on either side of an isolated cuticle (Schönherr and Baur, 1994), and therefore the amount within the isolated cuticle (or even determine the amount within the cuticle directly). However, quantifying the amount of active ingredient within just the cuticle in a whole plant system is not currently possible.

It is clear that the mass or mol applied per unit area is important (Liu *et al.*, 1996). A review by Knoche (1994) concluded that, for glyphosate, plant response consistently increased as carrier volume decreased, but for other herbicides performance generally decreased as carrier volume decreased. Caution should be applied when evaluating such trends, because overall herbicide efficacy is a combination of both retention by and uptake into plants, and such trends should not be confused with those which may be found for uptake (or retention) on their own.

It has been stated (Holloway *et al.*, 1992; Stock and Holloway, 1993; Stock *et al.*, 1993) that there is no general correlation between surfactant-enhanced uptake and the contact area of the corresponding deposit. This implies that any spreading effects are incidental, because a good surfactant may cause the formation of a deposit of the agrochemical over a small area, an intermediate area or even a large area of the leaf (Stock and Holloway, 1993).

Other researchers have found the dose of AI applied is important for better uptake, particularly in the case of hydrophilic actives. Liu and Zabkiewicz (1997) demonstrated that uptake of glyphosate varied with both surfactant and glyphosate concentration, with glyphosate mass per unit area being positively correlated with uptake.

Fick's first law of diffusion as modified for plant cuticles *in vitro* (eqn (3.4)) may not be appropriate for *in vivo* situations where the applied quantity is a finite dose (from a droplet deposit). Watanabe (2002) found that the models dealing with non-equilibrium transcuticular penetration kinetics did not fully quantify all the kinetic parameters involved in penetration from a droplet, and he has recently developed a non-steady state, non-equilibrium model (Watanabe, 2002), termed 'the logistic-kinetic penetration model'.

Although concentration has been used in such models, when the initial solution deposit (droplet) rapidly becomes a deposit residue due to solvent evaporation, the initial droplet concentration becomes irrelevant. In the present study, uptake is considered in relation to the

initial molar quantity applied per unit area, because this should be proportional to the initial driving force in eqn (3.4).

All research using isolated cuticles has, by necessity, used plants having thick cuticles that can readily be isolated. Many common weed species, such as *Chenopodium album* L, have thin cuticles and this plant has been studied as well as *Hedera helix* L and *Stephanotis floribunda* Brongn which are widely used in isolated cuticle work (Buchholz *et al.*, 1998).

The objective of our study was to determine whether the uptake of model xenobiotics differing in lipophilicity and in the presence of a range of surfactants could be described by a simple relationship involving the initial dose of the xenobiotic applied.

## MATERIALS AND METHODS

### Plant material

*Chenopodium album* (common lambsquarters; sourced from Valley Seed Service, Fresno, CA) plants were grown from seed in individual pots containing Bloom potting mix (Yates Ltd, NZ). Plants were raised under controlled environment conditions (70% RH and 14-h photoperiod, *ca* 500 μmol $m^{-2}$ $s^{-1}$). Growing conditions during the trials were: 23 °C/15 °C day/night. Plants were used at 3 weeks of age, from sowing. *Hedera helix* plants were grown from cuttings in individual pots containing Bloom potting mix. Plants were raised in a glass house and used at 6-9 months of age. *Stephanotis floribunda* plants were sourced from Arbours nursery NZ, and 1-year-old cuttings were used. Two weeks prior to use, the *H. helix* and *S. floribunda* plants were transferred into growth cabinets having controlled environment conditions that were the same as for the *C. album* plants.

### Chemicals

#### *Model compounds*

2-Deoxy-D-glucose (DOG; Aldrich Chemical Company, Inc.; 99% purity), 2,4-dichlorophenoxyacetic acid (2,4-D; Dow Agrosciences (NZ) Ltd.; 92% purity) and epoxiconazole [2RS,3SR)-1-[3-(2-chlorophenyl)-2,3-epoxy-2-(4-fluoro-phenyl)propyl]-1H-1,2,4-triazole; BASF; 96% purity] were studied over a wide range of concentrations (0.0088-148 g $litre^{-1}$ DOG, equating to 0.0129-217 nmol per 0.24-μl droplet; 0.034-50 g $litre^{-1}$ 2,4-D, equating to 0.037-54 nmol per 0.24-μl droplet; 0.039-4.34 g $litre^{-1}$ epoxiconazole, equating to 0.029-4.34 nmol per 0.24-μl droplet), including for each model compound a molar concentration (0.0045 *M*) close to that of the surfactants studied. The mass of the radiolabel was included in the calculation of the AI concentration when it accounted for > 1% of total AI

mass. All compounds were studied up to the limits of their solubility. The solubilities in water (20ºC) of DOG, 2,4-D and epoxiconazole are 100 (Fluka, 1999/2000), 0.620 (Kidd and James, 1993) and 0.0000663 (Kidd and James, 1993) g litre$^{-1}$ respectively; the Log P values are –2.69 (SRC's program online), 2.62 (SRC's program online) and 3.44 (Kidd and James, 1993), and the relative molecular masses are 164, 221 and 330.

***Surfactants***

Silwet L-77® (TSE7.5, a trisiloxane ethoxylate with mean EO of 7.5, supplied by GE Advanced Materials-Silicones), triethylene glycol monododecyl ether ($C_{12}EO_3$; > 99% purity) and hexaethylene glycol monododecyl ether ($C_{12}EO_6$; > 98% purity, both from Fluka) were used on all three plant species. Decaethylene glycol monododecyl ether ($C_{12}EO_{10}$; from Sigma, purified prior to use by HPLC to give > 90% purity) was used on *C. album* foliage only. All surfactants were studied at equimolar concentrations (0.0044 *M*, corresponding to 2.3 g litre$^{-1}$ TSE7.5, 1.4 g litre$^{-1}$ $C_{12}EO_3$, 2.0 g litre$^{-1}$ $C_{12}EO_6$ and 2.8 g litre$^{-1}$ $C_{12}EO_{10}$), approximating typical use rates. The relative molecular masses of the surfactants $C_{12}EO_3$, $C_{12}EO_6$, $C_{12}EO_{10}$ and TSE7.5 are 319, 451, 627 and 517 respectively. All xenobiotics were studied alone, and in the presence of each of the surfactants (excluding $C_{12}EO_{10}$ for *H. helix* and *S. floribunda*).

**Uptake**

2-Deoxy-D-(U-$^{14}$C)glucose (DOG; specific activity 11.5 GBq mmol$^{-1}$; Amersham UK), [*carboxy*-$^{14}$C]2,4-D (specific activity 0.751 GBq mmol$^{-1}$; Sigma) and [*chlorophenyl*-U-$^{14}$C]epoxiconazole (specific activity 1.09 GBq mmol$^{-1}$; BASF) were incorporated into treatments (added at *ca* 1400 dpm per droplet) prior to use. All solutions were made up in water + acetone (1:1 by volume). The use of 50% acetone:water for model uptake experiments is common, and this mixture is considered to have no significant effect on the uptake of the active ingredient (Stevens and Baker, 1987). This enabled higher concentrations of lipophilic xenobiotics to be studied, as well as the xenobiotic in the absence of a surfactant. Droplets of each solution (0.24 µl, ca. 770 µm diameter) were applied to the upper surface of the youngest fully expanded leaf of *C. album*, *H. helix* and *S. floribunda* (14 per leaf, with the exception of treatments containing TSE7.5 where 7 droplets were applied per leaf) on five separate plants per species, within 4 h of the start of the illumination period. The quantity of xenobiotic applied to each plant was determined by dispensing droplets (7 or 14) directly into scintillation vials (three replicates). Treated leaves were excised at 24 h after treatment.

Excised leaves were washed with water + ethanol (1 + 1 by volume; 2 x 4 ml) to recover unabsorbed DOG and epoxiconazole (both > 97% recovery on droplet dry-down), or water + acetone (1 + 3 by volume; 2 x 4 ml) to recover unabsorbed 2,4-D (95% recovery on droplet dry-down and >95% recovery from glass slides at 24 h). The washings were taken up in 13 ml of ACS II scintillant (Amersham International, UK) and the radioactivity quantified by scintillation counting (Packard 2100TR). Percentage uptake was determined as the proportion of the applied radiolabel not recovered by washing the treated leaves.

**Droplet spread area determination**

The droplet spread areas for the different formulations, on the three plant species, were measured under UV illumination using $V^{++}$ for Windows image analysis software (mean of 10 determinations). To visualise droplet spread, Blankophor-P fluor (5 g $litre^{-1}$; Bayer NZ) was incorporated into treatments containing DOG or epoxiconazole, while Uvitex NFW 450 (5 g $litre^{-1}$; Ciba Geigy) was incorporated into treatments containing 2,4-D.

**Statistical analyses**

The SAS general linear models (GLM) procedure was used to analyse the data, with least significant difference (LSD) tests used to compare treatments. Stabilising transformations were performed, where required, prior to analysis.

**RESULTS**

**Uptake of DOG formulations into *Chenopodium album***

All of the surfactants enhanced the uptake of DOG into *C. album* foliage (Table 3.1). The surfactants $C_{12}EO_6$ and $C_{12}EO_{10}$ showed the greatest enhancement. The results were variable and did not show a trend. It may be more appropriate, especially when comparing uptake among a range of chemicals, or when comparing different concentrations of a xenobiotic, to consider uptake in molar quantities. If uptake of DOG is calculated in nmol (Table 3.2), within each formulation uptake generally increases with increasing nmol applied. There is a definite trend, although some variability among results still exists. The major reason for this variability is that the addition of different surfactants to DOG caused the applied droplet to spread to a different extent (Table 3.3). Hence the dose applied (in nmol $mm^{-2}$) is different for each formulation and concentration. Spread areas of droplets for the different formulations can be taken into account by calculating the nmol applied per unit area.

This is calculated from the total amount applied in relation to the spread areas for the different formulations.

A plot of DOG applied against uptake (Fig 3.1) shows a strong relationship ($R^2 = 0.98$). The amount of DOG applied per unit area covered a wide range, and many of the lower values in Fig 1 overlap. To overcome this overlap, the data can be presented on a log scale (Fig 3.2). This is the format that is used in subsequent presentations of data.

**Table 3.1:** Percentage uptake of DOG from 0.24 µl droplet at 24 h into *Chenopodium album* foliage, applied in the presence or absence of surfactants (all at 1.056 nmol per 0.24 µl).

| DOG per droplet (nmol) | Uptake (%)[a] | | | | |
|---|---|---|---|---|---|
| | DOG alone | + $C_{12}EO_3$ | + $C_{12}EO_6$ | + $C_{12}EO_{10}$ | + TSE7.5 |
| 0.0128 | $18^{l}$ | $24^{ijkl}$ | $49^{de}$ | $82^{ab}$ | $37^{efgh}$ |
| 0.1108 | $9^{m}$ | $19^{kl}$ | $42^{efg}$ | $85^{a}$ | $30^{ghij}$ |
| 1.09 | $7^{m}$ | $35^{fghi}$ | $86^{a}$ | $88^{a}$ | $22^{jkl}$ |
| 10.86 | $5^{mn}$ | $28^{hijkl}$ | $96^{a}$ | $87^{a}$ | $28^{hijk}$ |
| 54.29 | $0.5^{o}$ | $46^{ef}$ | $96^{a}$ | $82^{ab}$ | $22^{jkl}$ |
| 108.58 | $0.4^{no}$ | $20^{hijkl}$ | $85^{a}$ | $67^{bc}$ | $22^{jkl}$ |
| 217.16 | ND[b] | $10^{m}$ | $28^{hijkl}$ | $64^{cd}$ | $30^{hij}$ |

[a]Treatments with no letter in common are significantly different ($P = 0.05$).

[b]ND = not done. This concentration of DOG, in the absence of surfactants, precipitated on application and was therefore withdrawn from the treatments.

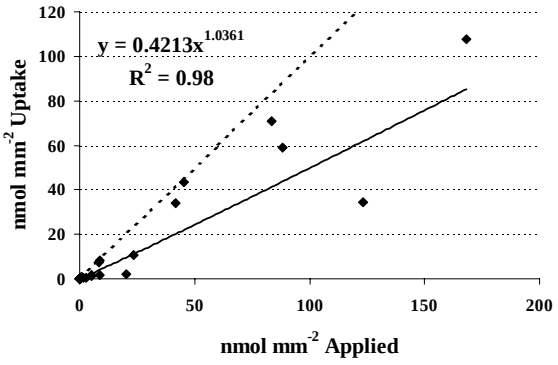


**Figure 3.1.** Uptake at 24 h of DOG into *Chenopodium album* foliage in the presence of the surfactants $C_{12}EO_3$, $C_{12}EO_6$, $C_{12}EO_{10}$ and TSE7.5 (--- maximum uptake line, representing 100% uptake over the initial dose range).

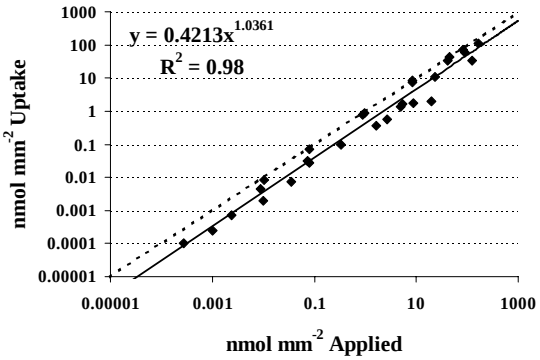


**Figure 3.2.** Uptake at 24 h of DOG into *Chenopodium album* foliage in the presence of the surfactants $C_{12}EO_3$, $C_{12}EO_6$, $C_{12}EO_{10}$ and TSE7.5 (--- maximum uptake line, representing 100% uptake over the initial dose range).

**Table 3.2:** Uptake of DOG from 0.24 µl droplet at 24 h into *Chenopodium album* foliage, applied in the presence or absence of surfactants (all at 1.056 nmol per 0.24 µl).

| | Uptake (nmol)[a] | | | | |
|---|---|---|---|---|---|
| DOG per droplet (nmol) | DOG alone | + $C_{12}EO_3$ | + $C_{12}EO_6$ | + $C_{12}EO_{10}$ | + TSE7.5 |
| 0.0128 | $0.0024^{s}$ | $0.0031^{s}$ | $0.0066^{qr}$ | $0.0112^{p}$ | $0.0048^{r}$ |
| 0.1108 | $0.01^{pq}$ | $0.0209^{o}$ | $0.0466^{mn}$ | $0.0937^{l}$ | $0.0329^{n}$ |
| 1.09 | $0.0722^{lm}$ | $0.3831^{j}$ | $0.9304^{h}$ | $0.9551^{h}$ | $0.2360^{k}$ |
| 10.86 | $0.5878^{ij}$ | $2.9933^{g}$ | $10.4680^{f}$ | $9.4370^{f}$ | $3.0526^{g}$ |
| 54.29 | $0.4656^{hi}$ | $24.7368^{e}$ | $52.0296^{cd}$ | $44.2595^{d}$ | $11.9861^{f}$ |
| 108.58 | $0.4250^{g}$ | $21.8469^{e}$ | $92.3712^{ab}$ | $72.3864^{bc}$ | $23.3985^{e}$ |
| 217.16 | ND | $21.6469^{e}$ | $60.5573^{cd}$ | $138.7680^{a}$ | $64.8939^{bcd}$ |

[a]Treatments with no letter in common are significantly different ($P = 0.05$).

[b]ND = not done. This concentration of DOG, in the absence of surfactants, precipitated on application and was therefore withdrawn from the treatments.

**Table 3.3:** Spread area of 0.24 µl droplets of DOG on the upper surface of *Chenopodium album* foliage, applied in the presence or absence of surfactants.

| | Spread area ($mm^2$)[a] | | | | |
|---|---|---|---|---|---|
| DOG per droplet (nmol) | DOG alone | + $C_{12}EO_3$ | + $C_{12}EO_6$ | + $C_{12}EO_{10}$ | + TSE7.5 |
| 0.0109 | $0.84^{no}$ | $12.76^{bcd}$ | $1.51^{h}$ | $1.34^{ij}$ | $47.32^{a}$ |
| 0.109 | $0.81^{o}$ | $10.92^{de}$ | $1.50^{h}$ | $1.36^{hi}$ | $46.51^{a}$ |
| 1.09 | $0.69^{q}$ | $13.76^{cd}$ | $1.21^{jk}$ | $1.11^{l}$ | $31.13^{abc}$ |
| 10.86 | $0.89^{n}$ | $2.16^{f}$ | $1.27^{ijk}$ | $1.29^{ijk}$ | $32.39^{abc}$ |
| 54.29 | $0.71^{p}$ | $2.31^{f}$ | $1.20^{kl}$ | $1.30^{ijk}$ | $32.5^{abc}$ |
| 108.58 | $0.96^{m}$ | $12.56^{bcd}$ | $1.30^{ijk}$ | $1.23^{jk}$ | $40.67^{ab}$ |
| 217.16 | ND[b] | $10.74^{e}$ | $1.76^{g}$ | $1.29^{ijk}$ | $40.61^{ab}$ |

[a]Treatments with no letter in common are significantly different ($P = 0.05$).

[b]ND = not done. This concentration of DOG, in the absence of surfactants, precipitated on application and was therefore withdrawn from the treatments.

### Uptake of 2,4-D formulations into *Chenopodium album*

All of the surfactants significantly enhanced ($P < 0.05$) the percentage uptake of 2,4-D, but unlike the case with DOG, there is little difference among the surfactant formulations. This is in agreement with published percentage uptake results for 2,4-D (Holloway *et al.*, 1989; Holloway and Stock, 1990; Stock, 1990; Stock *et al.*, 1993). The surfactant TSE7.5 enhanced the uptake of 2,4-D significantly ($P < 0.05$) more than $C_{12}EO_3$, while there was no significant difference among the other surfactants. The percent uptake of 2,4-D applied in the absence of surfactant was < 15% at all five concentrations studied, except at 1 g litre$^{-1}$ where 2,4-D uptake was 51%. Applied in the presence of the four surfactants, > 65% 2,4-D was taken up, except at the highest concentration of 2,4-D (50 g litre$^{-1}$), where uptake was between 21 and 32%). Again, the spread areas of the droplets on *C. album* foliage varied (0.87 – 44 mm$^2$) with formulation. Despite these variable effects, there was again a strong relationship ($R^2 = 0.98$) between molar uptake of 2,4-D per unit area and the amount applied per unit area (Fig 3.3).

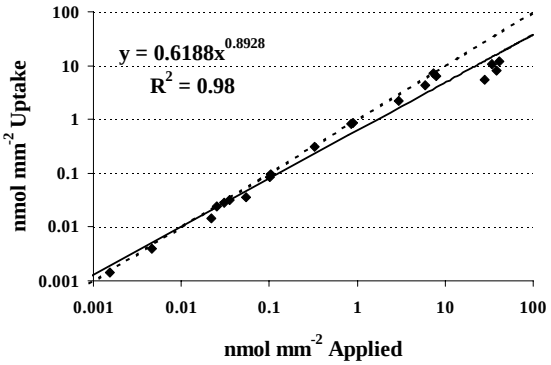


**Figure 3.3.** Uptake at 24 h of 2,4-D into *Chenopodium album* foliage in the presence of the surfactants $C_{12}EO_3$, $C_{12}EO_6$, $C_{12}EO_{10}$ and TSE7.5 (--- maximum uptake line, representing 100% uptake over the initial dose range).

### Uptake of epoxiconazole into *Chenopodium album*

All surfactants enhanced percentage uptake at the two lowest concentrations of epoxiconazole applied (59 and 17% uptake of epoxiconazole applied at 0.065 and 0.19 g litre$^{-1}$, respectively, in the absence of surfactant compared to >94% uptake for both concentrations of epoxiconazole in the presence of each of the four surfactants). However, only TSE7.5 enhanced uptake at the three highest concentrations of epoxiconazole (40, 17 and 30% uptake of epoxiconazole applied at 1.5, 2.99 and 5.97 g litre$^{-1}$, respectively, in the absence of surfactants compared to 92, 78 and 54% uptake in the presence of TSE7.5), except for $C_{12}EO_{10}$, which enhanced uptake (42%) at ca 2 nmol (2.99 g litre$^{-1}$) of epoxiconazole. As with DOG and 2,4-D, the formulations showed a range of droplet spread areas (0.69 – 37

$mm^2$) when applied to *C. album* foliage. The relationship ($R^2$ = 0.96) between uptake and dose applied for epoxiconazole is shown in Fig. 3.4.

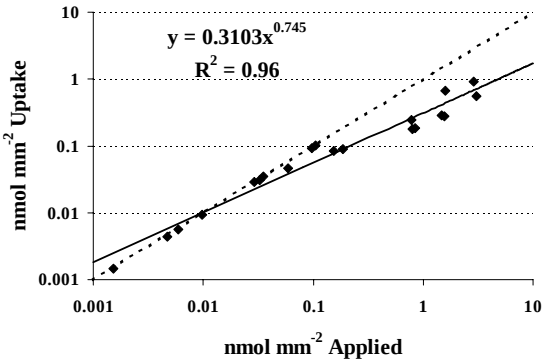


**Figure 3.4.** Uptake at 24 h of epoxiconazole into *Chenopodium album* foliage in the presence of the surfactants $C_{12}EO_3$, $C_{12}EO_6$, $C_{12}EO_{10}$ and TSE7.5 (--- maximum uptake line, representing 100% uptake over the initial dose range).

### Uptake of DOG, 2,4-D and epoxiconazole into *Hedera helix*

The surfactant $C_{12}EO_{10}$ was left out from this set of experiments, as the results with *C. album* had indicated that uptake and spread area of formulations containing $C_{12}EO_{10}$ and $C_{12}EO_6$ were similar.

Less than 53% of DOG was taken up into *H. helix* across all surfactants and concentrations, with less than 20% being taken up by DOG applied in the absence of surfactants. Taking the mean of all concentrations, only $C_{12}EO_6$ significantly ($P < 0.05$) enhanced the uptake of DOG. All surfactants significantly ($P < 0.05$) enhanced the uptake of 2,4-D into *H. helix*, with $C_{12}EO_6$ enhancing uptake to a greater extent than either $C_{12}EO_3$ or TSE7.5, which were not significantly different ($P > 0.05$). The highest uptake of 2,4-D applied in the absence of surfactants was 31%, while up to 98% was taken up in the presence of a surfactant. All surfactants significantly ($P < 0.05$) enhanced the uptake of epoxiconazole into *H. helix*, with $C_{12}EO_3$ enhancing uptake to a greater extent than TSE7.5, while there was no significant difference between $C_{12}EO_6$ and either $C_{12}EO_3$ or TSE7.5.

An excellent relationship was found to exist between the molar uptake of DOG per unit area into *H. helix* and dose of DOG applied in the presence of $C_{12}EO_3$, $C_{12}EO_6$ and TSE7.5 (Fig. 3.5; $R^2$ =0.98). This was also the case for 2,4-D ($R^2$ = 0.97; data not shown) and epoxiconazole ($R^2$ = 0.90; data not shown).

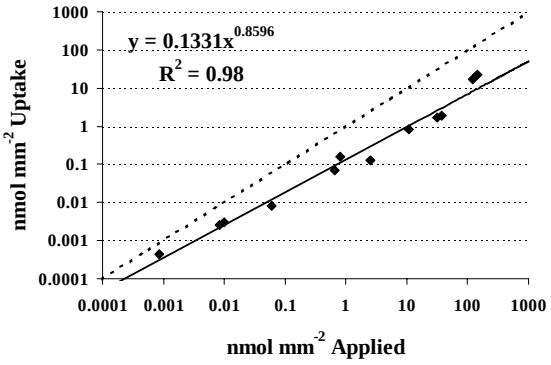


**Figure 3.5.** Uptake at 24 h of DOG into *Hedera helix* foliage in the presence of the surfactants $C_{12}EO_3$, $C_{12}EO_6$, and TSE7.5 (--- maximum uptake line, representing 100% uptake over the initial dose range).

## Uptake of DOG, 2,4-D and epoxiconazole into *Stephanotis floribunda*

Less than 47% of DOG was taken up into *S. floribunda* across all surfactants and concentrations. Taking the mean of all concentrations, there was no difference ($P > 0.05$) among formulations, including DOG applied in the absence of surfactants. The addition of surfactant only enhanced the uptake of DOG at one concentration (1.09 nmol per droplet in the presence of the three surfactants, among which there was no significant difference). Less than 43% of 2,4-D was taken up into *S. floribunda* across all formulations and concentrations. All surfactants enhanced ($P < 0.05$) the percentage uptake of 2,4-D, with overall no significant difference among the surfactants. All surfactants enhanced the uptake of epoxiconazole into *S. floribunda*, with $C_{12}EO_3$ and $C_{12}EO_6$ enhancing uptake the most. However, there was no significant difference between $C_{12}EO_6$ and TSE7.5. The highest uptake of epoxiconazole applied alone was 32%, compared to 84% in the presence of a surfactant. The addition of a surfactant did not enhance the uptake of epoxiconazole at the two highest concentrations (1.09 and 2.17 nmol per droplet) of epoxiconazole applied.

A good relationship was found to exist between the molar uptake of DOG per unit area into *S. floribunda* and dose of DOG applied in the presence of $C_{12}EO_3$, $C_{12}EO_6$ and TSE7.5 (Fig. 3.6; $R^2$ =0.87). This was also the case for 2,4-D ($R^2$ = 0.98; data not shown) and epoxiconazole ($R^2$ = 0.95; data not shown).

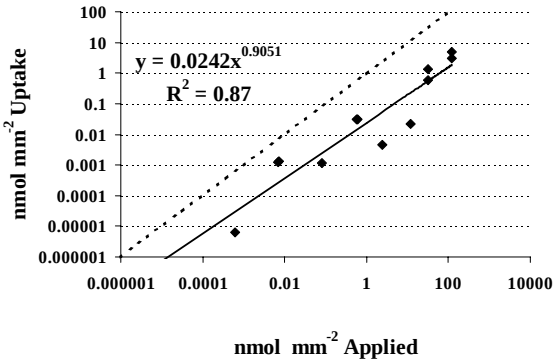


**Figure 3.6.** Uptake at 24 hours of DOG into *S. floribunda* foliage, in the presence of the surfactants $C_{12}EO_3$, $C_{12}EO_6$, and TSE7.5 (--- maximum uptake line, representing 100% uptake over the initial dose range).

## DISCUSSION

The failure to correlate physical properties of the AI with percentage uptake into a range of plant species is understandable with hindsight. Although the spray formulation may have distinct characteristics, after it is deposited on a leaf surface, dynamic interactions such as spreading and drying of the droplet, with concomitant changes in distribution and dose of both AI and formulants, lead to an initial 'spray deposit' that has little resemblance to the original solution. In particular the 'concentration' has been increased to a point such that the residue may be a quasi-solid and more akin to a deposit with minimal water content. Hence it may be appropriate to consider it as a mass of AI per unit area.

While the use of percent AI uptake is sufficient to compare uptake among formulations or to choose the 'best' formulation for a specific species, this can only be identified after actual experiments have been performed. Hence there is no predictive capability and this approach has not provided insights into the mechanisms of xenobiotic uptake into plants (Zabkiewicz and Forster, 2001). Attempting to relate uptake in percentage terms to the initial deposit (initial dose) also fails to provide any obvious correlation ($R^2 < 0.09$) as is illustrated by the data in Fig 3.7 for DOG formulations.

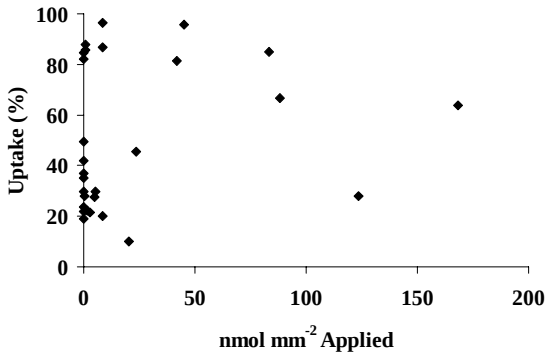


**Figure 3.7.** Percentage uptake of DOG vs. nmol $mm^{-2}$ applied into *Chenopodium album* foliage at 24 h, in the presence of the surfactants $C_{12}EO_3$, $C_{12}EO_6$, $C_{12}EO_{10}$ and TSE7.5.

The model developed by Stock (1990) was totally empirical, and was not based on Fick's law of diffusion. The criticism levelled at the Stock model, ie that the model was derived from uptake values at only one time interval, could also be levelled at the present approach as it only relates to one time period and does not take variations in uptake kinetics into account. These issues will be the focus of subsequent studies.

In the past, whole plant studies have not used Fick's law of diffusion as an approach by which to study cuticular uptake. Recently (Satchivi *et al.*, 2000a, 2000b) a dynamic non-linear simulation model has been developed for whole plant transport and allocation of foliar-applied xenobiotics. This model includes an equation describing the cuticular sorption process based on models developed by Schönherr and co-workers (eqn (4)) (Schönherr and Baur, 1994, 1996), with the addition of parameters describing surfactant effects and taking relative humidity into account. The difficulty with this model is the number of factors required, including many isolated cuticle factors (eg xenobiotic diffusion coefficient, wax/water partition coefficient, cuticle/water partition coefficient, thickness of the limiting skin, xenobiotic concentration in the formulation residue and in the cuticular membrane, and the critical micelle concentration of the surfactant). The validation of this model (Satchivi *et al.*, 2000b) did not distinguish among plant species, and a single cuticle thickness was used for all plant species. At present, a physical measurement cannot establish the 'thickness' of the limiting skin, as this is not simply the thickness of the cuticle, or the wax layer, but the length of the diffusion path through the limiting skin (Riederer and Schreiber, 1995).

