

SESSION 3.

CHEMICAL AND PHYSIOLOGICAL INTERACTION OF HERBICIDES

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This paper deals briefly with some of the ways in which chemical structure affects the three main phenomena concerned in the action of herbicides, viz. absorption, translocation, and toxicity.

As a generalisation it may be noted that with the exception of oils and certain chlorinated benzenes, most herbicides are acids, salts, or esters. This paper deals mainly with 2,4-D and related compounds, because most research has been done on such substances. In fact, the large amount of research done on growth regulators has until recently held up research on other types of herbicides.

Absorption can take place either through the roots or through the aerial portions (leaves in particular). If absorption does not occur in the vapour form, it must occur in the dissolved form, the latter