Another method of modelling foliar uptake of pesticides has recently been presented (Lamb *et al.*, 2001). Again numerous inputs are required for the model, such as diffusion coefficient, partition coefficient between droplet and cuticle and between cuticle and plant, cuticle thickness, droplet volume and diameter, and the duration of the experiment. Species

differences were taken into account in this model in the form of cuticle thickness, and prediction versus actual uptake appeared good.

In the current study, a simple relationship has been developed for uptake per unit area in relation to the initial dose of xenobiotic applied. The mass uptake of all three xenobiotics, in the presence of several surfactants, can be plotted on a common scale and format (Fig 3.8). Thus the uptake of DOG, 2,4-D or epoxiconazole into *C. album*, *H. helix* or *S. floribunda* in the presence of surfactants can be described by a simple equation involving the initial dose of the xenobiotic applied:

$$\text{Uptake per unit area} = a\,[ID]^b \text{ at time } t = 24 \text{ h};$$

where ID is the initial dose or the mass of xenobiotic applied per unit area, given by mass applied, $M$ (nmol) divided by droplet spread area $A$ ($mm^2$). Total mass uptake is then calculated from an equation of the form:

$$\text{Uptake} = a\,[ID]^b.A.$$

It should be noted that, as the graphs use a log-log scale, a single equation for all three 'actives' would give large errors for points lying any distance from the line. Although a universal model that applies to different xenobiotics has not yet been achieved, the present approach does illustrate the principle that initial dose is a strong determinant of uptake. However, in reality, different formulations will have individual lines, rather than fitting one line through all surfactants studied with each xenobiotic. A single regression line used for each AI (including all surfactants) would still be a good indicator of uptake within the AI concentration range commonly used. Differences in regression line y-axis intercept and slope have been observed for different surfactants and species treatments (Forster *et al.*, unpublished) indicating that the influence of species and surfactant factors can be calculated. Although the current study used model compounds and formulations, ie all model compounds were made up in water + acetone (1 + 1 by volume), other formulations made up in water have been studied and the relationship has held true for a wider range of xenobiotics, surfactants and plant species (Forster *et al.*, unpublished).

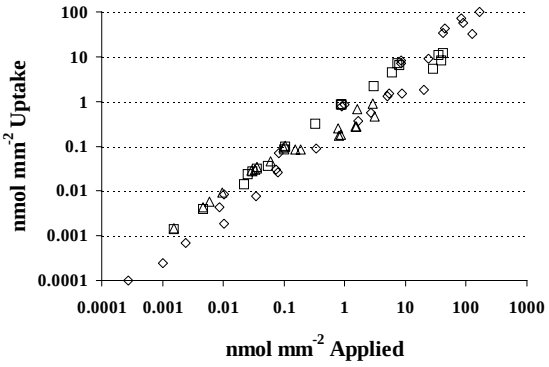


**Figure 3.8.** Uptake of (◇) DOG, (□) 2,4-D and (△) epoxiconazole into *Chenopodium album* foliage in the presence of the surfactants $C_{12}EO_3$, $C_{12}EO_6$, $C_{12}EO_{10}$ and TSE7.5.

## CONCLUSIONS

The current *in vivo* studies of xenobiotic uptake show clearly the dominance of the initial dose of xenobiotic applied in relation to its mass uptake. This positive determinant for uptake is applicable to hydrophilic and moderately lipophilic xenobiotics, in the presence of surfactants having a large range of lipophilicities.

Although a surfactant that causes a formulation to spread will decrease the dose of AI per unit area and therefore theoretically decrease the uptake per unit area compared to a formulation which spreads less, total uptake is the product of the uptake per unit area and spread area. Therefore total uptake may be less than, equal to, or greater than the formulation which spreads less. After taking into account differences among formulations due to spread area, differences in uptake due to the specific surfactant used are still significant. This also will be the subject of a future publication.

Few studies have considered such a range of concentrations, with most studies considering only a single xenobiotic concentration. Although the upper concentration limits used in this research are much higher than would be used in practice, it has provided a much better understanding of a significant determinant to uptake, i.e. initial dose. The lower concentrations studied here (for 2,4-D and epoxiconazole) are ones used operationally, and fit well with the current model.

The advantages of the approach presented here are that few variables are required, and they are simple to measure. In addition, the initial molar quantity applied per unit area has been shown to be proportional to the initial driving force, in line with Fick's law of diffusion modified for plants.

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# Cuticular uptake of xenobiotics into living plants. Part 2: Influence of the xenobiotic dose on the uptake of bentazone, epoxiconazole and pyraclostrobin, applied in the presence of various surfactants, into *Chenopodium album, Sinapis alba* and *Triticum aestivum* leaves

## INTRODUCTION

Although pesticide spray formulations may have distinct characteristics, once droplets are deposited on a leaf surface, dynamic interactions such as spreading and drying of the droplet cause changes in distribution and dose of both active ingredient (AI) and formulants. This 'spray deposit' has little resemblance to the original solution. In particular, the 'concentration' increases to a point where the residue may be a quasi-solid and more akin to a deposit with minimal water content. Hence, it is more appropriate to consider it as a mass of AI per unit area (initial dose).

A recent study (Forster *et al.*, 2004a) determined the uptake of three model compounds, applied in the presence or absence of four different surfactants, into the leaves of three plant species (*Chenopodium album* L., *Hedera helix* L. and *Stephanotis floribunda* Brongn). The mass uptake of 2-deoxy-D-glucose (DOG), 2,4-dichlorophenoxyacetic acid (2,4-D) and epoxiconazole {(2RS,3SR)-1-[3-(2-chlorophenyl)-2,3-epoxy-2-(4-fluorophenyl)propyl]-1H-1,2,4-triazole} in the presence of these surfactants demonstrated that the initial dose (in nmol $mm^{-2}$) of xenobiotic applied to plant foliage was a strong, positive determinant of uptake. This held true for all the xenobiotic formulations studied over a wide concentration range, even when they exceeded typical operational concentrations, although some anomalous results were obtained at higher dosages (Forster *et al.*, 2004b). The mass uptake on a per unit area basis could be related to the initial dose of xenobiotic applied by an equation of the form

$$\text{Uptake}_{(\text{nmol mm}^{-2})} = a\,[\text{ID}]^b \text{ at time t = 24 h}$$

where ID is the initial dose or the mass of xenobiotic applied per unit area ($M_{(\text{nmol xenobiotic applied})}/A_{(\text{mm}^2 \text{ droplet spread area})}$). The total mass uptake could be calculated from an equation of the form

$$\text{Total Uptake}_{(\text{nmol})} = a[\text{ID}]^b A$$

The objective of the current study was to confirm this relationship using representative, commercial pesticide formulations, made up in water (instead of water + acetone) at practical working concentrations (instead of very wide AI concentration ranges) with surfactants that were not pure homologues. Furthermore, the pesticides were in the form of water-dispersible, emulsifiable concentrate or suspension concentrate formulations. The AI dose per unit area generated in the present study was solely dependent on the surfactant and the surfactant

concentration used, rather than that obtained by changing the AI concentration. The present study also considered the influence of surfactant type and concentration relative to the AI product, as may be produced by adding surfactants to the initial tank mix formulation. The plants used, *Chenopodium album* L., *Triticum aestivum* L. and *Sinapis alba* L., were also representative of agricultural weeds or crops.

## MATERIALS AND METHODS

### Plant material

*Chenopodium album* (common lambsquarters; sourced from Valley Seed Service, Fresno, CA), *Sinapis alba* (*S. alba*; supplied by BASF Germany) and *Triticum aestivum* (wheat; supplied by BASF NZ) plants were grown from seed in individual pots containing Bloom potting mix (Yates Ltd., NZ). Plants were raised under controlled environment conditions (70% RH, 14 h photoperiod for *C. album* and *S. alba*, 12 h photoperiod for *T. aestivum*, ~ 400 μmol $m^{-2}$ $s^{-1}$). Growing conditions during the trials were: 25°C/15°C day/night for *C. album* and *S. alba* and 20°C/15°C day/night for *T. aestivum*. *Chenopodium album* and *T. aestivum* plants were used at 4 weeks of age, while *S. alba* plants were used at 2 weeks of age, from sowing.

### Chemicals

#### *Xenobiotics*

Bentazone (3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide was used as a commercial 480 $gL^{-1}$ SL (Basagran; BASF, Limburgerhof, Germany) at 1.9 g AI $L^{-1}$ (for uptake into *C. album*) and 0.25 g AI $L^{-1}$ (for uptake into *S. alba*). Bentazone formulations were prepared in phosphate buffer ($KH_2PO_4$, 2.632 g $L^{-1}$, + $Na_2HPO_4.12H_2O$, 7.163 g $litre^{-1}$, 5 + 95 by volume; pH 8). The pH was regulated in order to avoid producing different mixtures of undissociated and ionic molecules, depending on the pH of the water used. A higher carrier pH produces more anions, which have a higher water solubility and will stay in solution for a longer time.

Epoxiconazole [(2RS,3SR)-1-[3-(2-chlorophenyl)-2,3-epoxy-2-(4-fluorophenyl)propyl]-1H-1,2,4-triazole] was used as a commercial 125 g L-1 SC (Opus SC, BASF) at 0.83 g AI $L^{-1}$. Pyraclostrobin [methyl-N-{2-[1-(4-chlorophenyl)-1H-pyrazol-3-yloxymethyl]phenyl}-(N-methoxy)carbamate] was used as an experimental 250 g $L^{-1}$ EC supplied by BASF at 1.67 g AI $L^{-1}$.

### *Surfactants*

The surfactants and surfactant concentrations used with the different products and plants are listed in Table 4.1.

**Table 4.1.** Surfactants and surfactant concentrations used with the different products and plants

| Species | Product | Additional Adjuvants[a] | Structures (molecular mass range) |
|---|---|---|---|
| *C. album* | Bentazone SL (Basagran) | AO 3*, AO 5*, AO 7*, AO 10*, At 11*, At 25*, ON 30[x], ON 50[x], ON 60[x], ON 80[x], ON 110[x] | alkyl ethylene oxide (EO) polymers (290 - 1350) |
| | | O13E4P3[x], O13E4P9[x], O13E6P9[x], T16E6P3[x], T16E6P9[x], LF 300[x], LF 400[x], LF 401[x], LF 400s[x], LF 600[x], LF 1300[x], PE 6200*, PE 6400*, PE 6800*, XE 980[x] | alkyl ethylene oxide (EO)-propylene oxide (PO) block copolymers (620-8000) |
| | | AG 6202[x], Silwet L-77[x] | alkyl glycoside (ca. 292) trisiloxane ethoxylate (ca. 517) |
| *S. alba* | Bentazone SL (Basagran) | AO 3*, AO 5*, AO 7*, AO 10*, At 11*, At 25*, | alkyl ethylene oxide (EO) polymers (340-1350) |
| | | LF 400*, PE 6200*, PE 6400*, PE 6800*, XE 980* | alkyl ethylene oxide (EO)-propylene oxide (PO) block copolymers (650-8000) |
| *T. aestivum* | Epoxiconazole SC (Opus) | ON 30[#], ON 70[#], TO3[#], Emulan HE 50[#], Lutensol XL 500[#], Lutensol XL 700[#], | alkyl ethylene oxide (EO) polymers (290 - 500) |
| | | LF 300[•], XE 980[+], PE 6400[+], RPE 2035[#], Pluronic RPE 1740[#], | alkyl ethylene oxide (EO)-propylene oxide (PO) block copolymers (650 - 2900) |
| | | Agnique DAE 88 S/2013[#], | dioctyl ethylene oxide (EO) ether[b] |
| | | Agnique DAC 88[#], | dioctyl ethylene oxide (EO) carbonate[b] |
| | | Mapeg 400 DO PEG[#], ESA 49496[#] | polyethylene glycol ester[b] silicone surfactant[b] |
| *T. aestivum* | Pyraclostrobin EC (BAS 500 F) | ON 30[#], ON 70[#], TO 3[#], | alkyl ethylene oxide(EO) polymers (290-470) |
| | | LF 300[+], XE 980[#], PE 6400[+] | alkyl ethylene oxide (EO)-propylene oxide (PO) block copolymers (650-2900) |

[a: x] 1 and 5 g $L^{-1}$, * 5 g $L^{-1}$, [•] 0.417 and 0.833 g $L^{-1}$, [+] 0.417, 0.833 and 1.67 g $L^{-1}$, # 1.67 g $L^{-1}$, [b] undefined molecular mass

### Uptake

[*phenyl*-U-$^{14}$C]Bentazone (specific activity ca. 1.6 Gbq $mmol^{-1}$, BASF), was incorporated into treatments (at < 1% of mass) prior to use. Droplets of each solution (0.24

μL, ca 770 μm diameter) were applied to the upper surface of the fully expanded second leaf of *C. album* or the first pair of fully expanded true leaves of *S. alba* (8-16 droplets per leaf) within 4 h of the start of the illumination period. Each treatment contained five replicates (two or four leaves per replicate for *C. album*, and one or two leaves per replicate for *S. alba*)**.** The quantity of xenobiotic applied to each plant was determined by dispensing droplets (8-16) directly into scintillation vials (three replicates). Treated leaves were excised 24 h after treatment. Excised leaves were washed with water + methanol (1 + 1 by volume; 2 x 4 ml) to recover unabsorbed bentazone (> 95% recovery on droplet dry-down).

[*tolyl-ring*-U-$^{14}$C]Pyraclostrobin (specific activity ca 1.8 GBq mmol$^{-1}$) was incorporated into the pyraclostrobin EC formulation immediately prior to use. Droplets of each solution were applied to the third leaf of each *T. aestivum* plant (12 per leaf)**,** within 4 h of the start of the illumination period. Each treatment contained five replicates. The quantity of xenobiotic applied to each plant was determined by dispensing droplets (12) directly into scintillation vials (three replicates). Treated leaves were excised and washed as before to recover unabsorbed pyraclostrobin (100% recovery on droplet dry-down).

The washings from both the bentazone and the pyraclostrobin studies were taken up in 13 mL ACS II scintillant (Amersham International, UK) and the radioactivity quantified by scintillation counting (Packard 2100TR). The percentage uptake was determined as the proportion of the applied radiolabel not recovered by washing the treated leaves.

Water suspensions of epoxiconazole (Opus SC with added surfactants) were applied (20 droplets, 0.24 μL) onto the third leaf of *T. aestivum* plants. Every treatment consisted of five replicates. Treated leaves were excised 24 h after treatment and washed with water + acetonitrile (2 + 3 by volume; 2 x 4 mL) to recover unabsorbed epoxiconazole (> 96% recovery on droplet dry-down). Uptake of epoxiconazole was determined by HPLC using a Phenomenex column (150 x 4.6 mm, C18 5 μm), a water + acetonitrile (40 + 60 by volume) mobile phase, flow rate of 0.9 mL min$^{-1}$ and UV detection (225 nm).

**Droplet spread area determination**

The droplet spread areas for the different formulations, on the three plant species, were measured under UV illumination using V$^{++}$ for Windows image analysis software (mean of ten determinations). To visualise droplet spread, Blankophor-P fluor (5 g L$^{-1}$; Bayer NZ) was incorporated into treatments.

## RESULTS

### Uptake of bentazone formulations into *Chenopodium album*

The percentage uptake of bentazone into *C. album*, applied in the presence of different surfactants (28 surfactants at 5 g $L^{-1}$, 19 surfactants at 1 g $L^{-1}$; molecular masses from 290 to 8000), ranged from 27 to 98% (data not shown), with the droplet spread areas ranging from 0.55 to 72 $mm^2$ (data not shown). A plot of percentage uptake versus initial dose (nmol $mm^{-2}$) did not show any coherent relationship ($R^2 < 0.25$; data not shown). It has been shown recently (Forster *et al.*, 2004a) that mass uptake into plants can be represented in a different, more generic format by plotting nmol xenobiotic applied versus nmol uptake per $mm^{-2}$, which shows excellent correlation. However, a plot of nmol $mm^{-2}$ of bentazone applied versus uptake (nmol $mm^{-2}$) (Fig. 4.1) showed a relatively poor relationship ($R^2 = 0.57$).

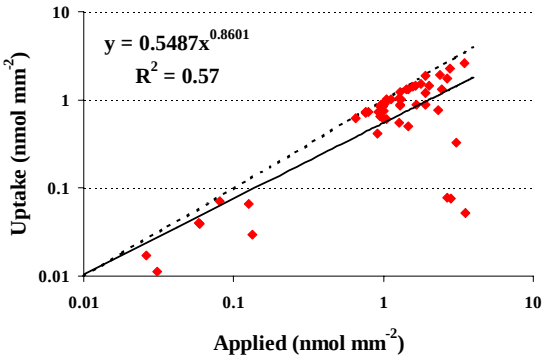


**Figure 4.1.** Uptake at 24 h of bentazone into *Chenopodium album* foliage in the presence of a range of surfactants (see Table 1) at 1 and 5 g $L^{-1}$ ( --- maximum uptake line, representing 100% uptake over the initial dose range). Each point represents the average of five replicates.

The reason for this was primarily the low uptake of bentazone when applied in the presence of the highest molecular mass surfactants. Removing the mass uptake results for bentazone applied in the presence of the four surfactants having a molecular mass ≥ 1350 (At 25, PE 6200, PE 6400 and PE 6800 of 1350, 2450, 2900 and 8000 molecular mass respectively) significantly improved the relationship ($R^2 = 0.94$) (Fig. 4.2). Removing the same results from the percentage uptake versus initial dose comparison reduces the relationship even further ($R^2 < 0.06$; data not shown). This is again in agreement with previous studies (Forster *et al.*, 2004a) where uptake in percentage terms relative to the initial dose of model compounds, applied in the presence of a range of surfactants, failed to provide any obvious correlation. For this reason, as in a previous paper by the present authors (Forster *et al.*, 2004), percentage uptake versus initial dose will not be considered further in this paper. The relationship between the amount of bentazone applied and the amount of uptake per unit area is even

stronger for formulations containing 5 g $L^{-1}$ surfactant ($R^2$ = 0.98) and lies closer to the maximum uptake line, compared with formulations containing only 1 g $L^{-1}$ surfactant ($R^2$ = 0.88) (Fig. 4.3). These results and trends confirm the relationship between nmol $mm^{-2}$ of xenobiotic applied and uptake per unit area presented previously (Forster *et al.*, 2004), but demonstrate also a surfactant concentration and surfactant molecular weight influence.

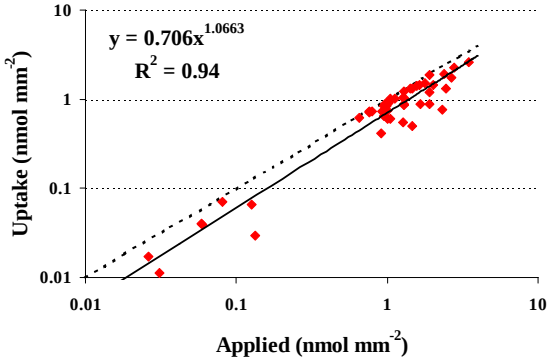


**Figure 4.2.** Uptake at 24 h of bentazone into *Chenopodium album* foliage in the presence of a range of surfactants (see Table 4.1) at 1 and 5 g $L^{-1}$, excluding surfactants with molecular masses of ≥ 1350 (--- maximum uptake line, representing 100% uptake over the initial dose range). Each point represents the average of five replicates.

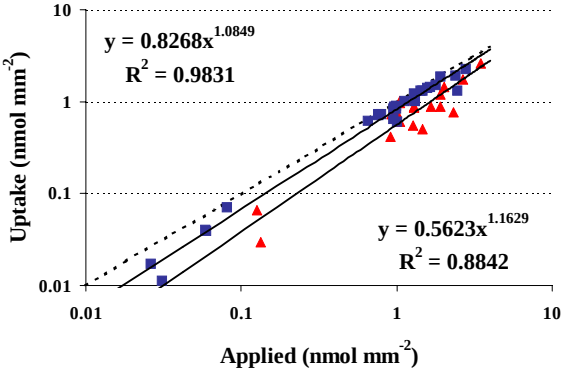


**Figure 4.3.** Uptake at 24 h of bentazone into *Chenopodium album* foliage, in the presence of a range of surfactants (see Table 4.1) at (▲) 1 and (■) 5 g $L^{-1}$ excluding surfactants with a molecular mass ≥ 1350 (--- maximum uptake line, representing 100% uptake over the initial dose range). Each point represents the average of five replicates**.**

### Uptake of bentazone formulations into *Sinapis alba*

The percentage uptake of bentazone into *S. alba*, applied in the presence of a range of surfactants (11 surfactants at 5 g $L^{-1}$; molecular mass 340-8000), was > 86% except for the highest molecular mass surfactant, which was only 57% (data not shown). Droplet spread

areas ranged from 1.28 to 2.13 $mm^2$ (data not shown). A plot of nmol $mm^{-2}$ of bentazone applied versus uptake (nmol $mm^{-2}$) (Fig. 4.4) showed a poor relationship ($R^2$ = 0.42). The poor relationship was due to the highest molecular mass surfactant (PE 6800, molecular mass 8000) causing a much lower uptake of bentazone, and, with this data point removed (Fig. 4.5), the relationship was again excellent ($R^2$ = 0.96). The three surfactants with a molecular mass of >1350 but < 8000, which gave much lower than predicted uptake into *C. album*, provided excellent uptake into *S. alba*. Surfactant molecular mass influences were not as evident in this plant species.

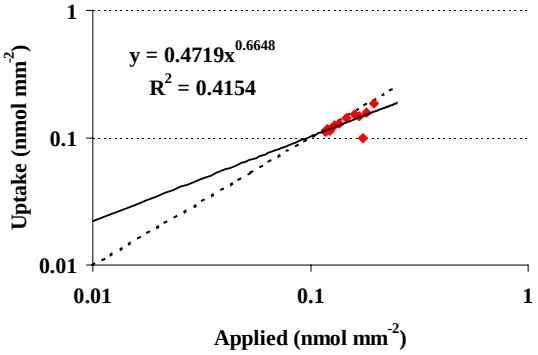


**Figure 4.4.** Uptake at 24 h of bentazone into *Sinapis alba* foliage in the presence of a range of surfactants (see Table 1) at 5 g $L^{-1}$ (--- maximum uptake line, representing 100% uptake over the initial dose range). Each point represents the average of five replicates.

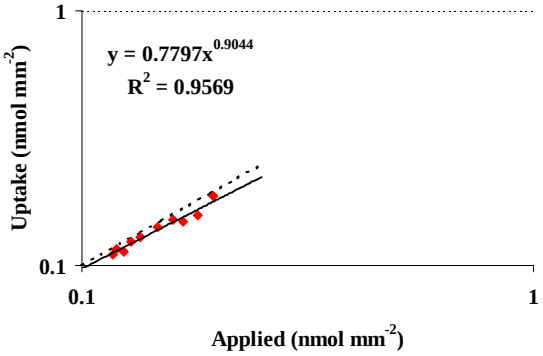


**Figure 4.5.** Uptake at 24 h of bentazone into *Sinapis alba* foliage in the presence of a range of surfactants (see Table 1) at 5 g $L^{-1}$, excluding the surfactant with a molecular mass of 8000 (--- maximum uptake line, representing 100% uptake over the initial dose range). Each point represents the average of five replicates.

## Uptake of epoxiconazole from SC formulations into *Triticum aestivum*

The percentage uptake into *T. aestivum* of epoxiconazole, applied in the presence of different surfactants (15 surfactants at 0.167-1.67 g $L^{-1}$, a total of 20 formulations; molecular mass 290-2900, ranged from 3 to 69% (data not shown). Droplet spread areas ranged from

0.64 to 19.83 $mm^2$ (data not shown). A plot of nmol $mm^{-2}$ of epoxiconazole applied versus uptake (nmol $mm^{-2}$) (Fig. 4.6) showed a relationship ($R^2$ = 0.71) which was again improved ($R^2$ = 0.92) (Fig. 4.7) by removing the highest molecular mass surfactant uptake data (PE 6400, molecular mass 2900; three concentrations).

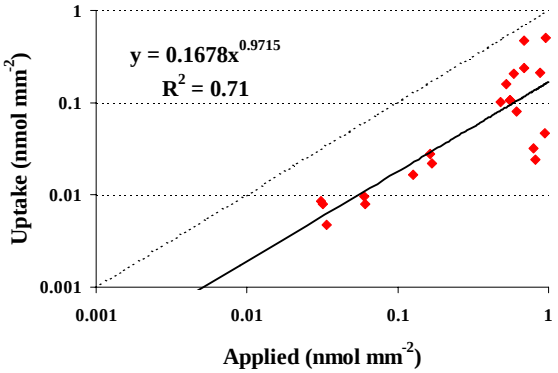


**Figure 4.6.** Uptake at 24 h of epoxiconazole into *Triticum aestivum* foliage in the presence of a range of surfactants (see Table 4.1) at 0.417 – 1.67 g $L^{-1}$ (--- maximum uptake line, representing 100% uptake over the initial dose range). Each point represents the average of five replicates.

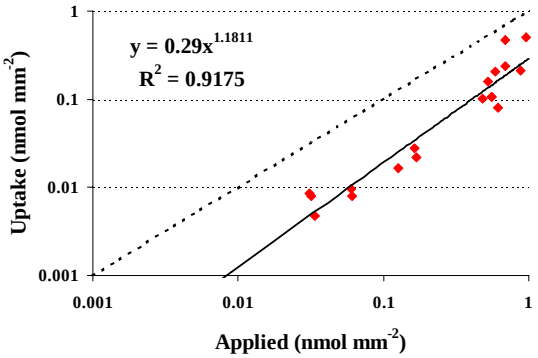


**Figure 4.7.** Uptake at 24 h of epoxiconazole into *Triticum aestivum* foliage in the presence of a range of surfactants (see Table 4.1) at 0.417 – 1.67 g $L^{-1}$, excluding formulations containing the surfactant with a molecular mass of 2900 (--- maximum uptake line, representing 100% uptake over the initial dose range). Each point represents the average of five replicates.

## Uptake of pyraclostrobin from EC formulations into *Triticum aestivum*

The percentage uptake into *T. aestivum* of pyraclostrobin, applied in the presence of different surfactants (six surfactants at 0.0417-0.167 g $L^{-1}$, a total of ten formulations; molecular mass 290-2900), ranged from 9 to 31% (data not shown). Droplet spread areas ranged from 0.79 to 9.09 $mm^2$ (data not shown). A plot of nmol $mm^{-2}$ of pyraclostrobin applied versus uptake (nmol $mm^{-2}$) (Fig. 4.8) showed an excellent relationship ($R^2$ = 0.96).

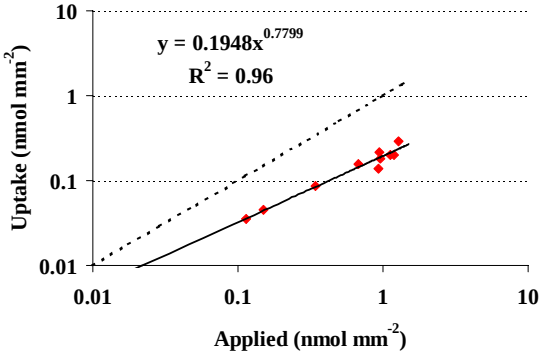


**Figure 4.8.** Uptake at 24 h of pyraclostrobin into *Triticum aestivum* foliage in the presence of a range of surfactants (see Table 4.1) at 0.417 – 1.67 g $L^{-1}$ (--- maximum uptake line, representing 100% uptake over the initial dose range). Each point represents the average of five replicates.

### Uptake of bentazone, epoxiconazole and pyraclostrobin formulations

The uptake of bentazone into *C. album* and *S. alba*, as well as epoxiconazole and pyraclostrobin into *T. aestivum*, can be shown on one graph (Fig. 4.9). This is an excellent presentation format where data covering a wide range of formulations, actives and plant species can be presented and compared, which is not possible in the conventional use and presentation employing percentage uptake.

### Droplet spread area influences

Although a surfactant causing a formulation to spread will decrease the dose of AI per unit area and therefore theoretically decrease the uptake per unit area, the total mass uptake is the product of the uptake per unit area and the spread area. Therefore, the total mass uptake from a formulation that spreads well may be less than, equal to or greater than that from a formulation which spreads less well. The general equation that can account for this behaviour is of the form

$$\text{Total Uptake}_{(\text{nmol})} = a[\text{ID}]^{b}A$$

as identified previously.[1] The application of this equation is illustrated by the data in Table 2, which shows the calculated mass uptake of bentazone (at 24 h) into *C. album* in the presence of four surfactants (XE 980, AO 3, ON 60 and ON 30) that provided completely different spread areas. The total amount applied was ~ 1.9 nmol of bentazone in this example, and, owing to droplet spread, the nmol $mm^{-2}$ applied ranged from 0.0311 to 1.4598. The equations used to calculate uptake are based on the average trend line for bentazone in the presence of

all of the surfactants used (excluding high molecular mass outliers as discussed previously). All data points between 0.01 and 0.1 nmol $mm^{-2}$ or 1 and 10 nmol $mm^{-2}$ were used, depending on the initial dose of bentazone. Since ON 30 provided a significantly greater spread area than XE 980 (by a factor of 47), it provided a much lower initial dose and the calculated uptake per unit area was much lower. When the total uptake was calculated (by multiplying the uptake per unit area by the spread area), XE 980 still provided significantly greater total uptake of bentazone (by a factor of ~2). In contrast, although AO 3 provided significantly greater spread area than XE 980 (by a factor of 18) and hence a much lower initial dose and lower calculated uptake per unit area, the calculated total uptake was similar to that with XE 980. Using this approach and these parameters, the calculated percentage uptake could also be obtained and compared with the actual percentage uptake (Table 4.2). There are some discrepancies that may again be due to specific surfactant structure, amount or interaction with the leaf structure. Hence, such equations based only on spread areas and initial dose are as yet inadequate, to provide a model for all types of xenobiotic formulation.

## DISCUSSION

The present study confirms that initial dose (which is influenced by the spread area) is a strong positive determinant for mass uptake per unit area, as demonstrated previously with model xenobiotic and plant systems (Forster *et al.*, 2004). Although the strong influence of the initial dose, equivalent to the 'driving force' in models of xenobiotic uptake in isolated cuticle studies (Schönherr and Baur, 1994), is being confirmed by the present results, other factors must have an influence. These will include species (or cuticular differences), xenobiotic structure (whether active ingredient or adjuvant) and adjuvant concentration. This study also shows the potential application of regression equations, derived from the mass uptake and initial dose relationships, to predict the uptake of an AI in the presence of a wide range of surfactants. These regression equations are based on the initial dose, with only the change in spread area caused by the different surfactants as a 'surfactant factor'. Calculated versus actual percentage uptake at 24 h was in good agreement overall. However, after taking into account spread area differences among formulations, the variation in uptake owing to the specific surfactants or concentrations used is still substantial.

Although it has been stated previously (Holloway *et al.*, 1992; Stock and Holloway, 1993; Stock *et al.*, 1993) that there is no general correlation between surfactant-enhanced uptake and the contact area of the corresponding deposit, this study demonstrates that (a) the

droplet spread area determines the initial dose produced, (b) the surfactant concentrations affect uptake and (c) the surfactant structures and / or molecular mass affect mass uptake.

**Table 4.2.** Actual and calculated uptake of bentazone into *Chenopodium album* at 24 h in the presence of four surfactants XE 980, AO 3, ON 60 and ON 30 at 5 g $L^{-1}$

| | Bentazone into *C. album* | | | |
|---|---|---|---|---|
| | XE 980 | AO 3 | ON 60 | ON 30 |
| Total applied (nmol) | 1.8978 | 1.8978 | 1.8978 | 1.8978 |
| Spread area (A) ($mm^2$) | 1.3 | 23.6 | 31.8 | 61 |
| Initial dose (nmol $mm^{-2}$) | 1.4598 | 0.0804 | 0.0598 | 0.0311 |
| Unit area uptake equation[a] (nmol $mm^{-2}$) | $y = 0.9297(ID)^{0.8809}$ | $y = 2.6403(ID)^{1.4722}$ | $y = 2.6403(ID)^{1.4722}$ | $y = 2.6403(ID)^{1.4722}$ |
| **Calculated** Unit area uptake (nmol $mm^{-2}$) | 1.297 | 0.0646 | 0.0418 | 0.0159 |
| Total uptake equation (nmol) | $y = 0.9297(ID)^{0.8809}.A$ | $y = 2.6403(ID)^{1.4722}.A$ | $y = 2.6403(ID)^{1.4722}.A$ | $y = 2.6403(ID)^{1.4722}.A$ |
| **Calculated** Total uptake (nmol) | 1.6866 | 1.5236 | 1.3262 | 0.9728 |
| **Calculated** Uptake (%) | 89 | 80 | 70 | 51 |
| **Actual** Uptake (%) | 89 | 89 | 66 | 36 |

[a] Based on the average trend line for bentazone in the presence of all of the surfactants used (excluding high molecular weight outliers as discussed previously). All data points between 0.01 and 0.1 nmol $mm^{-2}$ or 1 and 10 nmol $mm^{-2}$ were used, depending on the initial dose of bentazone.

The literature suggests that surfactants may enhance uptake of an AI by increasing either AI partitioning from the droplet deposit into the cuticular membrane (CM) or AI mobility in the CM (Schönherr *et al.*, 1999). Surfactant enhanced AI mobility in the CM requires surfactant penetration of the CM. In principle, rapid surfactant penetration may leave the AI in the droplet deposit, while slow surfactant penetration may result in a deposit which keeps the AI in solution and thus favours AI retention in the solution deposit rather than partitioning into the CM (Petracek *et al.*, 2004). Optimal effectiveness may thus require similar CM penetration rates for surfactant and AI. Stevens and Bukovac (1987) found that the percentage uptake of octylphenoxy surfactants approximately paralleled the uptake of DDT and atrazine (with the percentage uptake of the surfactants being significantly higher) and concluded that the enhanced uptake of these non-polar compounds was perhaps attributable to copenetration of the surfactants, although they could not postulate a mode of action for this effect.

Although the present studies were not designed to show the quantitative influence of surfactant concentration on AI uptake, there are strong indications (Forster and Zabkiewicz, unpublished) that surfactant concentrations need to be above a certain threshold to provide maximum uptake. This is in agreement with Stock *et al.* (1993), who found that, for some plant species, there was a critical threshold concentration of surfactant that needed to be exceeded before significant uptake enhancement could occur. They found that the nature of the uptake response to increasing surfactant concentration varied significantly according to both AI and plant species. Evidence from studies using radiolabelled surfactants may help to explain the differential response to surfactant concentration (Stock *et al.*, 1992). In some situations, particularly with lipophilic compounds (Stock *et al.*, 1993), poor uptake enhancement of the AI with a low surfactant concentration is associated with reduced surfactant penetration. An increase in surfactant concentration can, however, result in penetration of a greater amount of surfactant causing a concomitant increase in the uptake of the model compound. Studies with cuticular waxes have shown that the highest diffusion rates were associated with small molecules and the lowest diffusion rates were associated with the largest molecules (Bauer and Schönherr, 1992; Baur *et al.*, 1996). This would explain why the present study found that high molecular weight surfactants produced a much lower uptake enhancement than would be expected from the dose regression relationship, although behaviour was also species dependent. It can be hypothesised that the high molecular mass surfactants do not penetrate into the leaf to a sufficient extent (if at all) to enhance the uptake of the AI as much as the lower molecular mass surfactants may do. The species into which compounds can penetrate more easily (i.e. *Sinapis alba* in this study) may allow a higher molecular mass surfactant to penetrate better, compared to more difficult-to-penetrate species.

In contrast to the previous study (Forster *et al.*, 2004a), where a defined surfactant series was used, the present study used a wide range of surfactant structures. Such a wide range of surfactant structure is unusual in this type of study. Although there is information in the literature on the effect of surfactants on the uptake of actives, in particular with regard to ethylene oxide (EO) chain length (Stevens and Bukovac, 1987; Holloway *et al.*, 1989; Steurbaut *et al.*, 1989; Holloway and Stock, 1990; Stock, 1990; Chamel *et al.*, 1992; Gaskin and Holloway, 1992; Holloway and Edgerton, 1992; Holloway *et al.*, 1992; Kirkwood *et al.*, 1992; Stock and Holloway, 1993; Coret and Chamel, 1994), there is a lack of information on surfactant uptake, and the relative uptake of surfactants compared with actives. This will be the subject of future studies.

That there are species-AI interactions is also indicated by the similarity of the trend lines for bentazone uptake into *C. album* and *S. alba* versus the trend lines for uptake of epoxiconazole and pyraclostrobin into *T. aestivum* (Fig. 4.9). These interactions and their relative influence will also be the subject of a further study. A plethora of authors, in both the whole plant and isolated cuticle fields, have found significant differences in uptake among species (Stevens, 1984; Price and Anderson, 1985; Baker *et al.*, 1992; Chamel *et al.*, 1992; Gouret *et al.*, 1993; Santier and Chamel, 1996). Chamel and Vitton (1996) concluded that the structural characteristics of the cuticles are of major importance and must be given particular attention when attempting to establish models to predict the transfer of xenobiotics through plant cuticles. Price and Anderson (1985) concluded that the difference between the uptake patterns for the various species studied was large, which meant that it could not be assumed that a high uptake of one formulation into a particular species would lead to similar behaviour in another. However, Baur *et al.* (1997) concluded that, in the cuticular membranes of all of the plant species that they studied, the diffusion of all types of solutes proceeded in comparable chemical microenvironments, independent of plant species and possible differences in wax composition.

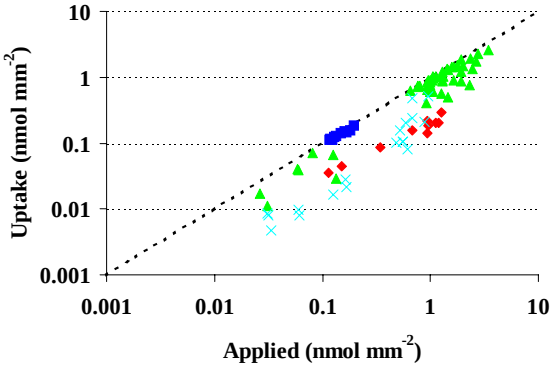


**Figure 4.9.** Uptake at 24 h of bentazone into (▲) C. album and (■) S. alba and of (x) epoxiconazole and (◆) pyraclostrobin into *T. aestivum* foliage in the presence of a range of surfactants (see Table 4.1), excluding high molecular mass surfactants as in previous figures (--- maximum uptake line, representing 100% uptake over the initial dose range). Each point represents the average of five replicates).

The present study has shown that the sole use of initial dose is too simplistic, and other factors need to be added to a model. Fick's law as modified for plant cuticles (Price, 1982; Schönherr and Baur, 1994; Schönherr and Baur, 1996) is more complex and although the variables (diffusion coefficient, partition coefficient between the formulation residue on the leaf surface and the cuticle, partition coefficient between the cuticle and the aqueous phase of

the epidermal cell wall, concentration of the AI on the leaf surface, concentration of the AI in the cuticle, tortuosity factor and effective cuticular thickness) have been determined using isolated cuticles, it would be extremely difficult to derive them all using whole-plant systems. Alternative variables need to be considered for whole-plant uptake, and this type of dataset is well suited to testing the relative influences of each of the factors involved in uptake, i.e. species, AI, AI concentration, surfactant etc. This will also be the subject of future publications.

## CONCLUSIONS

Using various formulation types (water, EC and SC) at typical working concentrations, the current *in vivo* studies of xenobiotic uptake confirmed the dominance of the initial dose of xenobiotic applied in relation to its mass uptake. This positive determinant of uptake is applicable to hydrophilic and moderately lipophilic xenobiotics in the presence of a wide range of surfactants and formulations. Surfactant concentration was also found to have an effect, with the lower surfactant concentrations showing a poorer relationship between the amount of xenobiotic applied and the amount of its uptake. High molecular mass surfactants produced a much lower uptake than expected from the dose uptake regressions, although this behaviour was very species dependent.

Although a surfactant that causes a formulation to spread will decrease the dose of AI per unit area and therefore decrease the uptake per unit area, the total uptake is the product of the uptake per unit area and the spread area. Therefore, the total uptake may be less than, equal to or greater than that of a formulation that spreads less well. After taking into account differences in spread area among formulations differences in uptake owing to the specific surfactant used are still significant.

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# Statistical Analysis to Determine the Relative Importance of Variables Involved in Foliar Uptake

## INTRODUCTION

A recent study (Forster *et al.*, 2004) determined the uptake of three model compounds that were applied in the presence and absence of four different surfactants into the leaves of three plant species (*Chenopodium album*, *Hedera helix* and *Stephanotis floribunda*). The mass uptake of 2-deoxy-D-glucose (DOG), 2,4-dichlorophenoxy-acetic acid (2,4-D), and (2RS,3SR)-1-[3-(2-chlorophenyl)-2,3-epoxy-2-(4-fluorophenyl)propyl]-1H-1,2,4-triazole (epoxiconazole) in the presence of these surfactants demonstrated that the initial dose (nmol $mm^{-2}$) of xenobiotic applied to plant foliage was a strong positive determinant of uptake. This held true for all the xenobiotic formulations studied over a wide concentration range, even when exceeding typical operational concentrations. The mass uptake on a per unit area basis could be related to the initial dose of xenobiotic applied by an equation of the form: $Uptake_{(nmol\ mm^{-2})} = a\ [ID]^b$ at time t = 24 h, where ID is the initial dose or the mass of xenobiotic applied per unit area ($M_{(nmol\ xenobiotic\ applied)}/A_{(droplet\ spread\ area)}$). To convert this mechanistically useful data into more commonly used terms, total mass uptake can be calculated from an equation of the form: $total\ uptake_{(nmol)} = a\ [ID]^b.A$.

Having established the importance and relationship of ID to uptake per unit area, this latest study uses the mass uptake relationship to establish the relative importance of each of the variables involved in uptake, i.e., species, AI, AI concentration (g $L^{-1}$) and surfactant.

## MATERIALS AND METHODS

The materials and methods used, except for the statistical analyses, have been described fully elsewhere (Forster *et al.*, 2004). However, the following is a summary of the most pertinent features.

### Plant material

*Chenopodium album* (common lambsquarters) plants were grown from seed and raised under controlled environment conditions. *Hedera helix* plants were grown from cuttings raised in a glass house and used at 6 to 9 months of age. *Stephanotis floribunda* plants were sourced from Arbours nursery NZ, and 1 year old cuttings were used. Two weeks prior to use the *H. helix* and *S. floribunda* plants were transferred into growth cabinets having controlled environment conditions that were the same as for the *C. album* plants.

## Chemicals

*Model Compounds*

The compounds 2-deoxy-D-glucose (DOG), 2,4-dichlorophenoxy-acetic acid (2,4-D) and (2RS,3SR)-1-[3-(2-chlorophenyl)-2-(4-fluoro-phenyl)oxiran-2-ylmethyl]-1H-1,2,4-triazole (epoxiconazole) were studied over a wide range of concentrations (0.0088 to 148 g $L^{-1}$ DOG, equating to 0.0129 to 217 nmol per 0.24 µl droplet; 0.034 to 50 g $L^{-1}$ 2,4-D, equating to 0.037 to 54 nmol per 0.24 µl droplet; 0.039 to 4.34 g $L^{-1}$ epoxiconazole, equating to 0.029 to 4.34 nmol per 0.24 µl droplet). All compounds were studied up to the limits of their solubility. The solubility in water (20ºC) of DOG, 2,4-D and epoxiconazole is 100, 0.620, and 0.0000663 g $L^{-1}$, respectively (Fluka, 1999/2000; Kidd and James, 1993), the Log P values are –2.69, 2.62 and 3.44 respectively (Kidd and James, 1993; SRC, 1995), and the molecular weights are 164, 221, and 330 respectively.

*Surfactants*

Silwet L-77® (TSE7.5, a trisiloxane ethoxylate with mean EO of 7.5, supplied by GE Advanced Materials-Silicones), triethylene glycol monododecyl ether ($C_{12}EO_3$), and hexaethylene glycol monododecyl ether ($C_{12}EO_6$; both from Fluka) were used on all three plant species. Decaethylene glycol monododecyl ether ($C_{12}EO_{10}$; from Sigma, purified prior to use by HPLC to give > 90% purity) was used on *C. album* foliage only. All surfactants were studied at equimolar concentrations (0.0044 mol $L^{-1}$, corresponding to 2.3 g $L^{-1}$ TSE7.5, 1.4 g $L^{-1}$ $C_{12}EO_3$, 2.0 g $L^{-1}$ $C_{12}EO_6$, and 2.8 g $L^{-1}$ $C_{12}EO_{10}$), approximating typical use rates. The molecular weights of the surfactants $C_{12}EO_3$, $C_{12}EO_6$, $C_{12}EO_{10}$, and TSE7.5 are 319, 451, 627, and 517, respectively. All xenobiotics were studied alone and in the presence of each of the surfactants (excluding $C_{12}EO_{10}$ for *H. helix* and *S. floribunda*).

## Uptake

Radiolabelled 2-deoxy-D-(U-$^{14}$C)glucose (DOG; specific activity 11.5 GBq $mmol^{-1}$; Amersham International, U.K.), 2,4-dichlorophenoxy-acetic acid-carboxy-$^{14}$C (2,4-D; specific activity 0.751 GBq $mmol^{-1}$; Sigma), and (2RS, 3SR)-3-(2-chlorophenyl)-2-(4-fluorophenyl_-2-[1H-1,2,4-triazol-1-yl)methyl]oxirane-[chlorophenyl-U-$^{14}$C] ($^{14}$C-epoxiconazole; specific activity 1.09 GBq $mmol^{-1}$; BASF) were incorporated into treatments (added at approx. 1400 dpm per droplet) prior to use. All solutions were made up in water + acetone (1:1 by volume). Droplets of each solution (0.24 µl, ca. 770 µm diameter) were applied to the upper surface of the youngest fully expanded leaf of *C. album*, *H. helix*, and *S. floribunda* (14 droplets per leaf,

with the exception of treatments containing TSE7.5, where 7 droplets were applied per leaf) on five separate plants per species, within 4 h of the start of the illumination period. Treated leaves were excised at 24 h after treatment. Excised leaves were washed with 2 x 4 mL water + ethanol (1:1 by volume) to recover unabsorbed DOG and epoxiconazole (both >97% recovery on droplet dry-down), or 2 x 4 mL water + acetone (1:3 by volume) to recover unabsorbed 2,4-D (95% recovery on droplet dry-down). The washings were taken up in 13 mL ACS II scintillant (Amersham International, U.K.), and the radioactivity quantified by scintillation counting (Packard 2100TR). Percent uptake was determined as the proportion of the applied radiolabel not recovered by washing the treated leaves.

**Droplet spread area determination**

The droplet spread areas for the different formulations, on the three plant species, were measured under UV illumination using a JVC model TK-1270 colour video camera in conjunction with $V^{++}$ for Windows image analysis software (mean of 10 determinations). To visualise droplet spread, Blankophor-P fluor (5 g $L^{-1}$; Bayer NZ) was incorporated into treatments containing DOG or epoxiconazole, while Uvitex NFW 450 (5 g $L^{-1}$; Ciba Geigy) was incorporated into treatments containing 2,4-D.

**Statistical analyses**

The uptake ratio, (AI uptake per unit area) / (ID), appeared best suited for analysis because this ratio was independent of applied AI per unit area over the experimental range studied, after accounting for experimental factors and, after fitting a model, the residuals were normally distributed. Logistic regression analysis employing the SAS procedure GENMOD (SAS, 2000) was used to analyse the uptake ratio. Included in the model were the experimental factors species, surfactant, AI, and AI concentration (g $L^{-1}$). The latter was fitted as log(AI concentration). All main effects and interactions were included in the model. Analyses of deviance were used to measure the effectiveness and statistical significance of each term in the model. Deviance is a measure of variation used in logistic regression models (McCullagh and Nelder, 1971), and the analysis of deviance is equivalent to the analysis of variance of a conventional regression model.

## RESULTS

The data from Forster *et al.* (2004) was modelled together, and then individually for each AI. An example showing the data modelled for DOG (but not distinguishing among surfactants) is given in Fig. 5.1. This relationship (nmol $mm^{-2}$ uptake versus ID) was termed the uptake ratio. The uptake ratio is equivalent to percent uptake but on a molar basis, and unlike percent uptake it provides insights into the mechanisms of xenobiotic uptake into plants (Forster *et al.*, 2004).

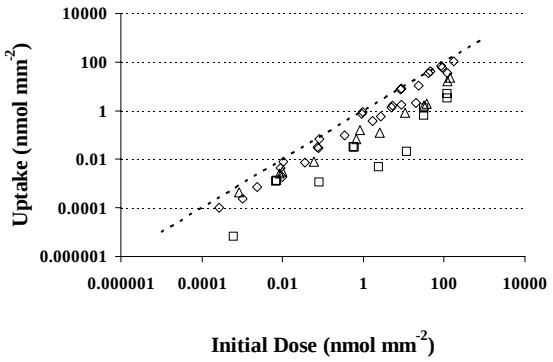


**Figure 5.1.** Uptake of DOG into *Chenopodium album* (◊), *Hedera helix* (Δ) and *Stephanotis floribunda* (□) foliage in the presence of the surfactants $C_{12}EO_3$, $C_{12}EO_6$, $C_{12}EO_{10}$ and TSE7.5 (dashed line = maximum uptake over the initial dose range).

### Analysis of Combined Model Xenobiotic Uptake

The analysis of deviance shown in Table 5.1 indicates the relative importance on the uptake ratio of each experimental factor and interaction in the model. Overall, 88% of the deviance could be explained. The analysis of deviance indicated that the main effects (species, AI, AI concentration, and surfactant) significantly affected the uptake ratio, explaining 51% of the deviance. No single effect was outstandingly predominant. The percentage variance explained by each main effect ranged from 8.9% (AI) to 17% (surfactant). Most of the interactions were also statistically significant, although they generally explained less of the variation than the main effects. The first-order interactions explained a further 25% of the deviance. The most important interaction was between AI concentration and AI, which explained 11.5% of the deviance. Other interactions were statistically significant although of less importance. Second-order interactions explained only 11% of the deviance, and the third-order interaction between all factors was not significant.

**Table 5.1.** Analysis of deviance of the uptake ratio [(AI uptake per unit area)/(ID)] at 24 h of DOG, 2,4-D, and epoxiconazole into *Chenopodium album*, *Hedera helix,* and *Stephanotis floribunda* in the absence or presence of the surfactants $C_{12}EO_3$, $C_{12}EO_6$, $C_{12}EO_{10}$, and TSE7.5.

| Source | d.f. | Deviance | F-ratio | Probability | % Deviance Explained | % Cumulative Deviance |
|---|---|---|---|---|---|---|
| Species | 2 | 13.1 | 62.64 | <.0001 | 13.0 | 13.0 |
| AI | 2 | 9.0 | 42.89 | <.0001 | 8.9 | 21.8 |
| Ln(AI Cn)[a] | 1 | 12.5 | 119.6 | <.0001 | 12.4 | 34.2 |
| Surfactant | 4 | 17.2 | 41.18 | <.0001 | 17.0 | 51.2 |
| Surfactant*Species | 8 | 4.8 | 5.7 | <.0001 | 4.7 | 55.9 |
| Ln(AI Cn)*AI | 2 | 11.6 | 55.73 | <.0001 | 11.5 | 67.5 |
| AI*Surfactant | 8 | 4.0 | 4.77 | <.0001 | 3.9 | 71.4 |
| Ln(AI Cn)*Surfactant | 4 | 1.0 | 2.33 | 0.061 | 1.0 | 72.4 |
| AI*Species | 4 | 2.1 | 5.12 | 0.0008 | 2.1 | 74.5 |
| Ln(AI Cn)*Species | 2 | 1.5 | 7.13 | 0.0012 | 1.5 | 76.0 |
| Ln(AI Cn)*AI*Species | 4 | 3.0 | 7.1 | <.0001 | 2.9 | 78.9 |
| AI*Surfactant*Species | 16 | 4.1 | 2.48 | 0.0029 | 4.1 | 83.0 |
| Ln(AI Cn)*AI*Surfactant | 8 | 3.8 | 4.51 | <.0001 | 3.7 | 86.7 |
| Ln(AI Cn)*Surfactant*Species | 8 | 0.6 | 0.67 | 0.72 | 0.6 | 87.3 |
| Ln(AI Cn)*AI*Surfactant*Species | 16 | 0.9 | 0.55 | 0.91 | 0.9 | 88.2 |
| Residual | 114 | 11.9 | | | | |

[a] AI Cn = active ingredient concentration.

Least squares means of the uptake ratio were performed to compare the levels within each main effect, and the results are given in Table 5.2. It can be seen that over all AI concentrations, species, and surfactants, the uptake ratio of DOG is significantly lower in comparison with 2,4-D and epoxiconazole, which are not significantly different from each other. Over all AI, AI concentrations, and surfactants, the uptake ratio into *C. album* is significantly higher than for *H. helix*, which in turn is significantly higher than *S. floribunda*. The addition of a surfactant to the AI significantly enhances the uptake ratio of the AI. Over all AI, AI concentrations, and species, $C_{12}EO_3$, $C_{12}EO_6$ and TSE7.5 do not provide significantly different uptake ratios, while $C_{12}EO_{10}$ provides a higher uptake ratio than $C_{12}EO_3$ and TSE7.5 but is not significantly different from $C_{12}EO_6$.

**Table 5.2.** Least squares means comparison of AI, species, and surfactant effect on the uptake ratio [(AI uptake per unit area)/(ID)] at 24 h.

| Main Effect | Level | Uptake ratio[a] |
|---|---|---|
| Active Ingredient | DOG | 0.22[b] |
| | 2,4-D | 0.45[a] |
| | Epoxiconazole | 0.39[a] |
| Species | *C. album* | 0.55[a] |
| | *H. helix* | 0.36[b] |
| | *S. floribunda* | 0.18[c] |
| Surfactant | Nil | 0.14[c] |
| | $C_{12}EO_3$ | 0.35[b] |
| | $C_{12}EO_6$ | 0.43[ab] |
| | $C_{12}EO_{10}$ | 0.49[a] |
| | TSE7.5 | 0.39[b] |

[a]Treatments followed by different letters within each main effect are significantly different (p=0.05)

The effect of AI concentration, an important primary factor explaining 12.4% of the deviance, cannot be shown using the least squares means comparisons, as it was incorporated in the model as a continuous variable rather than a class factor. However, the predicted uptake ratio from the model for a range of concentrations (g $L^{-1}$) is shown graphically in Fig. 5.2 for each AI. Fig. 5.2 shows that there was generally a decline in the uptake ratio with increasing concentration. However, this effect differed greatly for each AI. In other words, there was a strong interaction between concentration and AI. As shown in Table 5.1, this interaction explained 11.5% of the deviance.

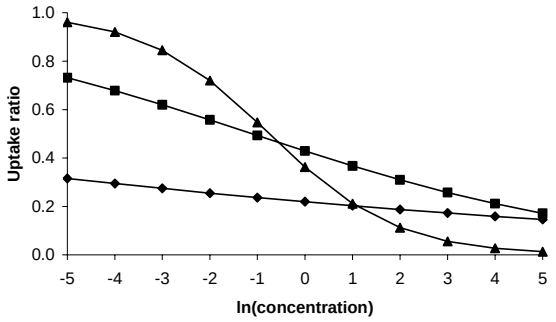


**Figure 5.2.** Predicted effect of AI and AI concentration on the uptake ratio [(AI uptake per unit area)/(ID)] at 24 h of DOG (♦), 2,4-D (■) and epoxiconazole (▲) into *Chenopodium album*, *Hedera helix* and *Stephanotis floribunda* in the absence or presence of the surfactants $C_{12}EO_3$, $C_{12}EO_6$, $C_{12}EO_{10}$, and TSE7.5.

The next most significant first-order interactions were between AI and surfactant, and between surfactant and species. Although the general ranking of surfactants shown in Table

5.4 holds overall, with the absence of a surfactant producing a much lower uptake ratio than in the presence of a surfactant, there are some differences in rankings of the surfactants for each AI as shown in Fig. 5.3. On the other hand, Fig. 5.4 shows that the interaction between species and surfactant arose chiefly because the uptake ratio was equally low for all species in the absence of surfactant, but the species rankings were very similar after addition of surfactant.

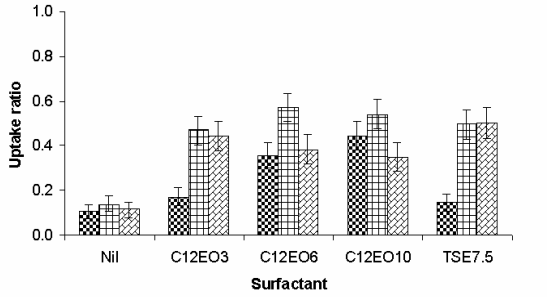


**Figure 5.3.** Effect of active ingredient (DOG, 2,4-D, and epoxiconazole) and surfactant on uptake ratio [(AI uptake per unit area)/(ID)]. Error bars are standard errors.

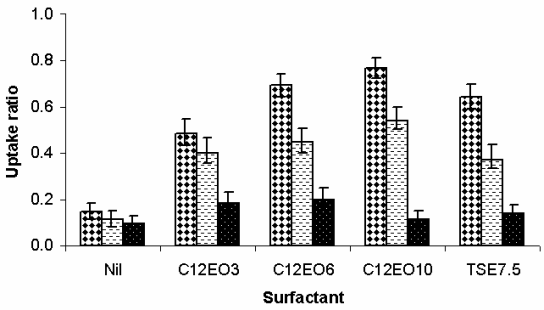


**Figure 5.4.** Effect of species (*C. album*, *H. helix*, *S. floribunda*) and surfactant on uptake ratio [(AI uptake per unit area)/(ID)]. Error bars are standard errors.

## Analysis of Uptake by Each Model Xenobiotic

To simplify the interpretation, separate analyses of deviance were performed also for each AI. These models explained 83%, 85% and 94% of the variance in the uptake ratio for DOG, 2,4-D, and epoxiconaxole, respectively (Table 5.3). In all cases, species, surfactant, and AI concentration significantly affected the uptake ratio. However, AI concentration explained the least (4.7%) of the main effects in the case of DOG. For 2,4-D, AI concentration again explained the least (17.3%) but explained much more than for DOG. For epoxiconazole, AI concentration explained the most (46.5%) of all the main effects. Species explained much less (5.2%) of the deviance in the case of epoxiconazole, while it explained 29.9% and 21.7%

respectively, for 2,4-D and DOG. Within the main effects, surfactant explained the most (32.7%) in the case of DOG, whereas in the case of 2,4-D and epoxiconazole the second highest percent deviance was explained by surfactant, with 25.2 and 16.8% of the deviance, respectively.

In the case of DOG, the only important interaction was between AI concentration and species, which explained 16.8% of the deviance (Table 5.3, Fig. 5.5). It can be seen (Fig. 5.6) that $C_{12}EO_{10}$ and $C_{12}EO_6$ produced the highest uptake ratio for DOG into *C. album*, while only $C_{12}EO_{10}$ produces a significantly higher uptake ratio for DOG into *H. helix*. None of the surfactants studied enhanced the uptake ratio for DOG into *S. floribunda*.

**Table 5.3.** Analysis of deviance of the uptake ratio [(AI uptake per unit area)/(ID)] at 24 h for DOG, 2,4-D and epoxiconazole into *Chenopodium album*, *Hedera helix* and *Stephanotis floribunda* in the absence or presence of the surfactants $C_{12}EO_3$, $C_{12}EO_6$, $C_{12}EO_{10}$, and TSE7.5.

| Active Ingredient | Source | d.f. | Deviance | F-ratio | Probability | % Deviance Explained | % Cumulative Deviance |
|---|---|---|---|---|---|---|---|
| DOG | Species | 2 | 5.96 | 27.46 | <.0001 | 21.7 | 21.7 |
| | Ln(AI Cn)[a] | 1 | 1.30 | 11.94 | 0.0012 | 4.7 | 26.4 |
| | Surfactant | 4 | 8.99 | 20.70 | <.0001 | 32.7 | 59.1 |
| | Ln(AI Cn)*Species | 8 | 4.61 | 5.31 | 0.0001 | 16.8 | 75.9 |
| | Surfactant*Species | 4 | 0.49 | 1.13 | 0.36 | 1.8 | 77.6 |
| | Ln(AI Cn)*Surfactant | 2 | 0.91 | 4.17 | 0.022 | 3.3 | 80.9 |
| | Species* Ln(AI Cn)*Surfactant | 8 | 0.47 | 0.54 | 0.82 | 1.7 | 82.6 |
| | Residual | 44 | 0.47 | | | | |
| 2,4-D | Species | 2 | 10.04 | 34.83 | <.0001 | 29.9 | 29.9 |
| | Ln(AI Cn) | 1 | 5.79 | 40.19 | <.0001 | 17.3 | 47.2 |
| | Surfactant | 4 | 8.46 | 14.68 | <.0001 | 25.2 | 72.4 |
| | Ln(AI Cn)*Species | 8 | 1.48 | 1.28 | 0.28 | 4.4 | 76.8 |
| | Surfactant*Species | 4 | 1.26 | 2.19 | 0.090 | 3.8 | 80.6 |
| | Ln(AI Cn)*Surfactant | 2 | 0.81 | 2.82 | 0.073 | 2.4 | 83.0 |
| | Species* Ln(AI Cn)*Surfactant | 8 | 0.65 | 0.56 | 0.80 | 1.9 | 85.0 |
| | Residual | 35 | 0.65 | | | | |
| Epoxiconazole | Species | 2 | 1.70 | 14.27 | <.0001 | 5.2 | 5.2 |
| | Ln(AI Cn) | 1 | 15.24 | 255.73 | <.0001 | 46.5 | 51.7 |
| | Surfactant | 4 | 5.50 | 23.07 | <.0001 | 16.8 | 68.5 |
| | Ln(AI Cn)*Species | 8 | 2.07 | 4.34 | 0.001 | 6.3 | 74.8 |
| | Surfactant*Species | 4 | 3.12 | 13.09 | <.0001 | 9.5 | 84.3 |
| | Ln(AI Cn)*Surfactant | 2 | 2.71 | 22.71 | <.0001 | 8.3 | 92.5 |
| | Species* Ln(AI Cn)*Surfactant | 8 | 0.36 | 0.75 | 0.64 | 1.1 | 93.6 |
| | Residual | 35 | 0.36 | | | | |

[a]AI Cn = active ingredient concentration.

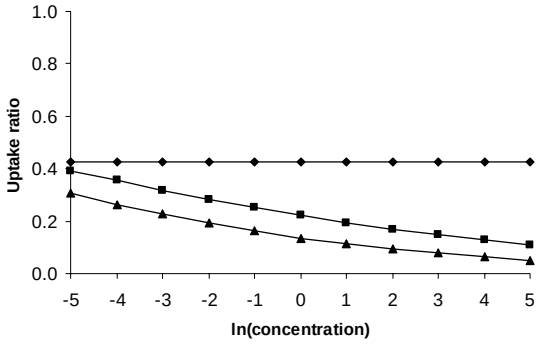


**Figure 5.5.** Predicted uptake ratio [(AI uptake per unit area)/(ID)] for DOG versus concentration and species (♦ *C. album*, ■ *H. helix* and ▲ *S. floribunda*).

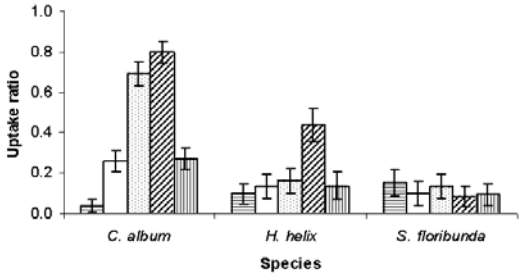


**Figure 5.6.** Effect of species and surfactant (Nil, $C_{12}EO_3$, $C_{12}EO_6$, $C_{12}EO_{10}$, TSE7.5) on the uptake ratio [(AI uptake per unit area)/(ID)] of DOG. Error bars show standard errors.

No interactions were important for 2,4-D (Table 5.3). All surfactants significantly enhanced the uptake ratio of 2,4-D into *C. album* and *H. helix*, while any enhancement into *S. floribunda* was minimal (Fig. 5.7).

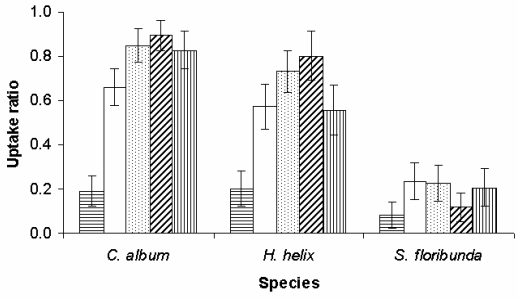


**Figure 5.7.** Effect of species and surfactant (Nil, $C_{12}EO_3$, $C_{12}EO_6$, $C_{12}EO_{10}$ TSE7.5) on the uptake ratio [(AI uptake per unit area)/(ID)] of 2,4-D. Error bars show standard errors.

In the case of epoxiconazole, all first-order interactions were significant (Table 5.3). However, the dominant effect was concentration, followed by surfactant, with interactions playing only a secondary role. All surfactants provided a significantly higher uptake ratio of

epoxiconazole into each species (Fig. 5.8) compared to epoxiconazole applied alone. It is interesting to note that TSE7.5, which spreads considerably more than the other surfactants studied, provided a significantly higher uptake ratio of epoxiconazole into *C. album* than the other surfactants, whereas $C_{12}EO_3$ provided a significantly higher uptake ratio of epoxiconazole into *H. helix* than TSE7.5. There was no significant difference among surfactants in uptake of epoxiconazole into *S. floribunda*.

Because the surfactant molar concentration was held constant, the effect of surfactant concentration on AI uptake cannot be determined from this study. The uptake ratio for DOG alone is the least and decreases with increasing concentration (Fig. 5.9), with similar trends in the presence of surfactants, but always greater than DOG alone.

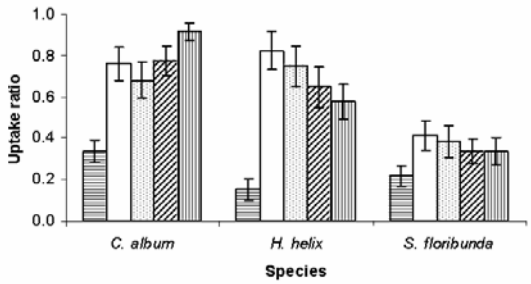


**Figure 5.8.** Effect of species and surfactant (Nil, $C_{12}EO_3$, $C_{12}EO_6$, $C_{12}EO_{10}$ TSE7.5) on the uptake ratio [(AI uptake per unit area)/(ID)] of epoxiconazole. Error bars show standard errors.

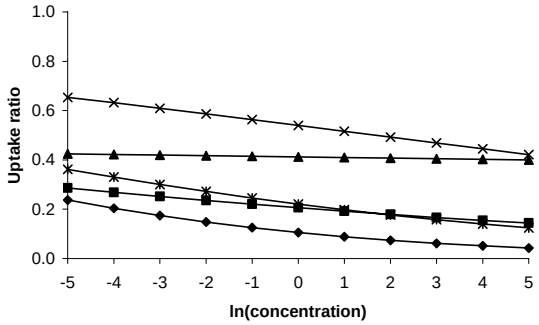


**Figure 5.9.** Effect of surfactant (♦ Nil, ■ C12EO3, ▲ C12EO6, x C12EO10, * TSE7.5) and AI concentration on the uptake ratio [(AI uptake per unit area)/(ID)] of DOG.

In contrast, there is much less difference among surfactants in the uptake ratio of 2,4-D (Fig. 5.10) versus ln(AI concentration), with the uptake ratio decreasing at a much greater rate with increasing concentration. The ln(concentration) for typical 2,4-D use rates would be in the

range of approximately 0.5 to 3.7. All surfactant trend lines are elevated well above the trend line for 2,4-D applied alone. The trend of the uptake ratio of epoxiconazole (Fig. 5.11) versus ln (AI concentration) is quite different from those for DOG and 2,4-D. The uptake ratio for epoxiconazole applied in the presence of surfactants, at very low concentrations, is near the maximum and significantly higher than for epoxiconazole applied alone. At very high concentrations of epoxiconazole applied in the presence of surfactants, the uptake ratio drops below that of epoxiconazole applied alone, which does not occur for either DOG or 2,4-D. However, ln(concentration) for typical use rates of epoxiconazole would be in the range of approximately -1 to 0.8.

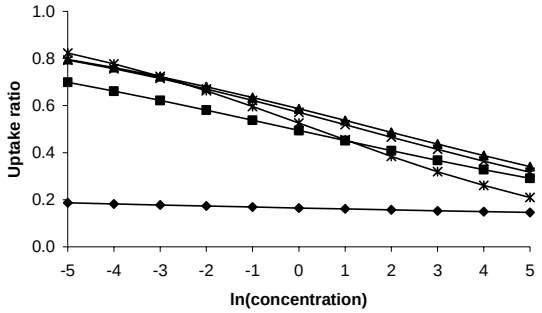


**Figure 5.10.** Effect of surfactant (♦ Nil, ■ C12EO3, ▲ C12EO6, x C12EO10, ∗ TSE7.5) and AI concentration on the uptake ratio [(AI uptake per unit area)/(ID)] of 2,4-D.

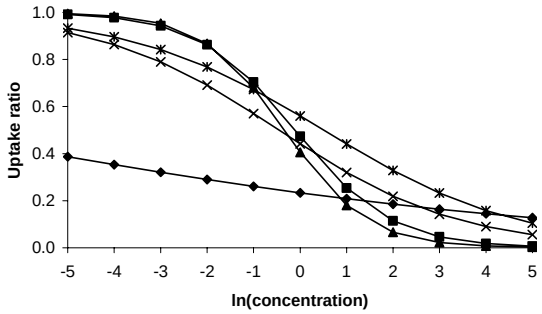


**Figure 5.11.** Effect of surfactant (♦ Nil, ■ C12EO3, ▲ C12EO6, x C12EO10, ∗ TSE7.5) and AI concentration on the uptake ratio [(AI uptake per unit area)/(ID)] of epoxiconazole.

The main effect means are shown in Table 5.4. Comparing species effects, the uptake ratio was the highest for all AI into *C. album*. However, in the case of 2,4-D this was not significantly different from the uptake ratio into *H. helix* (Table 5.4). In the case of 2,4-D and epoxiconazole, the uptake ratio was significantly higher into *H. helix* than *S. floribunda*, whereas there was no significant difference between the two species in the case of DOG. All

surfactants provided a significantly higher uptake ratio compared to 2,4-D or epoxiconazole applied alone, with no significant difference among the surfactants (Table 5.4). Surfactant $C_{12}EO_6$ and $C_{12}EO_{10}$ provide a significantly higher uptake ratio for DOG than either TSE7.5 or $C_{12}EO_3$, which were not significantly different to DOG applied alone.

**Table 5.4.** Least squares means comparison of species and surfactant effect on the uptake ratio [(AI uptake per unit area)/(ID)] for each AI.

| Main Effect | Level | DOG[a] | 2,4-D[a] | Epoxiconazole[a] |
|---|---|---|---|---|
| Species | *C. album* | 0.43$^{a}$ | 0.63$^{a}$ | 0.57$^{a}$ |
| | *H. Helix* | 0.21$^{b}$ | 0.55$^{a}$ | 0.52$^{b}$ |
| | *S. floribunda* | 0.14$^{b}$ | 0.19$^{b}$ | 0.37$^{c}$ |
| Surfactant | Nil | 0.11$^{b}$ | 0.17$^{b}$ | 0.25$^{b}$ |
| | $C_{12}EO_3$ | 0.20$^{b}$ | 0.49$^{a}$ | 0.56$^{a}$ |
| | $C_{12}EO_6$ | 0.41$^{a}$ | 0.58$^{a}$ | 0.52$^{a}$ |
| | $C_{12}EO_{10}$ | 0.52$^{a}$ | 0.58$^{a}$ | 0.52$^{a}$ |
| | TSE7.5 | 0.21$^{b}$ | 0.52$^{a}$ | 0.61$^{a}$ |

[a] Treatments followed by different letters within each main effect and AI column are significantly different (p = 0.05).

## DISCUSSION

Many studies have been performed attempting to relate the percent uptake of agrochemicals to the physicochemical properties of the AI and formulants (Stevens, 1984; Baker *et al.*, 1992; Stock, 1990). These have had varying success. However, the general consensus was that there was no simple relationship across all AI and formulants. Due to the empirical approaches used, it was thought that such equations would be of limited value in predicting actual uptake, as the inclusion of additional compounds of differing molecular properties would significantly influence the equations, necessitating re-analysis.

Although Fick's law of diffusion has been modified for plants (Price, 1982), this approach has historically not been used to study cuticular uptake into whole plants. Recently a dynamic non-linear simulation model has been developed for whole plant transport and allocation of foliar-applied xenobiotics (Satchivi *et al.*, 2000a, 2000b). This model includes an equation describing the cuticular sorption process based on models developed by Schönherr and co-workers, with the addition of parameters describing surfactant effects and taking relative humidity into account. The difficulty with this model is the number of factors required, including many isolated cuticle factors (e.g., xenobiotic diffusion coefficient, wax/water partition coefficient, cuticle/water partition coefficient, thickness of the limiting

skin, xenobiotic concentration in the formulation residue and in the cuticular membrane, and the critical micelle concentration of the surfactant). The validation of this model (Satchivi *et al.*, 2000b) did not distinguish among plant species, and a single cuticle thickness was used for all plant species. At present, a physical measurement cannot establish the "thickness" of the limiting skin, as this is not simply the thickness of the cuticle, or the wax layer, but the length of the diffusion path through the limiting skin (Riederer and Schreiber, 1995).

A method of modelling foliar uptake of pesticides using finite difference techniques has also been presented recently (Lamb *et al.*, 2001). Again, numerous inputs are required for the model, such as diffusion coefficient, partition coefficient between droplet and cuticle and between cuticle and plant, cuticle thickness, droplet volume and diameter, and the duration of the experiment. Species differences were taken into account in this model in the form of cuticle thickness, and prediction versus actual uptake appeared good.

The advantages of the uptake model we have presented (Forster *et al.*, 2004) are that few variables are required (molar concentration of the AI and spread area of the droplet) and they are simple to measure. This is quite different from previous attempts described in the literature. However, this was still too simplistic, as indicated in the present study establishing the relative importance of the principal factors involved in foliar uptake. This will enable subsequent studies to focus on the individual factors in an attempt to correlate measurable parameters to those factors. For example, it has been found that concentration of AI explains the highest percent deviance (46.5%) for the uptake ratio of epoxiconazole. Concentration can be used directly and needs no other parameters measured. However, surfactant explains the next highest percent deviance (16.8%), and in order to develop a universal equation we need to be able to measure some physical property, or more likely several properties, of each of the surfactants which will account for the differences among them. If we can account in some way for the differences among surfactants, then we will manage to explain > 60% of the deviance for a lipophilic compound. In the case of 2,4-D, to be able to account for a significant amount of the deviance, we need to be able to account for the differences among species, as well as AI concentration and surfactant. In the case of DOG, a hydrophilic compound, the two factors that account for the largest percent deviance are species and surfactant. Therefore, establishing a universal model for a lipophilic molecule appears to be the easiest place to begin. Rather than attempting to correlate the physical properties of the xenobiotics to percent uptake or total uptake, we would be attempting to correlate physical properties only to that proportion of the deviance of the uptake ratio which that factor

explains. This is quite different from other attempts described in the literature, and will be the subject of further studies.

## CONCLUSIONS

Although the uptake ratio is equivalent to percent uptake, unlike percent uptake it provides insights into the mechanisms of xenobiotic uptake into plants (Forster *et al.*, 2004). As the uptake ratio is uptake on a unit area basis and not total uptake, caution is required not to relate the results directly to total uptake. Total uptake is calculated by multiplying the uptake ratio by droplet spread area as stated earlier (Forster *et al.*, 2004).

This study has shown that species, AI, AI concentration, and surfactant all significantly affect the uptake ratio (explaining 51% of the deviance). The percentage variance explained by each factor ranged from 8.9% (AI) to 17% (surfactant). The most important interaction was between concentration and AI, which explained 11.5% of the deviance. Overall, 88% of the deviance could be explained.

More useful was the analysis of the individual xenobiotics, where the models explained 83%, 85% and 94% of the variance in uptake ratio for DOG, 2,4-D and epoxiconazole, respectively. In all cases, species, surfactant and AI concentration significantly affected the uptake ratio. However, there were differences in the relative importance of these factors among the xenobiotics studied. Concentration of AI increased in importance with increasing lipophilicity, while species was much less important for the most lipophilic compound. Surfactant became less important with increasing lipophilicity, although it was always important. The interaction between AI concentration and species was much more important for the most polar compound, while the interaction between surfactant and species increased in importance with increasing lipophilicity.

Now that the relative importance of the factors affecting the uptake ratio (excluding environmental factors and surfactant concentration) has been identified, future studies can attempt to correlate physical properties to the individual factors.

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# Mechanisms of Cuticular Uptake of Xenobiotics into Living Plants:

# Evaluation of a Logistic-Kinetic Penetration Model

## INTRODUCTION

Pesticide spray efficacy depends on several processes, namely, deposition, retention, uptake, and translocation. A spray droplet landing on foliage rapidly becomes a quasi-solid deposit due to solvent evaporation, but uptake into the leaf surface can occur over many hours. Total uptake after 24 h can be the same for a compound formulated with different surfactants, but rates of uptake (and therefore rain-fastness and subsequent translocation to target sites) can be quite different. Therefore, there is a requirement to be able to model uptake over time into whole plants.

There is general agreement that uptake of xenobiotics (e.g. pesticides) through the leaf cuticle is a diffusion process (Price, 1982). However, Fick's first law of diffusion as modified for plant cuticles *in vitro* (Price, 1982; Schönherr and Baur, 1994) may not be appropriate for *in vivo* situations when the applied quantity is a finite dose (from a droplet deposit). Watanabe (2002) reviewed uptake models presented in the literature and found that the models dealing with nonequilibrium transcuticular penetration kinetics did not fully represent all of the kinetic parameters involved in penetration from a droplet residue. This led him to develop a non-steady-state, nonequilibrium model (Watanabe, 2002), termed "the logistic-kinetic penetration model", using isolated cuticle measurements in the development of the model. The objective of our study was to determine the uptake of model xenobiotics differing in lipophilicity into two plant species over time, applied alone and in the presence of a range of surfactants, to determine whether the logistic-kinetic penetration model as described by Watanabe (2002) could be applied to *in vivo* uptake. In the current study, *Chenopodium album*, which has a thin cuticle, was compared with *Hedera helix*, which has a thick cuticle and has been widely used in isolated cuticle work (Buchholz *et al.*, 1998).

## MATERIALS AND METHODS

The materials and methods used have been described elsewhere (Forster *et al.*, 2004a). However, the following is a summary of the most pertinent features.

### Plant Material

*C. album* (common lambsquarters) plants were grown from seed and raised under controlled environment conditions. *H. helix* plants were grown from cuttings raised in a glass house and used at 6-9 months of age. Two weeks prior to use, the *H. helix* plants were transferred into growth cabinets having controlled environment conditions that were the same as for the *C. album* plants.

## Chemicals

### *Model Compounds*

2-Deoxy-D-glucose (DOG; Aldrich Chemical Company Co., Inc.; 99% purity), 2,4-dichlorophenoxyacetic acid (2,4-D; Dow Agrosciences (NZ) Ltd.; 92% purity) and (2RS,3SR)-1-[3-(2-chlorophenyl)-2-(4-fluoro-phenyl)oxiran-2-ylmethyl]-1H-1,2,4-triazole (epoxiconazole; BASF; 96% purity) were studied initially at one concentration each (0.75 g $L^{-1}$ DOG, 1.09 g $L^{-1}$ 2,4-D, 1.554 g $L^{-1}$ epoxiconazole) providing a molar concentration (0.0045 mol $L^{-1}$; 1.1 nmol per 0.24 µL droplet) close to that of the surfactant's concentration. A second experiment on *C. album* determined the uptake of DOG, 2,4-D, and epoxiconazole, each at two concentrations each (0.1107 and 54.29 nmol per 0.24 µL for DOG; 0.024 and 10.87 nmol per 0.24 µL for 2,4-D; 0.029; 2.19 nmol per 0.24 µL for epoxiconazole) in the presence of the three surfactants. The solubilities in water (20ºC) of DOG, 2,4-D, and epoxiconazole are 100, 0.620, and 0.0000663 g $L^{-1}$ respectively (Fluka, 1999/2000; Kidd and James, 1993); the log P values are –2.69, 2.62, and 3.44, respectively (Kidd and James, 1993; SRC's program online); and the molecular weights are 164, 221, and 330.

### *Surfactants*

Silwet L-77® [TSE7.5, a trisiloxane ethoxylate with mean ethylene oxide (EO) content of 7.5, supplied by GE Advanced Materials-Silicones], triethylene glycol monododecyl ether ($C_{12}EO_3$), and hexaethylene glycol monododecyl ether ($C_{12}EO_6$), both from Fluka, were used. All surfactants were studied at an equimolar concentration (0.0044 mol $L^{-1}$, corresponding to 2.3 g $L^{-1}$ TSE7.5, 1.4 g $L^{-1}$ $C_{12}EO_3$, and 2.0 g litre$^{-1}$ $C_{12}EO_6$), approximating typical use rates. The molecular weights of the surfactants $C_{12}EO_3$, $C_{12}EO_6$, and TSE7.5 are 319, 451, and 517, respectively. All xenobiotics were studied alone and in the presence of each of the surfactants.

## Uptake

Radiolabeled 2-deoxy-D-(U-$^{14}$C)glucose (DOG), 2,4-dichlorophenoxyacetic acid-carboxy-$^{14}$C(2,4-D), and (2RS,3SR)-3-(2-chlorophenyl)-2-(4-fluorophenyl_-2-[1H-1,2,4-triazol-1-yl)methyl]oxirane-[chlorophenyl-U-$^{14}$C] (epoxiconazole) were incorporated into treatments (added at ~ 1400 dpm per droplet) prior to use. All solutions were made up in water + acetone (1:1 by volume). The use of 50% acetone:water for model uptake experiments is common, and this mixture is considered to have no significant effect on the uptake of the active ingredient (AI) (Stevens and Baker, 1987). This enabled higher concentrations of lipophilic xenobiotics to be studied, as well as the xenobiotic in the absence

of a surfactant. Droplets of each solution (0.24 µL, ~770 µm diameter) were applied to the upper surface of the youngest fully expanded leaves of *C. album* and *H. helix* (14 per leaf) on five separate plants per species, within 4 h of the start of the illumination period. Treated leaves were excised at 0.5, 2, 4, and 6 h after treatment. A previous study (Forster *et al.*, 2004a) had already determined 24 h uptake. In the second experiment, treated leaves were excised at 2 and 6 h after treatment. Percentage uptake was determined as the proportion of the applied radiolabel not recovered by washing the treated leaves.

**Droplet Spread Area Determination**

The droplet spread areas for the different formulations, on the three plant species, were measured under UV illumination using $V^{++}$ for Windows image analysis software, with added Blankophor-P fluor (Bayer NZ) to treatments containing DOG or epoxiconazole and UVITEX NFW 450 (Ciba Geigy) to treatments containing 2,4-D.

**Statistical Analyses**

The statistical software package Statistix was used to analyze the data, with least significant difference (LSD) tests used to compare treatments. Stabilizing transformations were performed, when required, prior to analysis.

**RESULTS AND DISCUSSION**

The intention of this study was to validate the logistic-kinetic penetration model, and hence formulation differences due to the surfactants and xenobiotics studied are not discussed in the conventional sense. However, Tables 6.1 and 6.2 show actual percent uptake, along with statistical significance for all of the formulations tested.

**Validation of a logistic-kinetic penetration model to *in vivo* systems.** The model by Watanabe (2002) is

$$f = U[K/(K+e^{-qt})].(1-e^{-qt}) \qquad (6.1)$$

where $f$ is the amount penetrated at a given time, $U$ is the total amount of penetration (i.e., the maximum uptake), $K$ is the integral constant ($K$ = 0.6 or 0.7 is postulated for linearity or slight convexity, respectively, in the initial period of penetration), $q$ is the penetration rate factor, and t is time.

$$U = VCAP_u \quad (6.2)$$

where $V$ is the volume of the droplet applied, $C$ is the concentration (molar) of the xenobiotic, and $A$ is the contact (spread) area of the droplet. The unit partition ratio ($P_u$) of the pesticide is the ratio of the amount of pesticide partitioned from the droplet into the cuticular membrane (CM) relative to the amount applied ($VC$) per unit contact area, as defined by $P_u = U/(VCA)$. Thus, eqn 6.1 can be transformed to

$$f = VCAP_u[K/(K+e^{-qt})].(1-e^{-qt}) \quad (6.3)$$

All of the variables in eq 1, except $q$, are known for each rate study described in the methods. Rearranging eqn 1 to solve for $q$ produces

$$q = -1/t \ln(K(U-f)/UK+f) \quad (6.4)$$

In most cases, $U$ (total uptake) was taken as the uptake at 24 h. This was considered to be valid as the authors have found in most cases the majority of uptake into whole plants has occurred by 24 h. A value of 0.6 was used for $K$, and in the majority of cases $f$ (uptake at a given time) at 2 h was used. The exception was 2,4-D uptake into *C. album*, for which at 2 h most of the uptake had already taken place, necessitating $f$ to be taken at an earlier time (10 min, 600 s). In the case of DOG applied alone to *C. album*, uptake was very low, with no significant difference in uptake over time. However, although uptake was not significantly different, the quantity at 2 h (0.11 nmol or 10% uptake) was larger than that at 24 h (0.07 nmol or 7% uptake). Hence, $U$ was taken at 6 h, and $f$ at 30 min for illustrative purposes. After the value for $q$ had been derived, uptake over time ($f$) was calculated using eqn 3. The units used were $V$(μL), $C$ (nmol $\mu L^{-1}$), $A$ ($mm^2$), $P_u$ ($mm^{-2}$), $q$ ($s^{-1}$), and t (s). In all cases the volume applied was 0.24 μL, and the concentration was ~ 4.6 nmol $\mu L^{-1}$, except for 2,4-D with *H. helix* for which the concentration was ~5.1 nmol $\mu L^{-1}$. The values of $t$, $A$, $P_u$ and $q$ used in eqn 6.3 are shown in Tables 6.1 and 6.2, for uptake into *C. album* and *H. helix*, respectively. These tables also show calculated and actual uptake (both in nanomoles and by current convention, by percentage). Figures 6.1 – 6.6 graph the actual uptake (nanomoles) values over time, along with the calculated curves for the two species, three surfactants, and

three xenobiotics used. Overall, agreement between calculated and actual values is remarkably good, particularly where a plateau has been very obviously reached within 24 h (86400 s; Fig. 6.1-6.3).

**Table 6.1.** Watanabe model parameters[a] and calculated and actual uptake into *C. album* foliage over time of DOG, 2,4-D, and epoxiconazole, applied in the absence or presence of surfactants.

| formulation | time (s)* | total uptake (U)* (nmol) | spread area (A)* ($mm^2$) | unit partition ratio ($P_u$)* ($mm^{-2}$) | q* ($s^{-1}$) | calcd uptake (f) (nmol) | actual uptake (nmol) | calcd uptake (%) | actual uptake[b] (%) |
|---|---|---|---|---|---|---|---|---|---|
| DOG | 1800 | 0.0793 | 0.69 | 0.1058 | .00248 | .07688 | .07688 | 7.08 | 7.08 fgh |
| | 7200 | | | | | .07926 | .10564 | 7.30 | 9.73 efgh |
| | 14400 | | | | | .07926 | .05780 | 7.30 | 5.32 h |
| | 21600 | | | | | .07926 | .07926 | 7.30 | 7.30 fgh |
| | 86400 | | | | | .07926 | .07219 | 7.30 | 6.65 fgh |
| DOG + $C_{12}EO_3$ | 1800 | 0.3831 | 13.76 | 0.0256 | .00022 | .05808 | .13802 | 5.35 | 12.71 def |
| | 7200 | | | | | .22394 | .22394 | 20.62 | 20.62 bcd |
| | 14400 | | | | | .34095 | .25910 | 31.4 | 23.86 bcd |
| | 21600 | | | | | .37369 | .06577 | 34.4 | 6.06 gh |
| | 86400 | | | | | .38305 | .38305 | 35.3 | 35.28 b |
| DOG + $C_{12}EO_6$ | 1800 | 0.9304 | 1.21 | 0.7082 | .00036 | .23431 | .17778 | 21.58 | 16.37 cde |
| | 7200 | | | | | .76084 | .76084 | 70.07 | 70.07 a |
| | 14400 | | | | | .91577 | .92091 | 84.34 | 84.81 a |
| | 21600 | | | | | .92924 | .85980 | 85.58 | 79.19 a |
| | 86400 | | | | | .93038 | .93038 | 85.69 | 85.69 a |
| DOG + L-77 | 1800 | 0.3951 | 31.13 | 0.0117 | .00013 | .03673 | .24480 | 3.38 | 22.55 bcd |
| | 7200 | | | | | .14980 | .14980 | 13.80 | 13.80 defg |
| | 14400 | | | | | .27223 | .30842 | 25.07 | 28.411 bc |
| | 21600 | | | | | .34195 | .35798 | 31.49 | 32.97 bc |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 86400 | | | | | .39508 | .39172 | 36.39 | 36.39 b |
| 2,4-D | 300 | 0.6359 | 0.87 | 0.6533 | .0019 | .13961 | .08373 | 12.85 | 7.48 jk |
| | 600 | | | | | .27669 | .28511 | 25.48 | 25.48 ghi |
| | 1200 | | | | | .47644 | .51426 | 43.88 | 45.96 e |
| | 86400 | | | | | .61709 | .63589 | 56.83 | 57.82 de |
| 2,4-D + | 300 | 1.0552 | 3.4 | 0.2766 | .0025 | .30654 | .07219 | 27.32 | 33.16 g |
| $C_{12}EO_3$ | 600 | | | | | .58953 | .58953 | 52.55 | 52.55 ef |
| | 1200 | | | | | .92001 | .74633 | 82.00 | 66.52 cd |
| | 1800 | | | | | 1.0223 | .92756 | 91.11 | 82.67 b |
| | 7200 | | | | | 1.0552 | 1.0490 | 94.05 | 93.49 a |
| | 14400 | | | | | 1.0552 | 1.0592 | 94.05 | 94.4 a |
| | 21600 | | | | | 1.0552 | 1.0850 | 94.05 | 96.71 a |
| | 86400 | | | | | 1.0552 | 1.0552 | 94.05 | 94.05 a |
| 2,4-D + | 300 | 1.1005 | 1.31 | .7469 | .0007 | .09504 | .09504 | 8.45 | 6.79 k |
| $C_{12}EO_6$ | 600 | | | | | .19316 | .19316 | 17.17 | 12.89 ij |
| | 1200 | | | | | .38894 | .38894 | 34.58 | 26.51 ghi |
| | 1800 | | | | | .56880 | .56880 | 50.57 | 68.38 c |
| | 7200 | | | | | 1.0873 | 1.0873 | 96.67 | 92.59 a |
| | 14400 | | | | | 1.1005 | 1.1005 | 97.84 | 96.88 a |
| | 21600 | | | | | 1.1005 | 1.1005 | 97.85 | 97.47 a |
| | 86400 | | | | | 1.1005 | 1.1005 | 97.85 | 97.85 a |
| 2,4-D + | 300 | 1.0498 | 44.13 | .02111 | .0010 | .11871 | .20953 | 10.53 | 18.59 i |
| L-77 | 600 | | | | | .24123 | .24123 | 21.40 | 21.40 hi |
| | 1200 | | | | | .47741 | .35456 | 42.36 | 31.46 gh |
| | 1800 | | | | | .67433 | .78568 | 59.83 | 69.71 c |
| | 7200 | | | | | 1.0473 | 1.0046 | 92.92 | 89.13 ab |
| | 14400 | | | | | 1.0498 | 1.0302 | 93.14 | 91.40 ab |
| | 21600 | | | | | 1.0498 | 1.0555 | 93.14 | 93.69 a |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 86400 | | | | | 1.0498 | 1.0498 | 93.14 | 93.14 a |
| Epoxi | 1800 | .44237 | 0.69 | .57587 | .0001 | .04576 | .16735 | 4.11 | 15.03 gh |
| | 7200 | | | | | .18541 | .18541 | 16.65 | 16.65 fg |
| | 14400 | | | | | .32691 | .36499 | 29.36 | 32.7 cd |
| | 21600 | | | | | .39814 | .34440 | 35.76 | 30.94 cd |
| | 86400 | | | | | .44236 | .44237 | 39.73 | 39.73 bc |
| Epoxi + | 1800 | .52443 | 5.93 | .07933 | .0002 | .07304 | .23817 | 6.55 | 21.36 ef |
| $C_{12}EO_3$ | 7200 | | | | | .28602 | .28602 | 25.66 | 25.66 de |
| | 14400 | | | | | .45197 | .38638 | 40.54 | 34.66 bcd |
| | 21600 | | | | | .50596 | .36149 | 45.38 | 32.43 cd |
| | 86400 | | | | | .52443 | .52443 | 47.04 | 47.04 b |
| Epoxi + | 1800 | .24107 | 1.31 | .16515 | .0003 | .04406 | .14024 | 3.95 | 12.59 h |
| $C_{12}EO_6$ | 7200 | | | | | .1623 | .16230 | 14.57 | 14.57 gh |
| | 14400 | | | | | .22641 | .20564 | 20.32 | 18.45 fg |
| | 21600 | | | | | .23874 | .20600 | 21.42 | 18.49 fg |
| | 86400 | | | | | .24107 | .24107 | 21.63 | 21.63 ef |
| Epoxi + | 1800 | 1.0260 | 33.8 | .02731 | .0004 | .2818 | .39738 | 25.35 | 35.75 bcd |
| L-77 | 7200 | | | | | .87385 | .87385 | 78.61 | 78.61 a |
| | 14400 | | | | | 1.0158 | .97333 | 91.37 | 87.55 a |
| | 21600 | | | | | 1.0254 | .99095 | 92.24 | 89.14 a |
| | 86400 | | | | | 1.0260 | 1.0260 | 92.29 | 92.29 a |

[a]Required inputs for Watanabe model (2002) are marked with an asterisk.
[b]Treatments within each xenobiotic series with no letter in common are significantly different (p=0.05)

**Table 6.2.** Watanabe model parameters[a] and calculated and actual uptake into *H. helix* foliage over time of DOG, 2,4-D, and epoxiconazole, applied in the absence or presence of surfactants.

| formulation | time (s)* | total uptake (U)* (nmol) | spread area (A)* ($mm^2$) | unit partition ratio ($P_u$)* ($mm^{-2}$) | q ($s^{-1}$)* | calcd uptake (f) (nmol) | actual uptake (nmol) | calcd uptake (%) | actual uptake[b] (%) |
|---|---|---|---|---|---|---|---|---|---|
| DOG | 1800 | .04058 | 0.48 | .07785 | .00023 | .00644 | .02866 | .59348 | 2.64 ef |
| | 7200 | | | | | .02461 | .02461 | 2.2663 | 2.27 ef |
| | 14400 | | | | | .03668 | .09365 | 3.3781 | 7.93 bcd |
| | 21600 | | | | | .03977 | .03197 | 3.6631 | 2.94 ef |
| | 86400 | | | | | .04058 | .04058 | 3.7369 | 3.74 def |
| DOG + $C_{12}EO_3$ | 1800 | .11289 | 1.65 | .06301 | .00012 | .00922 | .04983 | .84908 | 4.59 cde |
| | 7200 | | | | | .03780 | .03780 | 3.4815 | 3.48 def |
| | 14400 | | | | | 6.5214 | .04409 | 6.5214 | 4.06 de |
| | 21600 | | | | | 8.4875 | .07016 | 8.4875 | 6.07 cde |
| | 86400 | | | | | 10.396 | .11289 | 10.396 | 10.40 bc |
| DOG + $C_{12}EO_6$ | 1800 | .20765 | 1.33 | .14379 | .00002 | .00255 | .02131 | .2343 | 1.96 ef |
| | 7200 | | | | | .01029 | .01029 | .9475 | 1.17 f |
| | 14400 | | | | | .02083 | .06638 | 1.9186 | 6.11 de |
| | 21600 | | | | | .03155 | .07252 | 2.9054 | 6.68 bcde |
| | 86400 | | | | | .12160 | .20765 | 11.199 | 17.38 a |
| DOG + L-77 | 1800 | .14901 | 18.23 | .00753 | .00013 | .01346 | .03608 | 1.2397 | 3.32 ef |
| | 7200 | | | | | .05498 | .05498 | 5.0636 | 5.06 cde |
| | 14400 | | | | | .10065 | .04691 | 9.2695 | 4.32 de |
| | 21600 | | | | | .12745 | .04236 | 11.738 | 3.90 de |
| | 86400 | | | | | .149 | .14901 | 13.723 | 13.72 ab |
| 2,4-D | 1800 | .37865 | .678 | .45046 | .00012 | .03011 | .02879 | 2.4288 | 2.32 j |
| | 7200 | | | | | .12357 | .12357 | 9.9668 | 9.97 ghij |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 14400 | | | | | .23271 | .0991 | 18.770 | 7.99 hij |
| | 21600 | | | | | .30496 | .08662 | 24.598 | 6.99 ij |
| | 86400 | | | | | .37860 | .37865 | 30.538 | 30.54 def |
| 2,4-D + | | | | | | | | | |
| $C_{12}EO_3$ | 1800 | .91370 | 1.557 | .48090 | .00011 | .07138 | .07219 | 5.8497 | 6.17 ij |
| | 7200 | | | | | .29305 | .29305 | 24.015 | 24.02 ef |
| | 14400 | | | | | .55382 | .61384 | 45.385 | 50.30 bcd |
| | 21600 | | | | | .72907 | .67957 | 59.746 | 55.69 abc |
| | 86400 | | | | | .91357 | .91371 | 74.865 | 74.88 a |
| 2,4-D + | 1800 | .7697 | 1.188 | .52572 | .00012 | .06128 | .07992 | 4.9720 | 6.48 ij |
| $C_{12}EO_6$ | 7200 | | | | | .25145 | .25145 | 20.402 | 22.54 fgh |
| | 14400 | | | | | .47353 | .29071 | 38.415 | 23.59 ef |
| | 21600 | | | | | .62029 | .50512 | 50.329 | 40.98 cde |
| | 86400 | | | | | .76964 | .76974 | 62.447 | 70.89 ab |
| 2,4-D + | 1800 | .30226 | 13.696 | .01812 | .00026 | .05580 | .11825 | 4.5810 | 9.71 ghij |
| L-77 | 7200 | | | | | .20493 | .20493 | 16.825 | 16.82 fghi |
| | 14400 | | | | | .28451 | .25624 | 23.358 | 21.04 fg |
| | 21600 | | | | | .29949 | .11328 | 24.588 | 9.30 ij |
| | 86400 | | | | | .30226 | .30226 | 24.815 | 24.82 def |
| Epoxi | 1800 | .28736 | .986 | .26011 | .00030 | .06104 | .20412 | 5.4476 | 18.22 bcdef |
| | 7200 | | | | | .21370 | .21369 | 19.072 | 19.07 bcdef |
| | 14400 | | | | | .27754 | .19194 | 24.770 | 17.13 bcdefg |
| | 21600 | | | | | .28622 | .20308 | 25.545 | 18.12 bcdef |
| | 86400 | | | | | .28736 | .28736 | 25.647 | 25.64 bcd |
| Epoxi + | 1800 | .52183 | 1.647 | .28107 | .00012 | .04180 | 3.7080 | 3.7080 | 15.56 defgh |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| $C_{12}EO_3$ | 7200 | | | | | .17148 | 15.213 | 15.213 | 15.21 cdefg |
| | 14400 | | | | | .32249 | 28.610 | 28.610 | 17.43 bcdefg |
| | 21600 | | | | | .42184 | 37.424 | 37.424 | 25.43 bc |
| | 86400 | | | | | .52177 | 46.289 | 46.289 | 46.29 a |
| Epoxi + | 1800 | .3200 | 1.44 | .19989 | .00021 | .04718 | .03891 | 4.2437 | 3.50 i |
| $C_{12}EO_6$ | 7200 | | | | | .18291 | .18291 | 16.453 | 16.93 bcdefg |
| | 14400 | | | | | .28198 | .13491 | 25.364 | 12.13 efgh |
| | 21600 | | | | | .31115 | .13294 | 27.988 | 11.96 efgh |
| | 86400 | | | | | .32000 | .32000 | 28.785 | 28.78 b |
| Epoxi + | 1800 | .24085 | 4.41 | .04864 | .00014 | .02297 | .02297 | 2.0460 | 5.52 hi |
| L-77 | 7200 | | | | | .09357 | .09357 | 8.3333 | 8.33 ghi |
| | 14400 | | | | | .16889 | .16889 | 15.041 | 10.96 fghi |
| | 21600 | | | | | .21059 | .21059 | 18.755 | 16.44 bcdefg |
| | 86400 | | | | | .24084 | .24084 | 21.449 | 21.45 bcde |

[a]Required inputs for Watanabe model (2002) are marked with an asterisk.
[b]Treatments within each xenobiotic series with no letter in common are significantly different (p=0.05)

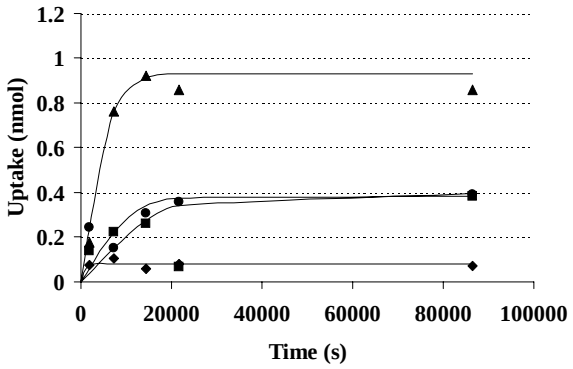


**Figure 6.1.** Uptake (nmol) over time of DOG into *C. album* foliage, in the absence (♦) and presence of the surfactants $C_{12}EO_3$ (■), $C_{12}EO_6$ (▲), and TSE7.5 (●). Symbols represent actual results; lines are calculated from eqn 6.3.

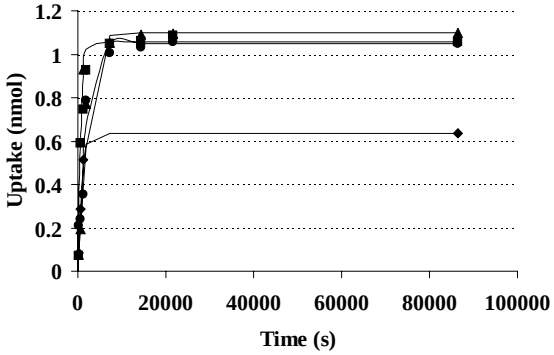


**Figure 6.2.** Uptake (nmol) over time of 2,4-D into *C. album* foliage, in the absence (♦) and presence of the surfactants $C_{12}EO_3$ (■), $C_{12}EO_6$ (▲), and TSE7.5 (●). Symbols represent actual results; lines are calculated from eqn 6.3.

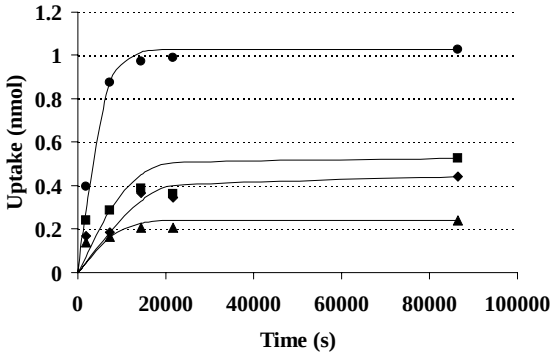


**Figure 6.3.** Uptake (nmol) over time of epoxiconazole into *C. album* foliage, in the absence (♦) and presence of the surfactants $C_{12}EO_3$ (■), $C_{12}EO_6$ (▲), and TSE7.5 (●). Symbols represent actual results; lines are calculated from eqn 6.3.

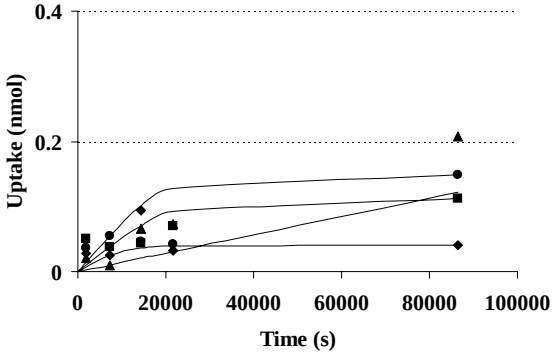


**Figure 6.4.** Uptake (nmol) over time of DOG into *H. helix* foliage, in the absence (♦) and presence of the surfactants $C_{12}EO_3$ (■), $C_{12}EO_6$ (▲), and TSE7.5 (●). Symbols represent actual results; lines are calculated from eqn 6.3.

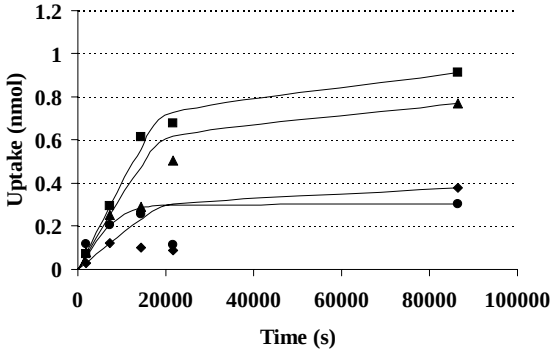


**Figure 6.5.** Uptake (nmol) over time of 2,4-D into *H. helix* foliage, in the absence (♦) and presence of the surfactants $C_{12}EO_3$ (■), $C_{12}EO_6$ (▲), and TSE7.5 (●). Symbols represent actual results; lines are calculated from eqn 6.3.

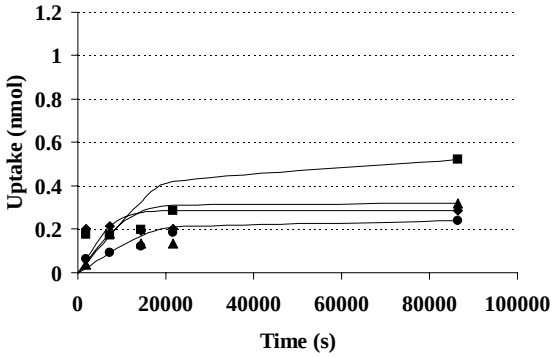


**Figure 6.6.** Uptake (nmol) over time of epoxiconazole into *H. helix* foliage, in the absence (♦) and presence of the surfactants $C_{12}EO_3$ (■), $C_{12}EO_6$ (▲), and TSE7.5 (●). Symbols represent actual results; lines are calculated from eqn 6.3.

This highlights the fact that *U* in the Watanabe (2002) model is defined as total uptake, meaning maximum uptake possible, and if maximum uptake, or a plateau, has not been reached at 24 h then $P_u$ and *q* cannot be derived correctly. This may be the case for uptake into *H. helix* of some of the formulations, in particular DOG, when applied in the presence of $C_{12}EO_6$ (Fig. 6.4). However, in this particular case, if *f* at 4 h is used to derive *q*, rather than *f* at 2 h as has been used throughout, then the data points correspond much better (data not shown) even though the calculated *f* at 2 h is not greatly different from the actual *f* (3 versus 1% uptake at 2 h). This changes *q* (the penetration rate factor) to 0.00006 rather than the 0.00002 in Table 6.2. This demonstrates the importance of obtaining very accurate uptake data for the steep portion of the curve when using the logistic-kinetic model to predict uptake at different times. The penetration rate factor, q, is overall markedly lower for DOG and 2,4-D

uptake into *H. helix*, compared with *C. album*, whereas the difference in *q* is much less for epoxiconazole (Tables 6.1 and 6.2).

These results show that the equation developed by Watanabe for uptake through isolated cuticles can be used to calculate uptake over time into whole plants, with either thin or thick cuticles. The logistic-kinetic transcuticular penetration model (2002) in most cases correctly predicts a linear increase in the penetration rate for the initial period, followed by a gradual decrease in penetration rate, with maximum penetration being approached asymptotically.

In comparison, a dynamic nonlinear simulation model that included an equation describing the cuticular sorption process, developed (Satchivi *et al.*, 2000a) and apparently validated (Satchivi *et al.*, 2000b) for whole plant transport of foliar-applied xenobiotics, predicts (Satchivi *et al.*, 2001) a steady (fairly linear) increase over a 72 h period for hydrophilic, intermediate polarity, and lipophilic compounds, which is not what is found in practice. However, the advantage of the model by Satchivi et al. (Satchivi *et al.*, 2000a, b, 2001) is that it can be used to examine how different chemical and plant properties, as well as environmental factors, might affect the absorption and translocation of xenobiotic compounds. The disadvantage is the number of factors required, including many derived from isolated cuticle studies (e.g. xenobiotic diffusion coefficient, wax/water partition coefficient, cuticle/water partition coefficient, thickness of the limiting skin, xenobiotic concentration in the formulation residue and in the cuticular membrane, and the critical micelle concentration of the surfactant). Another method of modelling foliar uptake of pesticides (Lamb *et al.*, 2001) also requires numerous inputs such as diffusion coefficient, partition coefficient between droplet and cuticle and between cuticle and plant, cuticle thickness, droplet volume and diameter, and duration of the experiment. Species differences are taken into account in this model in the form of cuticle thickness, and prediction versus actual uptake appears to be good. A more recent study (Brazee *et al.*, 2004) again using isolated cuticles developed a one-dimensional, membrane diffusion model for cuticular penetration of a bioregulator (1-naphthylacetic acid) applied as a finite dose to a plant surface. The authors found satisfactory agreement over the experimental time course of 120 h, but for the first 10 h of penetration, the model predicted an overestimate of penetration. They concluded that the cause may be that the model is of a uniformly decaying form from the time transport begins. The authors considered that a model is required such that diffusivity starts at a relatively low value, increases to a peak, then decays uniformly with time. This type of model would simulate an initial increase in solute concentration as solvent evaporated, followed by a gradual transition

to a hydrated residue state with a slower transport rate. The Watanabe logistic-kinetic penetration model describes this process. However, it cannot predict total or maximum uptake, and this value needs to be known, as well as uptake at a time on the steep portion of the uptake curve (e.g., uptake at 2 h), for all formulations, to derive *q* and use eqn 6.3.

**Application of a Logistic-Kinetic Penetration Model.** Watanabe (2002) states that an increase in AI concentration or an increase in contact area will have no effect on the penetration rate factor (*q*). A previous study (Forster *et al.*, 2004a) determined uptake at 24 h for the same formulations as used in the preceding sections, but with a wide range of concentrations for DOG, 2,4-D, and epoxiconazole. If the Watanabe concept is correct, and applicable to whole plant uptake, then the values for *q* derived in the current study, along with the 24 h uptake data determined in the previous study, should enable the uptake to be predicted at any given time prior to 24 h using eqn 6.1. This proposition was tested using two different concentrations of DOG, 2,4-D, and epoxiconazole, all applied in the presence of the surfactants $C_{12}EO_3$, $C_{12}EO_6$, and TSE7.5, onto *C. album*. The concentrations used were chosen to be well below and considerably above the original concentration used in the rate experiment described earlier. The concentrations chosen for DOG were 10 times lower and 50 times higher than the original concentration studied; for 2,4-D these were 100 times lower and 10 times higher; and for epoxiconazole they were 100 times lower and 2 times higher. Uptake at two intervals (2 and 6 h, 7200 and 21600 s) was predicted and then tested experimentally. The correlation between actual and predicted uptake was mainly poor (Table 6.3). Epoxiconazole showed the best correlation, followed by 2,4-D and then DOG. The largest discrepancy was with DOG uptake in the presence of $C_{12}EO_6$, particularly at the higher concentration. This formulation of DOG spreads the least, meaning that the dose is highest. It has been rationalized (Forster *et al.*, 2004b) that actual uptake can be much lower than predicted due to a significant amount of crystallization of xenobiotic on the surface of the leaf (Forster *et al.*, 2004b). However, in the current case, contact phytotoxicity to the leaf surface is observed when DOG is applied in the presence of $C_{12}EO_6$, whereas none is observed when DOG is applied in the presence of $C_{12}EO_3$ or TSE7.5. A much better correlation is found between predicted and actual uptake of the highest concentration of DOG formulated with TSE7.5, which spreads the most, meaning that the dose is much less. This lends weight to the postulate that the reason for DOG showing the poorest correlation between predicted and actual uptake is due to the 50 times higher concentration used. Considering the range of concentrations used, compared to the original concentration used to derive *q*, the correlations between actual and predicted uptake are remarkably good. More work is required, but the

conclusion at this stage is that the Watanabe theory is largely correct; that is, a change in AI concentration or contact area (i.e., a change in initial dose) does not alter the penetration rate factor, $q$. However, a caveat needs to be added, to limit the concentration ranges over which specific xenobiotics should be estimated.

If eqn 6.1 can be used to predict uptake, then can eqn 6.3 also be used to predict uptake? Total uptake needs to be known to utilize eqn 6.1, whereas total uptake is not required to utilize eqn 6.3 if $P_u$ is also constant across concentration or contact area. Using epoxiconazole data as an example (from Table 6.3), then Table 6.4 shows predicted uptake using eqn 6.3. It can be seen that the correlation between predicted and actual uptake is generally poor. This shows that although $q$ remains constant, $P_u$, the unit partition ratio of pesticide, changes when the concentration of the AI is changed. It would appear from these results that lowering the concentration of the AI increased $P_u$, that is, it would need a much higher $P_u$ to increase the predicted uptake to a value closer to the actual uptake. Increasing the concentration results in a similar, if not lower $P_u$. The lowest concentration of epoxiconazole used was 100 times less than that on which $P_u$ (and $q$) was based, whereas the highest concentration was only 2 times greater, which would explain why the differences are not as large for the higher concentration of epoxiconazole.

In the current study it has been possible to predict the rate of uptake for concentrations of active ingredients not already studied (for 2,4-D and epoxiconazole, but not DOG), at different time intervals. However, $q$ needs to be derived for one concentration of the formulation (into the specific species being considered), and maximum uptake needs to be known for all formulations and concentrations of interest. Recent studies (Forster *et al.*, 2004a; Forster *et al.*, 2006a) have shown that mass uptake at 24 h on a per unit area basis is related to the initial dose of xenobiotic applied, by an equation of the following form: $\text{uptake}_{(\text{nmol mm}^{-2})} = a\,[\text{ID}]^b$ at time t = 24 h, where ID is the initial dose or the mass of xenobiotic applied per unit area ($M_{\text{nmol xenobiotic applied}}/A_{\text{droplet spread area}}$) and a and b are constants specific to each xenobiotic applied to a specific species. Total mass uptake at 24 h could be calculated from an equation of the form: $\text{total uptake}_{\text{nmol}} = a\,[\text{ID}]^b.A$. Again, using epoxiconazole as the example, can this equation be used to predict $U$ (total uptake) and then use the derived q, and eqn 6.1. In the case of epoxiconazole, Forster *et al.* (2004a) found that: uptake (epoxiconazole in nmol) = $0.3103(\text{ID})^{0.745}$(spread area). When the predicted uptake value from this calculation is used in eqn 6.1, the overall correlation between actual and predicted uptake at 2 and 6 h is variable (Table 6.5).

**Table 6.3.** Calculated uptake into *C. album* foliage at 2 and 6 h of DOG, 2,4-D, and epoxiconazole, applied in the presence of surfactants, using *q* derived for another concentration of each AI, and 24 h uptake data for the predicted concentration.

| formulation | nmol applied per 0.24 µl | time*[a] (s) | total uptake (U)* (nmol) | spread area (A)* ($mm^2$) | q* ($s^{-1}$) | calcd uptake (f) (nmol) | actual uptake (nmol) | calcd uptake (%) | actual uptake (%) |
|---|---|---|---|---|---|---|---|---|---|
| DOG + | 0.1107 | 7200 | .02088 | 10.92 | .00022 | .01223 | .0066 | 11.04 | 5.96 |
| $C_{12}EO_3$ | | 21600 | | | | .02038 | .00892 | 18.40 | 8.05 |
| DOG + | 54.292 | 7200 | 24.736 | 2.31 | .00022 | 14.488 | 3.2502 | 26.69 | 6.29 |
| $C_{12}EO_3$ | | 21600 | | | | 24.137 | 6.3478 | 44.46 | 16.00 |
| DOG + | 0.1108 | 7200 | .04653 | 1.5 | .00036 | .03806 | .02755 | 34.34 | 25.37 |
| $C_{12}EO_6$ | | 21600 | | | | .04647 | .03296 | 41.94 | 29.74 |
| DOG + | 54.292 | 7200 | 52.034 | 1.2 | .00036 | 42.558 | 2.3440 | 78.39 | 4.32 |
| $C_{12}EO_6$ | | 21600 | | | | 51.97 | 8.30 | 95.72 | 15.29 |
| DOG + | 0.1106 | 7200 | .03284 | 46.51 | .00012 | .01134 | .03207 | 10.25 | 29.53 |
| L-77 | | 21600 | | | | .02723 | .06040 | 24.62 | 52.85 |
| DOG + | 54.292 | 7200 | 11.99 | 32.5 | .00012 | 4.1405 | 5.8707 | 7.63 | 10.81 |
| L-77 | | 21600 | | | | 9.9402 | 8.1237 | 18.31 | 14.96 |
| 2,4-D + | .02403 | 7200 | .01568 | 2.35 | .0025 | .01568 | .01372 | 65.26 | 57.09 |
| $C_{12}EO_3$ | | 21600 | | | | .01568 | .01695 | 65.26 | 70.53 |
| 2,4-D + | 10.87 | 7200 | 7.9976 | 1.81 | .0025 | 7.9976 | 9.3676 | 73.56 | 86.16 |
| $C_{12}EO_3$ | | 21600 | | | | 7.9976 | 10.186 | 73.56 | 94.27 |
| 2,4-D + | .02396 | 7200 | .02186 | 1.61 | .0007 | .02149 | .00657 | 89.68 | 60.31 |
| $C_{12}EO_6$ | | 21600 | | | | .02186 | .01891 | 91.24 | 78.91 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 2,4-D + | 10.87 | 7200 | 8.9402 | 1.37 | .0007 | 8.7876 | 8.956 | 80.83 | 82.38 |
| $C_{12}EO_6$ | | 21600 | | | | 8.9402 | 9.8229 | 82.23 | 90.36 |
| 2,4-D + | .02512 | 7200 | .02341 | 33.29 | .001 | .02336 | .00776 | 93.19 | 71.23 |
| L-77 | | 21600 | | | | .02341 | .00981 | 93.19 | 90.07 |
| 2,4-D + | 10.87 | 7200 | 8.1320 | 3.66 | .001 | 8.1158 | 7.9534 | 74.64 | 73.15 |
| L-77 | | 21600 | | | | 8.1320 | 9.1700 | 74.79 | 84.34 |
| Epoxi + | .02994 | 7200 | .02874 | 6.65 | .0002 | .01572 | .02822 | 52.51 | 94.27 |
| $C_{12}EO_3$ | | 21600 | | | | .02774 | .02858 | 92.66 | 95.45 |
| Epoxi + | 2.1915 | 7200 | .49965 | 2.73 | .0002 | .27333 | .49012 | 12.47 | 21.83 |
| $C_{12}EO_3$ | | 21600 | | | | .48231 | .3924 | 22.01 | 16.97 |
| Epoxi + | .02872 | 7200 | .02816 | 1.41 | .0003 | .02090 | .00810 | 72.76 | 74.28 |
| $C_{12}EO_6$ | | 21600 | | | | .02805 | .02774 | 97.65 | 96.60 |
| Epoxi + | 2.1901 | 7200 | .41874 | 1.47 | .0003 | .31073 | .36387 | 14.19 | 16.61 |
| $C_{12}EO_6$ | | 21600 | | | | .41703 | .33263 | 19.04 | 15.19 |
| Epoxi + | .02968 | 7200 | .02862 | 26.09 | .0004 | .02470 | .01004 | 83.23 | 92.08 |
| L-77 | | 21600 | | | | .02861 | .01055 | 96.39 | 96.56 |
| Epoxi + | 2.1906 | 7200 | 1.7234 | 36.65 | .0004 | 1.4875 | .93170 | 67.90 | 42.53 |
| L-77 | | 21600 | | | | 1.7226 | 1.5138 | 78.63 | 69.10 |

[a]Required inputs for Watanabe model (2002) are marked with an asterisk

**Table 6.4.** Calculated uptake into *C. album* foliage at 2 and 6 h of epoxiconazole, applied in the presence of surfactants, using q and $P_u$ derived from another concentration of epoxiconazole.

| formulation | nmol applied per 0.24 µl | time*[a] (s) | total uptake (U)* (nmol) | spread area (A)* ($mm^2$) | q ($s^{-1}$)* | calcd uptake (f) (nmol) | actual uptake (nmol) | calcd uptake (%) | actual uptake (%) |
|---|---|---|---|---|---|---|---|---|---|
| Epoxi + | .02994 | 7200 | .02874 | 6.65 | .0002 | .00864 | .02822 | 28.86 | 94.27 |
| $C_{12}EO_3$ | | 21600 | | | | .01525 | .02858 | 50.92 | 95.45 |
| Epoxi + | 2.1915 | 7200 | .49965 | 2.73 | .0002 | .25963 | .49012 | 11.84 | 21.83 |
| $C_{12}EO_3$ | | 21600 | | | | .45814 | .3924 | 20.91 | 16.97 |
| Epoxi + | .02872 | 7200 | .02816 | 1.41 | .0003 | .00496 | .00810 | 17.28 | 74.28 |
| $C_{12}EO_6$ | | 21600 | | | | .00666 | .02774 | 23.19 | 96.60 |
| Epoxi + | 2.1901 | 7200 | .41874 | 1.47 | .0003 | .39454 | .36387 | 18.01 | 16.61 |
| $C_{12}EO_6$ | | 21600 | | | | .52952 | .33263 | 24.18 | 15.19 |
| Epoxi + | .02968 | 7200 | .02862 | 26.09 | .0004 | .01825 | .01004 | 61.50 | 92.08 |
| L-77 | | 21600 | | | | .02114 | .01055 | 71.22 | 96.56 |
| Epoxi + | 2.1906 | 7200 | 1.7234 | 36.65 | .0004 | 1.8925 | .93170 | 86.39 | 42.53 |
| L-77 | | 21600 | | | | 2.1916 | 1.5138 | 100.04 | 69.10 |

[a]Required inputs for Watanabe model (2002) are marked with an asterisk.

**Table 6.5.** Calculated uptake of epoxiconazole, applied in the presence of surfactants, into *C. album* foliage at 2 and 6 h, using *q* derived for another concentration of epoxiconazole and 24 h uptake data predicted from alternative (Forster *et al.*, 2004) dose uptake equation.

| formulation | nmol applied per 0.24 µl | time*[a] (s) | total uptake (U)* (nmol) | spread area (A)* ($mm^2$) | q* ($s^{-1}$) | calcd uptake (f) (nmol) | actual uptake (nmol) | calcd uptake (%) | actual uptake (%) |
|---|---|---|---|---|---|---|---|---|---|
| Epoxi + | .02994 | 7200 | .03685 | 6.65 | .0002 | .02016 | .02822 | 67.33 | 94.27 |
| $C_{12}EO_3$ | | 21600 | | | | .03557 | .02858 | 118.80 | 95.45 |
| Epoxi + | 2.1915 | 7200 | .71920 | 2.73 | .0002 | .39344 | .49012 | 17.95 | 21.83 |
| $C_{12}EO_3$ | | 21600 | | | | .69424 | .3924 | 31.68 | 16.97 |
| Epoxi + | .02872 | 7200 | .02405 | 1.41 | .0003 | .01785 | .00810 | 62.15 | 74.28 |
| $C_{12}EO_6$ | | 21600 | | | | .02396 | .02774 | 83.41 | 96.60 |
| Epoxi + | 2.1901 | 7200 | .61388 | 1.47 | .0003 | .45553 | .36387 | 20.80 | 16.61 |
| $C_{12}EO_6$ | | 21600 | | | | .61138 | .33263 | 27.92 | 15.19 |
| Epoxi + | .02968 | 7200 | .05188 | 26.09 | .0004 | .04477 | .01004 | 150.86 | 92.08 |
| L-77 | | 21600 | | | | .05185 | .01055 | 174.70 | 96.56 |
| Epoxi + | 2.1906 | 7200 | 1.3943 | 36.65 | .0004 | 1.2034 | .93170 | 54.93 | 42.53 |
| L-77 | | 21600 | | | | 1.3936 | 1.5138 | 63.62 | 69.10 |

[a]Required inputs for Watanabe model (2002) are marked with an asterisk.

There are some large discrepancies, and when predicted uptake is well over 100%, actual uptake is close to 100%. Obviously, the equation using initial dose (ID) to predict uptake at 24 h needs to be refined further. The relative importance of each of the variables involved in uptake, that is, species, AI, AI concentration (g $L^{-1}$), and surfactant has also been established recently (Forster *et al.*, 2006b), and surfactant has been shown to be highly significant, even after taking spread area into account. Progress needs to continue in this area to produce a more accurate model for uptake at 24 h. However, using the equations based on initial dose provides a good rule of thumb for uptake at 24 h (Forster *et al.*, 2004c), and using these in conjunction with the Watanabe model has significantly advanced our understanding and ability to model xenobiotic uptake. The advantages of these models and equations are that few variables are required and they are simple to measure.

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# DISCUSSION AND CONCLUSIONS

A very limited number of extremely detailed models for xenobiotic uptake into plant foliage do exist (Satchivi *et al.*, 2000a, 2000b, 2001) but by their very nature they have a large number of parameters that tend to be plant and active ingredient specific that may be difficult, if not impossible to determine in most cases. What was lacking were intermediate models that had enough of the physical mechanisms incorporated into them to be realistic but not so many that the parameters were too numerous and their values impossible to determine. Because there is general agreement that uptake through the leaf cuticle is a diffusion process, and a simple way of considering the flux per unit area is the amount of xenobiotic that diffuses through a unit area per unit of time, it was hypothesised that uptake should be related to moles of xenobiotic per unit area. Therefore, the objective of initial studies (Chapter 3) was to determine whether the uptake of model xenobiotics differing in lipophilicity and in the presence of a range of surfactants could be described by a simple relationship involving the initial dose of the xenobiotic applied. The uptake of three model compounds, applied in the presence and absence of surfactants, into the leaves of three plant species (*Chenopodium album*, *Hedera helix* and *Stephanotis floribunda*) was determined. The results with 2-deoxy-D-glucose (DOG), 2,4-dichlorophenoxy-acetic acid (2,4-D) and epoxiconazole ((2RS,3SR)-1-[3-(2-chlorophenyl)-2-(4-fluoro-phenyl)oxiran-2-ylmethyl]-1H-1,2,4-triazole) in the presence of surfactants (the polyethylene glycol monododecyl ethers $C_{12}EO_3$, $C_{12}EO_6$, $C_{12}EO_{10}$, and a trisiloxane ethoxylate with mean EO of 7.5 (TSE7.5), all used at one equimolar concentration and therefore different percentage concentrations) illustrated that the initial dose (nmol $mm^{-2}$) of xenobiotic applied to plant foliage was a strong positive determinant of uptake. This held true for all the xenobiotic formulations studied over a wide concentration range, in the presence of these surfactants. Using this new approach for whole plant uptake, uptake on a per unit area basis could be related to initial dose of xenobiotic applied, by an equation of the form: $Uptake_{(nmol\ mm^{-2})} = a\ [ID]^b$ at time t = 24 hours, where ID is the initial dose or the mass of xenobiotic applied per unit area ($M_{(nmol\ xenobiotic\ applied)}/A_{(droplet\ spread\ area)}$). Total mass uptake could then be calculated from an equation of the form: $Total\ Uptake_{(nmol)} = a\ [ID]^b.A$.

Few studies have considered such a range of concentrations, with most studies considering only a single xenobiotic concentration. Although the upper concentration limits used in this research are much higher than would be used in practice, it has provided a much better understanding of a significant determinant to uptake, i.e. initial dose. The lower

concentrations studied here (for 2,4-D and epoxiconazole) are ones used operationally, and fit well with the current model.

The advantages of the approach presented here are that few variables are required, and they are simple to measure. In addition, the initial molar quantity applied per unit area has been shown to be proportional to the initial driving force, in line with Fick's law of diffusion modified for plants.

Extensions of the studies outlined in Chapter 3 were presented at the 7th International Symposium on Adjuvants for Agrochemicals (Appendix I and II). These papers illustrated how the equations developed in Chapter 3 could be applied to estimate uptake per unit area, total mass uptake or percent uptake (Appendix I), and related anomalies in the mass uptake relationship found at high concentrations to the appearance of precipitates (Appendix II). It was demonstrated (Appendix I) that although a surfactant causing a formulation to spread will decrease the dose of AI per unit area and therefore theoretically decrease the uptake per unit area, compared to a formulation which spreads less, total uptake is the product of uptake per unit area and spread area. Therefore total uptake may be less than, equal to, or greater than for a formulation which spreads less. It was shown (Appendix I) that equations based only on spread areas could not be used as stand alone models for all types of xenobiotics, but do give an estimate of uptake from the spread area of the formulation on the specified species. It was found (Appendix II) that "anomalies" in the uptake of epoxiconazole and 2,4-D could be explained by precipitation behaviour at the high concentrations studied. The appearance of precipitates from the droplet solutions related well to the pattern of epoxiconazole and 2,4-D uptake into *Chenopodium album*. However, in the case of the more polar DOG the precipitates observed on the leaf surfaces did not relate well to the uptake trends.

In order to verify the relationship developed in Chapter 3, further studies (Chapter 4) determined the uptake of three pesticides, applied at practical working concentrations as commercial and model formulations, in the presence of a wide range of surfactants, into the leaves of three plant species (bentazone into *Chenopodium album* and *Sinapis alba*, epoxiconazole and pyraclostrobin into *Triticum aestivum*). The pesticides were in the form of water-dispersible, emulsifiable concentrate or suspension concentrate formulations. The AI dose per unit area generated was solely dependent on the surfactant and the surfactant concentration used, rather than that obtained by changing the AI concentration. The study also considered the influence of surfactant type and concentration relative to the AI product, as may be produced by adding surfactants to the initial tank mix formulation. The results confirmed the finding that the initial dose (nmol $mm^{-2}$) of xenobiotic applied to plant foliage

is a strong, positive determinant of uptake. This held true for all the pesticide formulations studied, though surfactant concentration was found to have an effect. The lower surfactant concentrations studied showed an inferior relationship between the amount of xenobiotic applied and uptake. High molecular mass surfactants also produced much lower uptake than expected from the dose uptake equations although behaviour was also species dependent. It could be hypothesised that the high molecular mass surfactants do not penetrate into the leaf to a sufficient extent (if at all) to enhance the uptake of the AI as much as the lower molecular mass surfactants may do. The species into which compounds can penetrate more easily (i.e. *Sinapis alba* in this study) may allow a higher molecular mass surfactant to penetrate better, compared to more difficult-to-penetrate species. In contrast to the previous studies (Chapter 3), where a defined surfactant series was used, the present studies used a wide range of surfactant structures. Such a wide range of surfactant structure is unusual in these type of studies. Although there is information in the literature on the effect of surfactants on the uptake of actives, in particular with regard to ethylene oxide (EO) chain length (Stevens and Bukovac, 1987; Holloway *et al.*, 1989; Steurbaut *et al.*, 1989; Holloway and Stock, 1990; Stock, 1990; Chamel *et al.*, 1992; Gaskin and Holloway, 1992; Holloway and Edgerton, 1992; Holloway *et al.*, 1992; Kirkwood *et al.*, 1992; Stock and Holloway, 1993; Coret and Chamel, 1994), there is a lack of information on surfactant uptake, and the relative uptake of surfactants compared with actives. This should be the subject of future studies.

These studies (Chapters 3 and 4) have shown that the sole use of initial dose is too simplistic, and other factors need to be added to a model. Fick's law as modified for plant cuticles (Price, 1982; Schönherr and Baur, 1994, 1996) is more complex and although the variables (diffusion coefficient, partition coefficient between the formulation residue on the leaf surface and the cuticle, partition coefficient between the cuticle and the aqueous phase of the epidermal cell wall, concentration of the AI on the leaf surface, concentration of the AI in the cuticle, tortuosity factor and effective cuticular thickness) have been determined using isolated cuticles, it would be extremely difficult to derive them all using whole-plant systems. Alternative variables need to be considered for whole-plant uptake, and the dataset described in Chapter 3 was well suited to testing the relative influences of each of the factors involved in uptake.

Therefore, in a novel approach, further studies (Chapter 5) used this relationship (nmol $mm^{-2}$ uptake versus ID; termed the uptake ratio) to establish the relative importance of species, AI, AI concentration (g $L^{-1}$) and surfactant to uptake. Species, AI, its concentration, and surfactant all significantly affected the uptake ratio (together explaining 51% of the

deviance). The percentage variance explained by each factor ranged from 8.9% (AI) to 17% (surfactant). Overall, 88% of the deviance could be explained. More useful was the analysis of the individual xenobiotics, where the models explained 83%, 85%, and 94% of the variance in uptake ratio for DOG, 2,4-D, and epoxiconazole, respectively. In all cases, species, surfactant, and AI concentration significantly affected the uptake ratio. However, there were differences in the relative importance of these factors among the xenobiotics studied. Concentration of AI increased in importance with increasing lipophilicity of AI, while species was much less important for the most lipophilic compound. Surfactant became less important with increasing lipophilicity, although it was always important. The interaction between AI concentration and species was much more important for the most polar compound, while the interaction between surfactant and species increased in importance with increasing lipophilicity. Now that the relative importance of the factors affecting the uptake ratio (excluding environmental factors and surfactant concentration) has been identified, future studies can attempt to correlate physical properties to the individual factors. For example, it has been found that concentration of AI explains the highest percent deviance (46.5%) for the uptake ratio of epoxiconazole. Concentration can be used directly and needs no other parameters measured. However, surfactant explains the next highest percent deviance (16.8%), and in order to develop a universal equation we need to be able to measure some physical property, or more likely several properties, of each of the surfactants which will account for the differences among them. If we can account in some way for the differences among surfactants, then we will manage to explain > 60% of the deviance for a lipophilic compound. In the case of 2,4-D, to be able to account for a significant amount of the deviance, we need to be able to account for the differences among species, as well as AI concentration and surfactant. In the case of DOG, a hydrophilic compound, the two factors that account for the largest percent deviance are species and surfactant. Therefore, establishing a universal model for a lipophilic molecule appears to be the easiest place to begin. Rather than attempting to correlate the physical properties of the xenobiotics to percent uptake or total uptake, we would be attempting to correlate physical properties only to that proportion of the deviance of the uptake ratio which that factor explains. This is quite different from other attempts described in the literature, and will be the subject of further studies. The effect of surfactant concentration has not been considered in these studies, and will also be the subject of future work.

The preceding studies all considered uptake at only one time interval (24 hours). Total uptake after 24 hours can be the same for a compound formulated with different surfactants, but rates of uptake (and therefore rainfastness and subsequent translocation to target sites) can

be quite different. Therefore there was a requirement to be able to model uptake over time into whole plants. Hence the objective of further studies (Chapter 6) was to determine whether a logistic-kinetic penetration model could be applied to whole plant uptake. Uptake over 24 hours was determined for three model compounds (DOG, 2,4-D and epoxiconazole), applied in the presence and absence of surfactants ($C_{12}EO_3$, $C_{12}EO_6$ and TSE7.5), into the leaves of two plant species (*C. album* and *H. helix*). Data for two time intervals was used in the model, to predict uptake at intermediate intervals, and compared with experimental results. Overall, the model fitted the whole plant uptake data well. These results showed that the equation developed by Watanabe (2002) for uptake through isolated cuticles can be used to calculate uptake over time into whole plants, with either thin or thick cuticles. The logistic-kinetic transcuticular penetration model (Watanabe, 2002) in most cases correctly predicted a linear increase in the penetration rate for the initial period, followed by a gradual decrease in penetration rate, with maximum penetration being approached asymptotically. However, it cannot predict total or maximum uptake, and this value needs to be known, as well as uptake at a time on the steep portion of the uptake curve (e.g., uptake at 2 h), for all formulations, to derive $q$. The study confirmed that an increase (or decrease) in AI concentration or an increase in contact area will have no effect on the penetration rate factor, q, within the normal working concentration range. This enabled uptake to be predicted at different times for concentrations of AI not already studied, having first derived q for one concentration of the formulation of interest, and having 24 hour (maximum) uptake results for all formulations and concentrations of interest. This study highlighted the fact that $U$ in the Watanabe (2002) model is defined as total uptake, meaning maximum uptake possible, and if maximum uptake, or a plateau, has not been reached at 24 h then $P_u$ and $q$ cannot be derived correctly. It also demonstrated the importance of obtaining very accurate uptake data for the steep portion of the curve when using the logistic-kinetic model to predict uptake at different times. Using the equations developed (Chapter 3) based on initial dose to calculate uptake at 24 hours, in conjunction with the logistic-kinetic model (Watanabe, 2002), has significantly progressed our understanding and ability to model uptake. The advantages of the models and equations described are that few variables are required, and they are simple to measure.

Further work is still required in order to explain differences in uptake due to species, active ingredient or surfactant, and build these into the model.

Chamel and Vitton (1996) concluded that the structural characteristics of the cuticles are of major importance and must be given particular attention when attempting to establish models to predict the transfer of xenobiotics through plant cuticles. Diffusion of molecules is

thought to be mainly limited to the amorphous regions while the crystalline domain should represent an essentially excluded zone (Schreiber *et al.*, 1996a). It has been postulated that the relative size and spatial arrangement of crystalline and amorphous wax fractions will govern the mobility of permeating water and solute molecules (Riederer and Schreiber, 1995; Schreiber *et al.*, 1996a; Merk *et al.*, 1998). Therefore, building up a database quantifying the ratio of crystalline to amorphous region (along with cuticle thickness) across a large number of species may help to determine differences in uptake among species. Methods that may be used for this are solid-state nuclear magnetic resonance (NMR) (Reynhardt and Riederer, 1991, 1994; Deshmukh *et al.*, 2005) and Fourier transform infrared spectroscopy (FTIR) (Merk *et al.*, 1998). Further studies investigating the interaction of surfactants with the cuticle or wax, such as those by Schreiber *et al.* (1996b) using ESR-spectroscopy, would be useful.

While the use of percent AI uptake is sufficient to compare uptake among formulations or to choose the 'best' formulation for a specific species, this can only be identified after actual experiments have been performed. Hence there is no predictive capability and this approach has not provided insights into the mechanisms of xenobiotic uptake into plants (Zabkiewicz and Forster, 2001). Although the spray formulation may have distinct characteristics, after it is deposited on a leaf surface, dynamic interactions such as spreading and drying of the droplet, with concomitant changes in distribution and dose of both AI and formulants, lead to an initial 'spray deposit' that has little resemblance to the original solution. In particular the 'concentration' has been increased to a point such that the residue may be a quasi-solid and more akin to a deposit with minimal water content. It has been shown (Mitchell *et al.*, 1983; Dimitrova *et al.*, 1995; Håkansson *et al.*, 1998) that non-ionic surfactants in aqueous solutions form a variety of liquid crystalline phases depending on concentration and temperature. In pharmaceutical studies different surfactant phases have been shown to exclude, enhance or retard the uptake of medicinal compounds through mammalian skin (Brinon *et al.*, 1999). However, little has been recorded on how the liquid crystalline phases affect the diffusion of active compounds through the plant cuticle. This would appear to be an area that should be investigated further to discover whether these different crystalline phases help explain differences among surfactants in enhancing uptake, as well as possible interactions between the surfactant and AI. The different phases can be detected using a variety of instrumental techniques such as differential scanning calorimetry, rheometry, 2H NMR spectrometry, x-ray and neutron scattering, and plane polarising light microscopy (Dimitrova *et al.*, 1996; Wang *et al.*, 2005; Siddig *et al.*, 2006).

Previous attempts to correlate AI physical properties with uptake into whole plants have found that there was no simple relationship (Stevens, 1984). However trends have been shown both in terms of the physical properties of the AI (Baker *et al.*, 1992) and of the surfactants (Stock, 1990). It is now worth revisiting the physical properties of both AI and surfactant in terms of the uptake ratio.

## SUMMARY

There is a worldwide drive to reduce the quantities used and improve the field effectiveness of agrichemicals. This can be achieved by improving overall spray formulation efficacy, which is determined by the complex interactions of spray: deposition, retention to and uptake into the plant, as well as translocation to the site of biological activity within the plant. Such interactions are being addressed by developing models, based on fundamental processes. Models exist for deposition and retention, but uptake of agrichemicals into living plant foliage is complex and not well understood. There have been no suitable models available to predict the uptake of agrichemicals into living plant foliage. Predictive models cannot be formulated without prior knowledge of the variables and mechanisms involved.

The objective of this PhD was to progress the understanding of the mechanisms of cuticular uptake into living plant foliage, thereby enabling uptake of important compounds such as pesticides and pollutants to be modelled. Intermediate or simple models were required that incorporated enough of the physical mechanisms of uptake to be realistic, but not so many mechanisms that the parameters were too numerous and their values impossible to determine.

The majority of studies available in the literature have considered uptake into whole plants in terms of percentage values. To enable a true and mechanistically more relevant comparison among xenobiotics, the use of moles was considered a more appropriate unit of measure than percent or mass. Because there is general agreement that uptake through the leaf cuticle is a diffusion process, it was proposed that calculating the dose applied to the leaf surface may provide some perspective into the differences in uptake among formulations. The dose applied was derived from a measurement of the spread area and molar amount of xenobiotic applied per droplet, to give moles per unit area of xenobiotic applied.

The uptake of three model compounds, applied in the presence and absence of surfactants, into the leaves of three plant species (*Chenopodium album* L., *Hedera helix* L. and *Stephanotis floribunda* Brongn) was determined. The results with 2-deoxy-D-glucose (DOG), 2,4-dichlorophenoxy-acetic acid (2,4-D) and epoxiconazole ((2RS,3SR)-1-[3-(2-chlorophenyl)-2-(4-fluoro-phenyl)oxiran-2-ylmethyl]-1H-1,2,4-triazole) in the presence of surfactants (the polyethylene glycol monododecyl ethers $C_{12}EO_3$, $C_{12}EO_6$, $C_{12}EO_{10}$, and a trisiloxane ethoxylate with mean ethylene oxide (EO) content of 7.5, all used at one equimolar concentration and therefore different percentage concentrations) illustrated that the initial dose (nmol $mm^{-2}$) of xenobiotic applied to plant foliage was a strong positive determinant of

uptake. This held true for all the xenobiotic formulations studied over a wide concentration range, in the presence of these surfactants. Using this new approach for whole plant uptake, uptake on a per unit area basis was found to be related to initial dose of xenobiotic applied, by an equation of the form: $Uptake_{(nmol\ mm^{-2})} = a\ [ID]^b$ at time t = 24 hours, where ID is the initial dose or the mass of xenobiotic applied per unit area ($M_{(nmol\ xenobiotic\ applied)}/A_{(droplet\ spread\ area)}$). Total mass uptake can then be calculated from an equation of the form: $Total\ Uptake_{(nmol)} = a\ [ID]^b.A$.

In order to verify this relationship, further studies determined the uptake of three pesticides, applied as commercial and model formulations in the presence of a wide range of surfactants, into the leaves of three plant species (bentazone [(3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide] into *Chenopodium album* L. and *Sinapis alba* L., epoxiconazole and pyraclostrobin [methyl-N-{2-[1-(4-chlorophenyl)-1H-pyrazol-3-yloxymethyl]phenyl}-(N-methoxy)carbamate] into *Triticum aestivum* L.). The results confirmed the finding that the initial dose (nmol $mm^{-2}$) of xenobiotic applied to plant foliage is a strong, positive determinant of uptake. This held true for all the pesticide formulations studied, though surfactant concentration had an effect. The lower surfactant concentrations studied showed an inferior relationship between the amount of xenobiotic applied and uptake. High molecular weight surfactants also produced much lower uptake than expected from the dose uptake equations in specific situations.

In a novel approach, further studies used this relationship (nmol $mm^{-2}$ uptake versus ID; termed the uptake ratio) to establish the relative importance of species, active ingredient (AI), AI concentration (g $L^{-1}$) and surfactant to uptake. Species, AI, its concentration, and surfactant all significantly affected the uptake ratio (explaining 51% of the deviance). The percentage variance explained by each factor ranged from 8.9% (AI) to 17% (surfactant). Overall, 88% of the deviance could be explained. More useful was the analysis of the individual xenobiotics, where the models explained 83%, 85%, and 94% of the variance in uptake ratio for DOG, 2,4-D, and epoxiconazole, respectively. In all cases, species, surfactant, and AI concentration significantly affected the uptake ratio. However, there were differences in the relative importance of these factors among the xenobiotics studied. Concentration of AI increased in importance with increasing lipophilicity of AI, while species was much less important for the most lipophilic compound. Surfactant became less important with increasing AI lipophilicity, although it was always important. The interaction between AI concentration and species was much more important for the most polar compound, while the interaction between surfactant and species increased in importance with increasing lipophilicity of AI.

The preceding studies considered uptake at only one time interval (24 hours). Total uptake after 24 hours can be the same for a compound formulated with different surfactants, but rates of uptake (and therefore rain-fastness and subsequent translocation to target sites) can be quite different. Therefore, there was a requirement to be able to model uptake over time into whole plants. Hence, the objective of further studies was to determine whether a logistic-kinetic penetration model, developed using isolated plant cuticles, could be applied to whole plant uptake. Uptake over 24 hours was determined for three model compounds, applied in the presence and absence of surfactants, into the leaves of two plant species. Data for two time intervals was used in the model to predict uptake at intermediate intervals and compared with experimental results. Overall, the model fitted the whole plant uptake data well. The study confirmed that an increase (or decrease) in AI concentration or an increase in contact area would have no effect on the penetration rate factor, q, within the normal working concentration range. This enabled uptake to be predicted at different times for concentrations of AI not already studied, having first derived q for one concentration of the formulation of interest, and having 24 hour (maximum) uptake results for all formulations and concentrations of interest.

Using the equations developed, based on initial dose, to calculate uptake at 24 hours, in conjunction with the logistic-kinetic model, has significantly progressed our understanding and ability to model uptake. The advantages of the models and equations described are that few variables are required, and they are simple to measure.

# ZUSAMMENFASSUNG

Weltweit ist man bestrebt, die Aufwandsmengen von Agrochemikalien zu reduzieren und ihre Wirksamkeit im Feld zu verbessern. Dies kann durch die allgemeine Verbesserung der Wirkung von Sprühmittelformulierungen erreicht werden, die durch die komplexen Wechselwirkungen des Sprühmittels bestimmt werden: Ablagerung, Retention auf und Aufnahme in die Pflanze sowohl als auch die Translokation an die Stelle der biologischen Aktivität innerhalb der Pflanze. Solche Wechselwirkungen werden durch die Entwicklung von Modellen behandelt, die auf fundamentalen Verfahren beruhen. Es bestehen zwar Modelle zur Ablagerung und Retention, die Aufnahme von Agrochemikalien in die Blätter von lebenden Pflanzen ist jedoch kompliziert und noch nicht gut verstanden. Es stehen derzeit keine geeigneten Modelle zur Verfügung, um die Aufnahme von Agrochemikalien in die Blätter einer lebenden Pflanze abzuschätzen. Modelle zur Abschätzung können ohne vorherige Kenntnis der beteiligten Variablen und Mechanismen nicht formuliert werden.

Das Ziel dieser Dissertation war es, das Verständnis der Mechanismen der Aufnahme von Wirkstoffen über die Kutikula in die Blätter einer lebenden Pflanze zu verbessern und es dadurch möglich zu machen, die Aufnahme von wichtigen Verbindungen wie z.B. von Pestiziden und Schadstoffen zu modellieren. Mittlere und einfache Modelle waren notwendig, die eine ausreichende Anzahl von physikalischen Mechanismen zur Aufnahme berücksichtigten, um die Wirklichkeit beschreiben zu können, jedoch nicht so viele Mechanismen, dass die Parameter zu zahlreich und deren Werte nicht zu erfassen waren.

Die Mehrheit der in der Literatur zur Verfügung stehenden Studien hat die Aufnahme in ganze Pflanzen in Prozentsätzen behandelt. Um einen wahren und mechanistisch relevanteren Vergleich von Xenobiotika zu ermöglichen, erschien in dieser Arbeit die Verwendung von Mol als Maßeinheit besser geeignet als Prozent oder Masse. Da allgemein Übereinstimmung darüber besteht, dass es sich bei der Aufnahme durch die Kutikula eines Blattes um einen Diffusionsprozess handelt, wurde vorgeschlagen, dass die Berechnung der auf die Blattoberfläche aufgetragenen Dosis Hinweise auf die Unterschiede in der Aufnahme verschiedener Formulierungen geben könnte. Die aufgetragene Dosis wurde von einer Messung der benetzten Fläche und der pro Tropfen aufgetragenem Xenobiotikum Molmenge abgeleitet, um die Anzahl von Mol an aufgetragenem Xenobiotikum pro Einheitsfläche zu erhalten.

Es wurde die Aufnahme von drei Modellverbindungen ermittelt, die in Anwesenheit und Abwesenheit von oberflächenaktiven Stoffen in die Blätter von drei Pflanzenarten

(*Chenopodium album* L., *Hedera helix* L. und *Stephanotis floribunda* Brongn.) aufgetragen wurden. Das Ergebnis mit 2-Deoxy-D-Glucose (DOG), 2,4-Dichlorphenoxy-Essigsäure (2,4-D) und Epoxiconazol ((2RS,3SR)-1-[3-(2-Chlorphenyl)-2-(4-Fluoro-phenyl)Oxiran-2-Ylmethyl]-1H-1,2,4-Triazol) in Anwesenheit der oberflächenaktiven Stoffe (die Polyethylenglycolmonododecylether $C_{12}EO_3$, $C_{12}EO_6$, $C_{12}EO_{10}$, und ein Trisiloxanethoxylat mit einem EO-Mittelwert von 7,5; wobei alle in einer äquimolaren Konzentration und daher in verschiedenen prozentualen Konzentrationen verwendet wurden) zeigte, dass die auf die Pflanzenblätter aufgetragene anfängliche Dosis (nmol $mm^{-2}$) an Xenobiotikum ein starker, positiv bestimmender Faktor für die Aufnahme war. Dies traf auf alle Formulierungen, die über einen breiten Konzentrationsbereich in Anwesenheit dieser oberflächenaktiven Stoffe untersucht wurden, zu. Verwendet man diese neue Beschreibung der Aufnahme von Xenobiotica in ganze Pflanzen, so kann man feststellen, dass die Aufnahme pro Einheitsfläche von der anfänglichen Dosis von aufgetragenem Xenobiotikum abhängig ist, und zwar nach folgender Gleichung: $Aufnahme_{(nmol\ mm^{-2})} = a\ [ID]^b$ bei einer Zeit t = 24 Stunden, wobei ID für die anfängliche Dosis oder die Masse an pro Einheitsfläche aufgetragenem Xenobiotikum steht ($M_{(nmol\ aufgetragenes\ Xenobiotikum)}/A_{(Tropfenausbreitungsbereich)}$). Die Gesamtaufnahme der Masse kann dann aus einer Gleichung der Formel: $Gesamtaufnahme_{(nmol)} = a\ [ID]^b.A$ errechnet werden.

Um diese Beziehung zu bestätigen, wurde in zusätzlichen Studien die Aufnahme von drei Pestiziden ermittelt, die als gewerbliche und Modellformulierungen in Anwesenheit einer großen Auswahl von oberflächenaktiven Stoffen in die Blätter von drei Pflanzenarten (Bentazon [(3-Isopropyl-1H-2,1,3-Benzothiadiazin-4(3H)-one-2,2-Dioxid] in *Chenopodium album* L. und *Sinapis alba* L., Epoxiconazol und Pyraclostrobin [Methyl-N-{2-[1-(4-Chlorphenyl)-1H-Pyrazol-3-Yloxymethyl]phenyl}-(N-Methoxy)Carbamat] in *Triticum aestivum* L.) aufgetragen wurden. Die Ergebnisse bestätigten die Feststellung, dass die anfängliche, auf die Pflanzenblätter aufgetragene Dosis (nmol $mm^{-2}$) an Xenobiotikum ein starker, positiv bestimmender Faktor der Aufnahme ist. Dies galt für sämtliche untersuchten Pestizidformulierungen, jedoch hatte die Konzentration der Tenside einen Einfluss. Wurden niedrigere Konzentrationen an oberflächenaktiven Stoffe verwendet, so führte das zu einem schlechteren Verhältnis zwischen der Menge an aufgetragenem Xenobiotikum und der Aufnahme. Oberflächenaktive Stoffe mit hohem Molekulargewicht ergaben ebenfalls eine weit geringere Aufnahme als von den Dosisaufnahmegleichungen in den jeweiligen Situationen erwartet wurde.

Bei einem neuartigen Ansatz verwendeten zusätzliche Studien dieses Verhältnis (nmol $mm^{-2}$ Aufnahme pro ID; genannt Aufnahmeverhältnis), um die relative Bedeutung der Arten, der Wirksubstanz (AI), der AI-Konzentrationen (g $L^{-1}$) und der oberflächenaktiven Stoffe für die Aufnahme zu ermitteln. Die Art, AI, ihre Konzentration und die oberflächenaktiven Stoffe hatten alle einen erheblichen Einfluss auf das Aufnahmeverhältnis (was eine Erklärung für die 51 % an Abweichung ist). Die durch jeden Faktorwert erklärte prozentuale Varianz lag in einem Bereich von 8,9 % (AI) bis 17 % (oberflächenaktiver Stoff). Insgesamt konnte 88 % der Abweichung erklärt werden. Noch nützlicher war die Analyse der einzelnen Xenobiotika, bei denen die Modelle 83 %, 85 % und 94 % der Varianz im Aufnahmeverhältnis jeweils für DOG, 2,4-D und Epoxiconazol erklärten. In allen Fällen hatten die Arten, der oberflächenaktive Stoff und die AI-Konzentration einen erheblichen Einfluss auf das Aufnahmeverhältnis. Es gab jedoch Unterschiede bei der relativen Bedeutung dieser Faktoren unter den untersuchten Xenobiotika. Die Konzentration von AI gewann größere Bedeutung mit einer erhöhten Fettlöslichkeit von AI, während die Art eine weit geringere Rolle für die meisten lipophilen Verbindungen spielte. Oberflächenaktive Stoffe verloren an Bedeutung mit zunehmender AI-Fettlöslichkeit, obwohl diese stets von Bedeutung waren. Die Wechselwirkung zwischen der AI-Konzentration und der Art hatte eine weit wichtigere Bedeutung für die polarste Verbindung, während die Wechselwirkung zwischen dem oberflächenaktiven Stoff und der Art mit zunehmender Fettlöslichkeit von AI an Bedeutung gewann.

Die bisher dargestellten Studien zogen die Aufnahme bei nur einem Uhrzeitintervall (24 Stunden) in Betracht. Die Gesamtaufnahme nach 24 Stunden kann zwar bei einer Verbindung, die mit verschiedenen oberflächenaktiven Stoffen formuliert ist, die gleiche sein, die Aufnahmeraten (und daher die Regenfestigkeit und anschließende Translokation an Zielstellen) können dabei jedoch völlig verschieden sein. Es bestand daher die Notwendigkeit, die Aufnahme in vollständigen Pflanzen im Zeitablauf modellieren zu können. Infolgedessen war es das Ziel zusätzlicher Studien festzustellen, ob ein logistisch-kinetisches Penetrationsmodell, das unter Anwendung isolierter pflanzlicher Kutikeln entwickelt wurde, bei der Gesamtpflanzenaufnahme zum Einsatz kommen könnte. Die Aufnahme über 24 Stunden wurde für drei Modellverbindungen ermittelt, die in Anwesenheit und Abwesenheit von oberflächenaktiven Stoffen in die Blätter von zwei Pflanzenarten aufgetragen wurden. Die Daten von zwei Zeitintervallen wurden in dem Modell verwendet, um die Aufnahme in Zwischenintervallen abzuschätzen, und diese wurden dann mit den experimentellen Ergebnissen verglichen. Insgesamt gesehen entsprach das Modell den Pflanzenaufnahmedaten

sehr gut. Die Studie bestätigte, dass ein Anstieg (oder Abfall) der AI-Konzentration oder eine Zunahme der Kontaktfläche keinen Einfluss auf den Penetrationsratenfaktor, q, innerhalb des normalen Arbeitskonzentrationsbereichs haben würde. Dies ermöglichte es, die Aufnahme zu verschiedenen Zeiten für AI-Konzentrationen abzuschätzen, für die keine experimentellen Daten vorliegen, wobei zunächst q für eine Konzentration der betreffenden Formulierung abgeleitet wurde und wobei die Aufnahmeergebnisse aller betreffenden Formulierungen und Konzentrationen innerhalb von (maximal) 24 Stunden erhalten wurden.

Die Anwendung der entwickelten Gleichungen basierend auf der anfänglichen Dosis zur Errechnung der Aufnahme bei 24 Stunden, in Verbindung mit dem logistisch-kinetischen Modell, hat zu einer erheblichen Erweiterung unseres Verständnisses und unserer Fähigkeiten zur Modellierung der Aufnahme geführt. Die Vorteile der beschriebenen Modelle und Gleichungen sind, dass nur wenige Variablen erforderlich und diese einfach zu messen sind.

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# MECHANISMS OF CUTICULAR UPTAKE INTO LIVING PLANTS: INFLUENCE OF XENOBIOTIC DOSE AND SURFACTANT ON UPTAKE

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## INTRODUCTION

Many studies have been performed in an attempt to relate the uptake of agrochemicals to the physicochemical properties of the active ingredients (AIs) and formulants. These studies have provided empirical guidelines but there are currently no suitable models available to predict the uptake of agrochemicals into plant foliage.

There is general agreement that uptake (flux) through the leaf cuticle is a diffusion process described by Fick's law (Price, 1982), where the flux is the product of the diffusion coefficient and the concentration gradient of the AI through the cuticle. A simple way of considering the flux per unit area is the amount of a solute that diffuses through a unit area per unit of time, i.e. J = M/At, where M is the amount of solute (mol), A the area across which diffusion occurs ($m^2$) and t is time (s). Uptake (U; mol $m^{-2}$) over a specific time (t) can be determined in terms of mol uptake per unit area, i.e.: U = Jt = M/A. When the initial solution deposit (droplet) rapidly becomes a deposit residue due to solvent evaporation, the initial droplet concentration becomes irrelevant. It is clear that the mass or mol applied per unit area is important (Zabkiewicz and Forster, 2001).

It has been shown (Forster *et al.*, 2004) recently that uptake on a per unit area basis can be related to the initial dose of xenobiotic applied, by an equation of the form: $Uptake_{(nmol\ mm^{-2})} = a\ [ID]^b$ at time t = 24 hours; where ID is the initial dose or the mass of xenobiotic applied per unit area ($M_{(nmol\ xenobiotic\ applied)}/A_{(droplet\ spread\ area)}$). This paper considers the effect of surfactants on the spread area of droplets and illustrates the importance to uptake of initial dose of xenobiotic applied.

## MATERIALS AND METHODS

Full experimental details have been given elsewhere (Forster *et al.*, 2004) and the following is a summary of the procedures.

### Plant material

*Chenopodium album* (*C. album*; common lambsquarters) plants were grown from seed in individual pots (Bloom potting mix, Yates Ltd., NZ). Plants were raised under controlled environment conditions (70% RH, 14 hour photoperiod, ~ 500 μmol $m^{-2}$ $s^{-1}$, 23 °C/15 °C day/night temperature) and used at 3 weeks of age. *Hedera helix* (*H. helix*) plants were grown from cuttings in individual pots in a glasshouse and used at 6 to 9 months of age. *Stephanotis*

*floribunda* (*S. floribunda*) plants were also grown from cuttings and 1 year old plants were used. Two weeks prior to use the *H. helix* and *S. floribunda* plants were transferred into growth cabinets with controlled environment conditions as for the *C. album* plants.

**Chemicals**

2-Deoxy-D-glucose (DOG), 2,4-dichlorophenoxy-acetic acid (2,4-D) and epoxiconazole were studied over a wide range of concentrations (0.0088 to 148 g $L^{-1}$ DOG, equating to 0.0129 to 217 nmol per 0.24 µl droplet; 0.034 to 50 g $L^{-1}$ 2,4-D, equating to 0.037 to 54 nmol per 0.24 µl droplet; 0.039 to 4.34 g $L^{-1}$ epoxiconazole, equating to 0.029 to 4.34 nmol per 0.24 µl droplet), including a molar concentration (0.0045 mol $litre^{-1}$) close to that of the surfactants studied. Log P values are -2.69, 2.62 and 3.44 respectively. All compounds were studied up to the limits of their solubility.

Surfactants used were Silwet L-77® (TSE7.5, a trisiloxane ethoxylate with mean EO of 7.5), triethylene glycol monododecyl ether ($C_{12}EO_3$), hexaethylene glycol monododecyl ether ($C_{12}EO_6$) and decaethylene glycol monododecyl ether ($C_{12}EO_{10}$). All surfactants were studied at equimolar concentrations (0.0044 mol $litre^{-1}$, corresponding to 2.3 g $litre^{-1}$ TSE7.5, 1.4 g $litre^{-1}$ $C_{12}EO_3$, 2.0 g $litre^{-1}$ $C_{12}EO_6$ and 2.8 g $litre^{-1}$ $C_{12}EO_{10}$), approximating typical use rates.

**Uptake**

Radiolabelled 2-deoxy-D-(U-$^{14}$C)glucose (DOG), 2,4-dichlorophenoxyacetic acid-carboxy-$^{14}$C (2,4-D) and $^{14}$C-epoxiconazole were incorporated into treatments prior to use. All formulations were made up in water + acetone (1:1 by volume). This enabled higher concentrations of lipophilic xenobiotics to be studied, as well as the xenobiotic in the absence of a surfactant. Suitable numbers of droplets of each solution (0.24 µl, ca. 770 µm diameter) were applied to the upper surface of the youngest fully expanded leaf of *C. album*, *H. helix* and *S. floribunda*, on five separate plants per species within 4 h of the start of the illumination period. Excised leaves were washed with 2 x 4 ml water + ethanol (1:1 by volume) to recover unabsorbed DOG and epoxiconazole, or 2 x 4 ml water + acetone (1:3 by volume) to recover unabsorbed 2,4-D. The washings were taken up in 13 ml ACS II scintillant (Amersham International, UK) and the radioactivity quantified by scintillation counting (Packard 2100TR). Percentage uptake was determined as the proportion of the applied radiolabel not recovered by washing the treated leaves.

### Droplet Spread Area Determination

The droplet spread areas for the different formulations, on the three plant species, were measured under UV illumination using $V^{++}$ for Windows image analysis software (mean of 10 determinations). To visualise droplet spread, Blankophor-P fluor (5 g $litre^{-1}$; Bayer NZ) was incorporated into treatments containing DOG or epoxiconazole, while UVITEX NFW 450 (5 g $litre^{-1}$; Ciba Geigy) was incorporated into treatments containing 2,4-D.

## RESULTS

### Uptake of Epoxiconazole, 2,4-D and DOG Formulations into *Chenopodium Album*

*Epoxiconazole*

The percentage uptake of epoxiconazole at 24 hours into *C. album* foliage, applied in the absence or presence of surfactants ($C_{12}EO_3$, $C_{12}EO_6$, $C_{12}EO_{10}$, TSE7.5) is shown in Fig. AI.1.

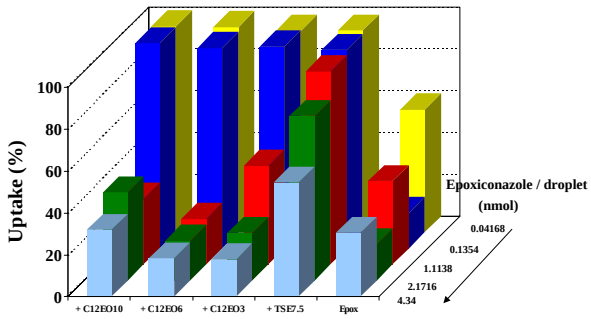


**Figure AI.1.** Percentage uptake of epoxiconazole (nmol/0.24 µl droplet) at 24 hours into *C. album* foliage, applied in the presence or absence of surfactants (all at 1.056 nmol/0.24 µl droplet).

The spread area of a droplet of epoxiconazole on *C. album* varied depending on formulation (Fig. AI.2) and this affected the amount (nmol) applied per unit area (Fig. AI.3). The uptake of epoxiconazole per unit area (U = Jt = M/A) at 24 hours (Fig. AI.4) gives quite a different trend compared to percent uptake (Fig. AI.1). The trends show clearly: increasing uptake in each formulation with increasing dose; increasing uptake among surfactant formulations in accordance with dose applied (Fig. AI.3); and relatively high uptake of epoxiconazole without surfactant at high applied dose. These mass uptake results can be represented more simply and clearly by plotting the amount of epoxiconazole applied per unit area (from Fig. AI.3) against the uptake per unit area (from Fig. AI.4) to give Fig. AI.5.

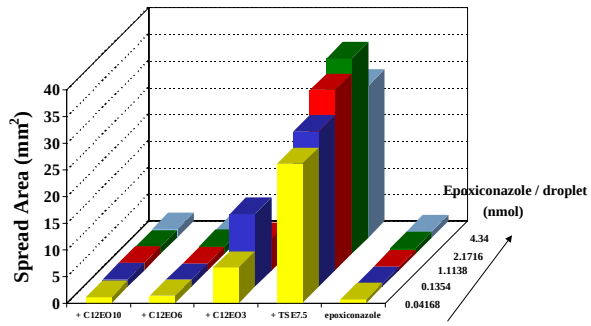


**Figure AI.2.** Spread area of 0.24 µl droplets of epoxiconazole on the upper surface of *C. album* foliage, applied in the presence or absence of surfactants.

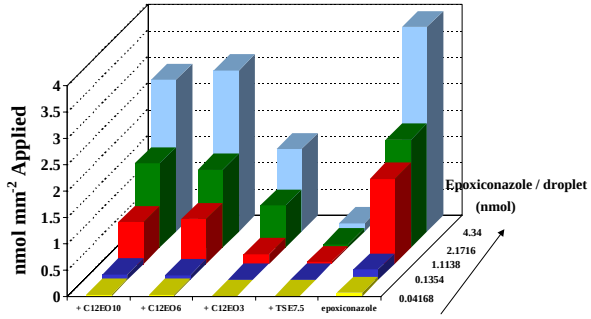


**Figure AI.3.** Amount of epoxiconazole (nmol $mm^{-2}$) applied to *C. album* foliage, in the presence or absence of surfactants.

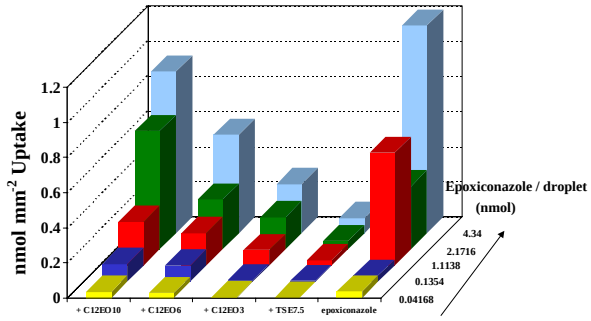


**Figure AI.4.** Uptake (nmol $mm^{-2}$) of epoxiconazole (  nmol/0.24 µl droplet) at 24 hours into *C. album* foliage, applied in the presence or absence of surfactants (all at 1.056 nmol/0.24 µl droplet).

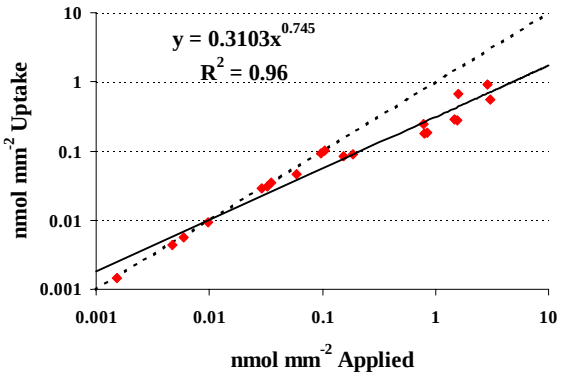


**Figure AI.5.** Uptake (nmol $mm^{-2}$ vs. nmol $mm^{-2}$ applied) at 24 hours of epoxiconazole into *C. album* foliage, applied in the presence or absence of the surfactants $C_{12}EO_3$, $C_{12}EO_6$, $C_{12}EO_{10}$ and TSE7.5 (--- maximum uptake line, representing 100% uptake over the initial dose range).

This provides an excellent relationship ($R^2$ = 0.99) at lower applied dosages, but there is divergence from the maximum uptake possible at higher applied dosages. Overall an excellent relationship ($R^2$ = 0.96) can be obtained.

*2,4-D*

2,4-D is less lipophillic than epoxiconazole, and the percentage uptake (Fig. AI.6) was quite different compared to epoxiconazole, with little difference among the surfactant formulations. However, there was again a strong relationship ($R^2$ = 0.98) between molar uptake per $mm^2$ of 2,4-D and the amount of 2,4-D applied per $mm^2$ (Fig. AI.7). The equation describing the relationship took the same form (a power relationship).

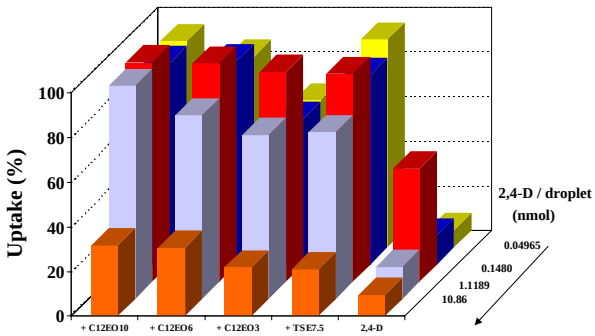


**Figure AI.6.** Percentage uptake of 2,4-D (nmol/0.24 µl droplet) at 24 hours into *C. album* foliage, applied in the presence or absence of surfactants (all at 1.056 nmol/0.24 µl droplet).

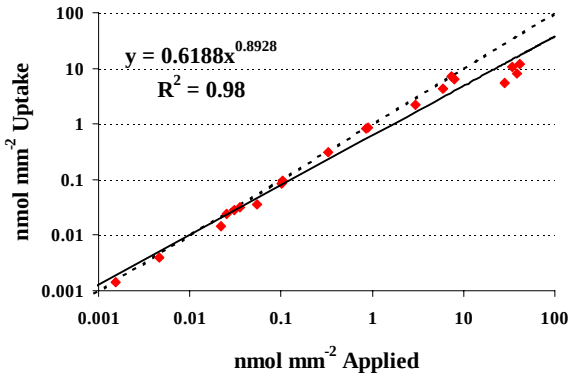


**Figure AI.7.** Uptake (nmol $mm^{-2}$ vs. nmol $mm^{-2}$ applied) at 24 hours of 2,4-D into *C. album* foliage, applied in the presence or absence of the surfactants $C_{12}EO_3$, $C_{12}EO_6$, $C_{12}EO_{10}$ and TSE7.5 (--- maximum uptake line, representing 100% uptake over the initial dose range).

*DOG*

DOG is a polar compound, and the pattern in percentage uptake (Fig. AI.8) was very different to both epoxiconazole and 2,4-D, but again there was an excellent relationship ($R^2$ = 0.98) between molar uptake per $mm^2$ and the amount of DOG applied per $mm^2$ (Fig. AI.9). The equation describing the relationship again took the same form (a power relationship) as for the previous two xenobiotics.

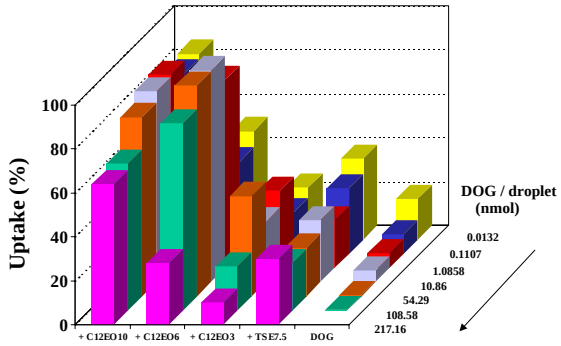


**Figure AI.8.** Percentage uptake of DOG (nmol/0.24 µl droplet) at 24 hours into *C. album* foliage, applied in the presence or absence of surfactants (all at 1.056 nmol/0.24 µl droplet).

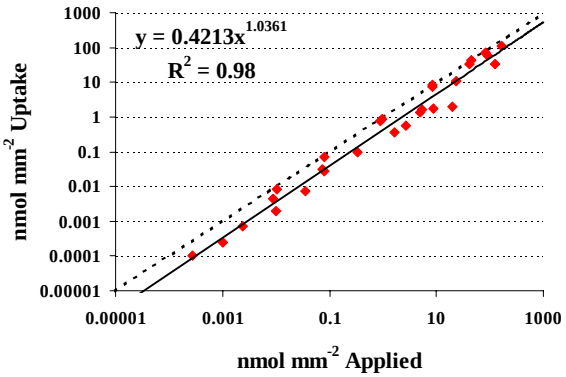


**Figure AI.9.** Uptake (nmol $mm^{-2}$ vs. nmol $mm^{-2}$ applied) at 24 hours of DOG into *C. album* foliage, applied in the presence or absence of the surfactants $C_{12}EO_3$, $C_{12}EO_6$, $C_{12}EO_{10}$ and TSE7.5 (--- maximum uptake line, representing 100% uptake over the initial dose range).

### Uptake of Epoxiconazole, 2,4-D and DOG Formulations into *Hedera Helix*

An excellent relationship was found to exist between the molar uptake of DOG per unit area into *H. helix* and dose applied in the presence of $C_{12}EO_3$, $C_{12}EO_6$ and TSE7.5 ($R^2$ =0.98; data not shown). This was also the case for 2,4-D ($R^2$ = 0.97; data not shown) and epoxiconazole ($R^2$ = 0.90; data not shown).

### Uptake of Epoxiconazole, 2,4-D and DOG Formulations into *Stephanotis Floribunda*

A good relationship was found to exist between the molar uptake of DOG per unit area into *S. floribunda* and dose of DOG applied in the presence of $C_{12}EO_3$, $C_{12}EO_6$ and TSE7.5 ($R^2$ =0.87; data not shown). This was also the case for 2,4-D ($R^2$ = 0.98; data not shown) and epoxiconazole ($R^2$ = 0.95; data not shown).

## DISCUSSION

It has been stated (Holloway *et al.*, 1992; Stock and Holloway, 1993; Stock *et al.*, 1993) that there is no general correlation between surfactant-enhanced uptake and the contact area of the corresponding deposit. This implies that any spreading effects are irrelevant, despite the fact that a surfactant may cause the formation of a deposit of the agrochemical over a small area, an intermediate area or even a large area of the leaf (Stock and Holloway, 1993). The current study illustrates that initial dose (which is affected by spread area) is a strong positive determinant for uptake per unit area. Although a surfactant causing a formulation to spread will decrease the dose of AI per unit area and therefore theoretically decrease the uptake per unit area, compared to a formulation which spreads less, total uptake is the product of the

uptake per unit area and spread area. Therefore total uptake may be less than, equal to, or greater than for a formulation which spreads less. This mass uptake relationship can be used to calculate uptake across different formulations. Using the two contrasting surfactants TSE7.5 and $C_{12}EO_6$, which have very different spreading behaviour, Table AI.1 illustrates how these equations (for each xenobiotic) can be applied to estimate uptake per unit area, total mass uptake or percent uptake.

The total amount applied was ~ 1.1 nmol in this example, and due to droplet spread, the nmol $mm^{-2}$ applied ranged from 0.0255 to 0.8974. The equation used for each model compound is based on the average trend line for that compound in the presence of the four surfactants (TSE7.5, $C_{12}EO_3$, $C_{12}EO_6$ and $C_{12}EO_{10}$), and used all data points between 0.01 nmol and 1.0 nmol $mm^{-2}$, for each of the 3 model compounds. Since TSE7.5 provided a significantly greater spread area than $C_{12}EO_6$, and hence a much lower initial dose for all three model compounds, the calculated uptake per unit area was much lower. However, when total uptake was calculated by multiplying the uptake per unit area by spread area, TSE7.5 provided significantly greater total uptake of epoxiconazole than $C_{12}EO_6$, slightly less of 2,4-D and substantially less of DOG. Comparing calculated percentage uptake with actual percentage uptake (Table AI.1) there are some large discrepancies, particularly with DOG. Hence the equations based only on spread areas cannot be used as stand alone models for all types of xenobiotics, but do give an estimate of uptake from the spread area of the formulation on the specified species.

**Table AI.1.** Actual and calculated uptake of model compounds in the presence of TSE7.5 and $C_{12}EO_6$

| | Epoxiconazole | | 2,4-D | | DOG | |
|---|---|---|---|---|---|---|
| | TSE7.5 | $C_{12}EO_6$ | TSE7.5 | $C_{12}EO_6$ | TSE7.5 | $C_{12}EO_6$ |
| Total Applied (nmol) | 1.1 | 1.1 | 1.1 | 1.1 | 1.09 | 1.09 |
| Spread Area (A) ($mm^2$) | 33.8 | 1.31 | 44.13 | 1.31 | 31.13 | 1.21 |
| Initial Dose (nmol $mm^{-2}$) | 0.0329 | 0.8506 | 0.0255 | 0.8586 | 0.0349 | 0.8974 |
| Uptake Equation (nmol $mm^{-2}$) | $y = 0.2677(ID)^{0.6298}$ | | $y = 0.9876(ID)^{1.0574}$ | | $y = 0.6711(ID)^{1.1603}$ | |
| Calculated Uptake (nmol $mm^{-2}$) | 0.0312 | 0.2418 | 0.0204 | 0.8406 | 0.0137 | 0.5919 |
| Total Uptake Equation (nmol) | $y = 0.2677(ID)^{0.6298}.A$ | | $y = 0.9876(ID)^{1.0574}.A$ | | $y = 0.6711(ID)^{1.1603}.A$ | |
| **Calculated** Total Uptake (nmol) | 1.0536 | 0.3167 | 0.9003 | 1.1 | 0.4258 | 0.7162 |
| **Calculated** Uptake (%) | 96 | 29 | 82 | 100 | 39 | 66 |
| **Actual** Uptake (%) | 92 | 22 | 93 | 98 | 22 | 86 |

## CONCLUSIONS

The use of percent AI uptake is sufficient to compare uptake among formulations or to choose the "best" formulation for a specific species. However there is no predictive capability and this approach has not provided insights into the mechanisms of xenobiotic uptake into plants (Zabkiewicz and Forster, 2001).

Uptake on a per unit area basis can be related to initial dose of xenobiotic applied, by an equation of the form: $Uptake_{(nmol\ mm^{-2})} = a\ [ID]^b$ at time t = 24 hours; where ID is the initial dose or the mass of xenobiotic applied per unit area ($M_{(nmol\ xenobiotic\ applied)}/A_{(droplet\ spread\ area)}$). Total mass uptake is then calculated from an equation of the form: $Total\ Uptake_{(nmol)} = a\ [ID]^b.A$.

Formulations with different spreading ability will have total uptake that should reflect this behaviour. However, after taking into account differences among formulations due to spread area, differences in uptake due to the specific surfactant used are still significant ($p < 0.05$, data not shown).

The current work has established the importance and relationship of dose applied, to uptake per unit area. This will enable the relationship to be used in further work, both in progressing towards a universal uptake model, and establishing the relative importance of each of the variables involved in uptake, i.e. species, AI, AI concentration, surfactant etc. These will be the subjects of future publications.

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# SPRAY FORMULATION DEPOSITS ON LEAF SURFACES AND XENOBIOTIC MASS UPTAKE

**W. Alison Forster, Jerzy A. Zabkiewicz and Markus Riederer**

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## INTRODUCTION

Organic compounds with a wide range of physicochemical properties are used as agrichemicals and formulated with different adjuvants. After the spray formulation is deposited on a leaf surface, spreading and drying of the droplets, with concomitant changes in concentration of both AI and formulants, leads to an initial "spray deposit" that has little resemblance to the original solution or concentration. In particular the "concentration" has been increased to a point such that the residue may be a quasi-solid and akin to a deposit with minimal water content. This may result in quite different behaviour of the xenobiotic, particularly in relation to uptake, which may be enhanced or reduced, depending on formulation.

The objective of this study was to examine the effect of a range of surfactants on xenobiotic dose and physical leaf deposit, in relation to xenobiotic uptake.

## MATERIALS AND METHODS

Full experimental details have been given elsewhere (Forster *et al.*, 2004a,b) and the following is a summary of the procedures.

### Plant material

*Chenopodium album* (*C. album*; common lambsquarters; sourced from Valley Seed Service, Fresno, CA.) plants were grown from seed in individual pots containing Bloom potting mix (Yates Ltd., NZ). Plants were raised under controlled environment conditions (70% RH and 14 hour photoperiod, ~ 500 μmol $m^{-2}$ $s^{-1}$, 23°C/15°C day/night) and used at 3 weeks of age.

### Chemicals

#### *Xenobiotics*

2-Deoxy-D-glucose (DOG; Aldrich Chemical Company Inc.; 99% purity), 2,4-dichlorophenoxy-acetic acid (2,4-D; Dow Agrosciences (NZ) Ltd.; 92% purity) and (2RS, 3SR)-1-[3-(2-chlorophenyl)-2-(4-fluoro-phenyl)oxiran-2-ylmethyl]-1H-1,2,4-triazole (epoxiconazole; BASF; 96% purity) were studied over a wide range of concentrations. All xenobiotics were studied alone, and in the presence of each of the four surfactants.

***Surfactants***

Silwet L-77® (TSE7.5, a trisiloxane ethoxylate with a mean EO of 7.5; supplied by OSi Specialities), triethylene glycol monododecyl ether (E3, $C_{12}EO_3$; > 99% purity), hexaethylene glycol monododecyl ether (E6, $C_{12}EO_6$; > 98% purity, both from Fluka); decaethylene glycol monododecyl ether (E10, $C_{12}EO_{10}$; from Sigma, purified prior to use by HPLC to give > 90% purity). All surfactants were studied at equimolar concentrations (0.0044 mol litre$^{-1}$, corresponding to 0.23% TSE7.5, 0.14% E3, 0.2% E6 and 0.28% E10).

**Uptake**

Radiolabelled 2-deoxy-D-(U-$^{14}$C)glucose (DOG; Amersham UK), 2,4-dichlorophenoxyacetic acid-carboxy-$^{14}$C (2,4-D; Sigma) and (2RS, 3SR)-3-(2-chlorophenyl)-2-(4-fluorophenyl)-2-[1H-1,2,4-triazol-1-yl)methyl]oxirane-[chlorophenyl-U-$^{14}$C] ($^{14}$C-epoxiconazole; BASF) were incorporated into treatments prior to use. All formulations were made up in water + acetone (1:1 by volume). Droplets of each solution (0.24 µl, ca. 770 µm diameter) were applied to the upper surface of the youngest fully expanded leaf of *C. album* (14 per leaf, or 7 for TSE7.5), on five separate plants within 4 h of the start of the illumination period. The quantity of radiolabelled xenobiotic applied to each plant was determined by dispensing droplets (14 or 7) directly into scintillation vials (3 replicates). Treated leaves were excised at 24 h after treatment. Excised leaves were washed with 2 x 4 ml water + ethanol (1:1 by volume) to recover unabsorbed DOG and epoxiconazole (both > 97% recovery on droplet dry-down), or 2 x 4 ml water + acetone (1:3 by volume) to recover unabsorbed 2,4-D (95% recovery on droplet dry-down). The washings were taken up in 13 ml ACS II scintillant (Amersham International, UK) and the radioactivity quantified by scintillation counting (Packard 2100TR). Percentage uptake was determined as the proportion of the applied radiolabel not recovered by washing the treated leaves.

**Droplet Residue Photographs**

A Nikon Coolpix digital camera (Model 990) was fitted to a Leica MZ12.5 stereo microscope with a planapochromatic 1.0X interchangeable objective lens. The droplet residue was illuminated with a Zeiss KL1500 LCD with two arm lights focused on the detached leaf.

## RESULTS AND DISCUSSION

### Epoxiconzole

The percentage uptake of epoxiconazole into *C. album* at 24 hours, applied in the presence of E3, E6, E10 or TSE7.5, generally decreased with increasing concentration of epoxiconazole (Fig. AII.1).

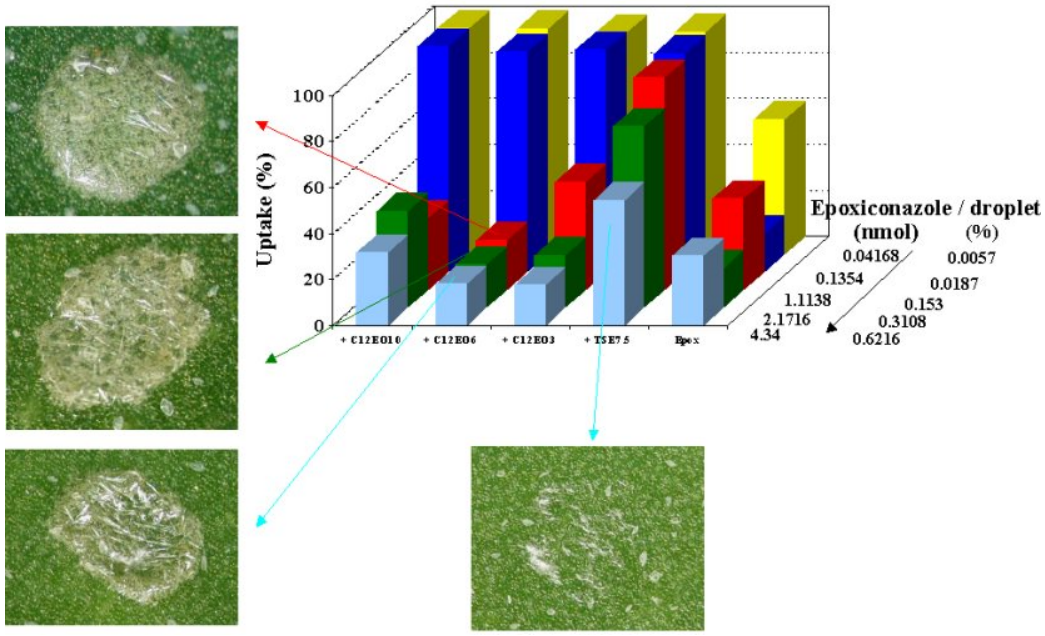


**Figure AII.1.** Uptake (%) of epoxiconazole applied alone and in the presence of E3, E6, E10 and TSE7.5 into *C. album* foliage at 24 hours, and representative droplet deposit photographs.

It has been shown (Forster *et al.*, 2004a,b) that mass uptake (nmol $mm^{-2}$) into foliage is positively correlated to the initial dose (in nmol $mm^{-2}$) of xenobiotic applied, which takes into account the droplet spreading caused by the surfactant. At very low doses of applied epoxiconazole (<0.1 nmol $mm^{-2}$) the relationship is excellent (Fig. AII.2; $R^2$ = 0.99). However, a single regression line, across all dosages would cause some discrepancy between predicted and actual results (Fig. AII.3), although the correlation is still strong ($R^2$ = 0.96). A study of the droplet residues helps to explain such uptake behaviour. In the presence of E3, crystallisation occurred at the two highest concentrations. Although all lower concentrations "marked" the leaf surface, beginning with an annulus that filled in with increasing epoxiconazole concentration, this did not affect uptake. Uptake of epoxiconazole applied in the presence of E6, into *C. album* foliage, showed a similar trend to that of epoxiconazole in

the presence of E3, though there appears to be significantly more precipitate, with marked crystallisation occurring at 0.153% of applied epoxiconazole. This is possibly because at equivalent application concentrations of epoxiconazole, the dose per unit area is higher, when in the presence of E6, compared to E3, due to the smaller spread area caused by the E6 formulations. Epoxiconazole shows very similar results in the presence of E10 compared to E6. In contrast, when epoxiconazole was applied in the presence of TSE7.5 there was no evidence of any droplet deposit on the leaf surface at 24 hours, except for a small amount of crystallisation at the highest concentration of epoxiconazole applied. This relates well with the fact that the total (nmol) uptake of epoxiconazole in the presence of TSE7.5, into *C. album*, was higher than any other epoxiconazole formulation studied.

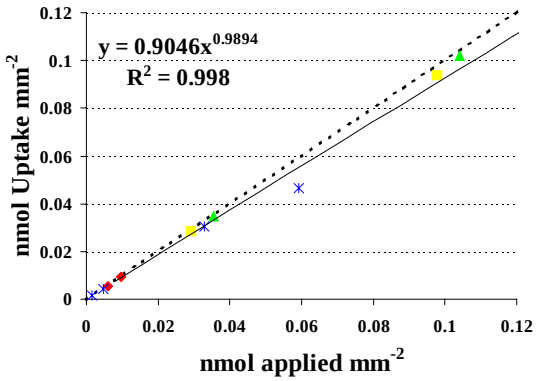


**Figure AII.2.** Uptake (nmol mm$^{-2}$) of epoxiconazole in the presence of E3 (◆), E6 (■), E10 (▲) and TSE7.5 (*) into *C. album* foliage at 24 hours (--- maximum uptake line, representing 100% uptake over the initial dose range).

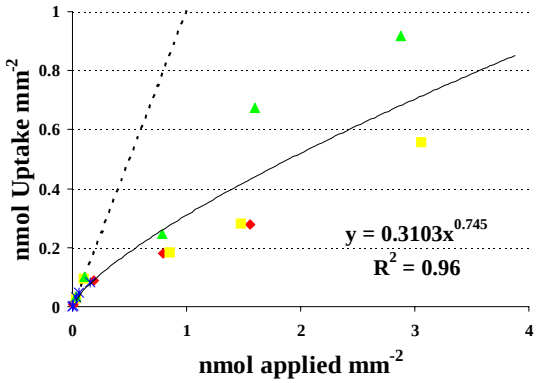


**Figure AII.3.** Uptake (nmol mm$^{-2}$) of epoxiconazole in the presence of E3 (◆), E6 (■), E10 (▲) and TSE7.5 (*) into *C. album* foliage at 24 hours (--- maximum uptake line, representing 100% uptake over the initial dose range).

## 2,4-D

2,4-D is less lipophillic than epoxiconazole, and therefore higher concentrations can be studied (up to 5.45%). Again there was a strong relationship between dose applied and dose uptake of 2,4-D into *C. album* foliage in the presence of surfactants (Fig. AII.4). Predicted uptake would be in excellent agreement with actual uptake ($R^2$ = 0.99) when less than 10 nmol $mm^{-2}$ of 2,4-D are applied (up to 1.09% 2,4-D), which is well within the operational (field) application rate (Fig. AII.4). However, when more than 10 - 20 nmol $mm^{-2}$ of 2,4-D are applied (Fig. AII.5), there is a break from linearity, which suggests some difference in behaviour of the concentrated spray deposit.

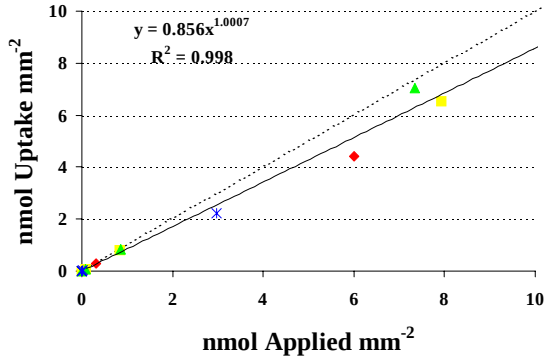


**Figure AII.4.** Uptake (nmol $mm^{-2}$) of 2,4-D in the presence of E3 (◆), E6 (■), E10 (▲) and TSE7.5 (*) into *C. album* foliage at 24 hours (--- maximum uptake line, representing 100% uptake over the initial dose range).

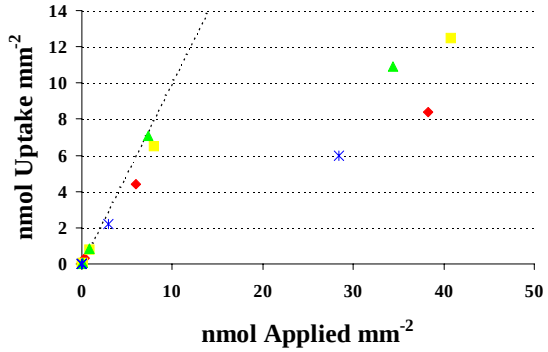


**Figure AII.5.** Uptake (nmol $mm^{-2}$) of 2,4-D in the presence of E3 (◆), E6 (■), E10 (▲) and TSE7.5 (*) into *C. album* foliage at 24 hours (--- maximum uptake line, representing 100% uptake over the initial dose range).

In the presence of E3, uptake increased with increasing dose applied and only departed significantly from the maximum dose uptake line above a concentration of 1.09% (6 nmol

$mm^{-2}$) 2,4-D applied (Fig. AII.6), when some precipitate was visible. At 5.45% (38 nmol $mm^{-2}$) considerable crystallisation was evident.

The pattern of uptake in the presence of E6 was very similar to that in the presence of E3, except that uptake was slightly higher for an equivalent dose applied (Fig. AII.7). Crystallisation was only evident at the highest rate of 2,4-D applied (5.45%, 40 nmol $mm^{-2}$), where there was considerable divergence from the trend line.

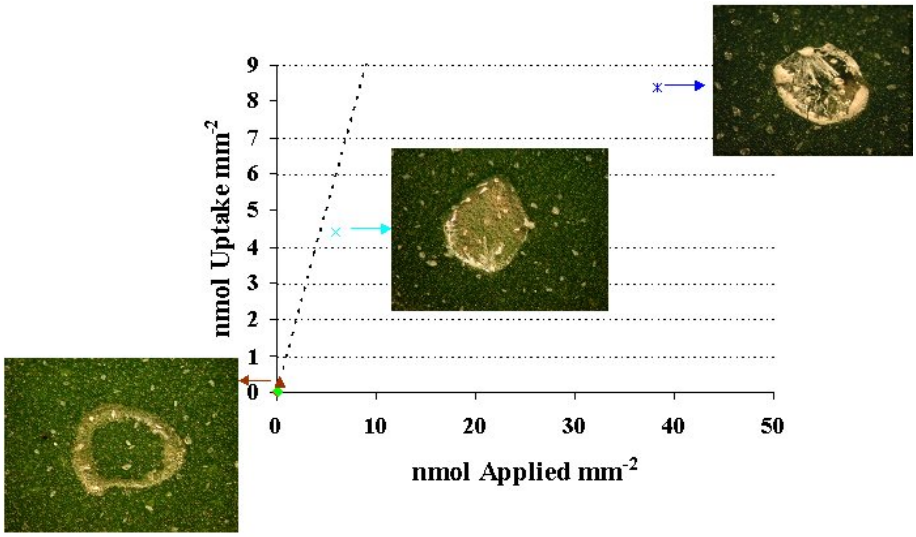


**Figure AII.6.** Uptake (nmol $mm^{-2}$) of 2,4-D, applied at 0.00457% (◆), 0.0135% (◆), 0.1034% (▲), 1.09% (x) and 5.45% (*), in the presence of E3, into *C. album* foliage at 24 hours (--- maximum uptake line, representing 100% uptake over the initial dose range). Photographs show droplet deposits.

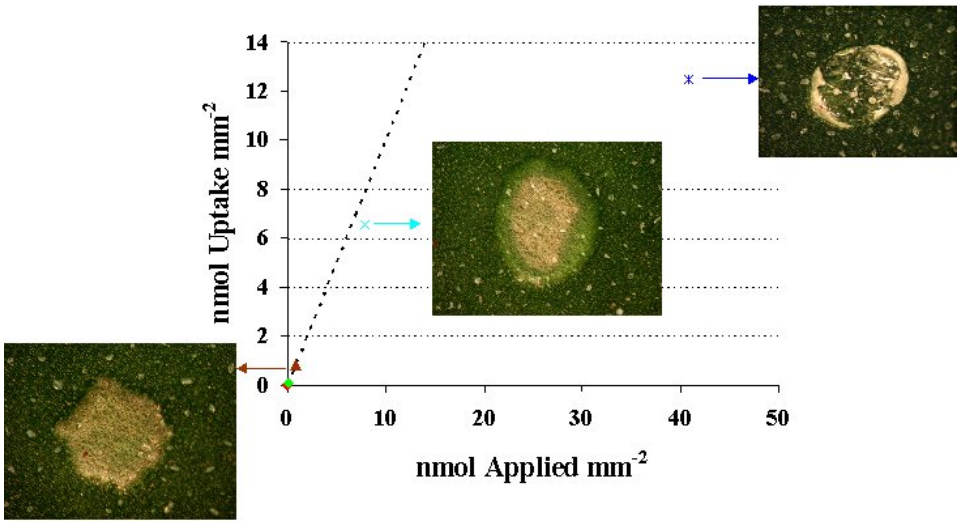


**Figure AII. 7.** Uptake (nmol $mm^{-2}$) of 2,4-D, applied at 0.0046% (◆), 0.0135% (◆), 0.1034% (▲), 1.09% (x) and 5.45% (*), in the presence of E6, into *C. album* foliage at 24 hours (--- maximum uptake line, representing 100% uptake over the initial dose range), and representative droplet deposit photographs.

In the presence of E10 uptake and behaviour was nearly identical to that in the presence of E6; a precipitate was only evident at the highest application rate, although this precipitate was somewhat less. However the droplet application area is obvious for all concentrations of 2,4-D in this formulation (Fig. AII.8).

When 2,4-D was applied in the presence of TSE7.5, crystallisation was only evident at the highest application rate of 2,4-D (5.45%, 28.4 nmol $mm^{-2}$).

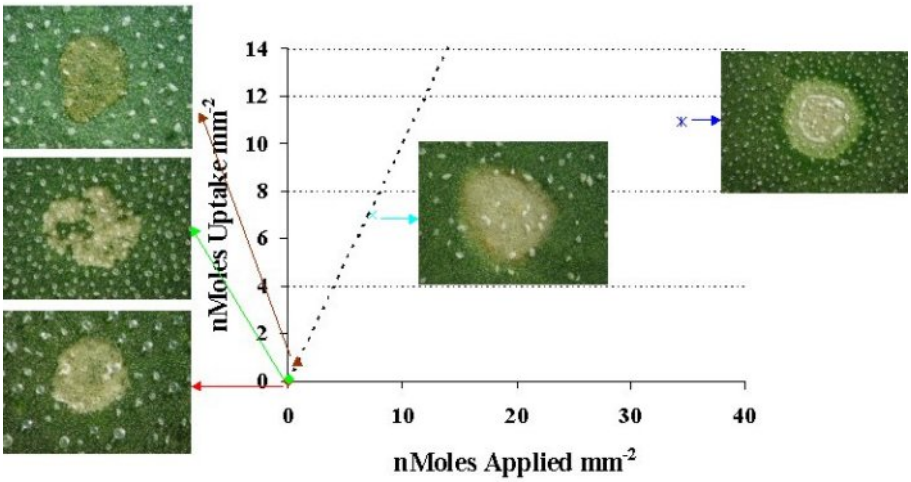


**Figure AII.8.** Uptake (nmol $mm^{-2}$) of 2,4-D, applied at 0.0046% (◆), 0.0135% (◆), 0.1034% (▲), 1.09% (x) and 5.45% (*), in the presence of E10, into *C. album* foliage at 24 hours (--- maximum uptake line, representing 100% uptake over the initial dose range), and representative droplet deposit photographs.

**DOG**

DOG is a polar compound, which enables a much wider concentration range to be studied (up to 15% giving an initial dose of 168 nmol $mm^{-2}$) than for either 2,4-D or epoxiconazole. In contrast to epoxiconazole and 2,4-D, although the $R^2$ relationship of DOG is good ($R^2$ = 0.97, Fig. AII.9) at low initial doses there would be a significant difference between predicted and actual results. Although the relationship between dose applied and uptake per unit area is strong over the entire dose range the predicted dose uptake could again be significantly different from the actual uptake.

The uptake of DOG in the presence of E10, into *C. album*, was linear over the entire range (Fig. AII.10); in the presence of E6 an aberrant data point appeared at the highest dose applied. Furthermore, uptake of DOG in the presence of E3 appeared to be inhibited even at lower initial dosages. These trends are somewhat different to those observed with the more lipophilic epoxiconazole and 2,4-D.

In the case of DOG, the deposit behaviour observed on the leaf surface does not relate well to its uptake behaviour. This suggests that there is a fundamental difference in behaviour

between very hydrophilic compounds and more lipophilic compounds that cannot be attributed to surface deposit characteristics.

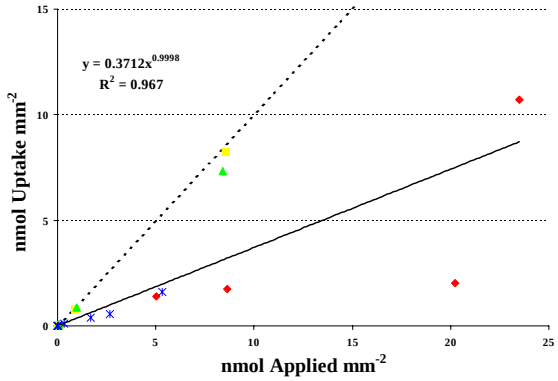


**Figure AII.9.** Uptake (nmol mm-2) of DOG in the presence of E3 (◆), E6 (■), E10 (▲) and TSE7.5 (*) into *C. album* foliage at 24 hours (--- maximum uptake line, representing 100% uptake over the initial dose range).

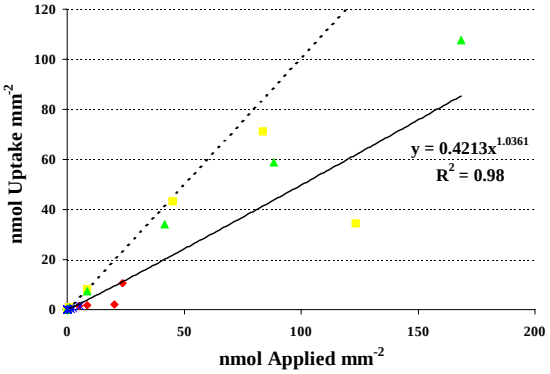


**Figure AII.10.** Uptake (nmol mm-2) of DOG in the presence of E3 (◆), E6 (■), E10 (▲) and TSE7.5 (*) into *C. album* foliage at 24 hours (--- maximum uptake line, representing 100% uptake over the initial dose range).

## CONCLUSIONS

Uptake related well to initial dose of xenobiotic applied in most cases within normal operational concentrations. This relationship failed at high concentrations. It was found that "anomalies" in the uptake of epoxiconazole and 2,4-D could be explained by precipitation behaviour at the high concentrations studied. The appearance of precipitates from the droplet solutions related well to the pattern of epoxiconazole and 2,4-D uptake into *C. album*. However, in the case of the more polar deoxyglucose the precipitates observed on the leaf surfaces did not relate well to the uptake trends. Precipitation of the xenobiotic is not only dependent on its water solubility, but in practice, in the formulation residue on the leaf surface, which results in some very specific interactions.

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# ERKLÄRUNG

**Erklärung nach §4 Abs. 3 der Promotionsordnung**

1. Ich habe vorliegende Dissertation selbständig angefertigt und keine anderen als die angegebenen Hilfsmittel dafür benutzt.

2. Die Vorliegende Dissertation lag bisher in keinen anderen Prüfungsverfahren vor

3. Auβer dem Diplom in Biologie have keine weiteren akademischen Grade erworben oder zu erwerben versucht.

W. Alison Forster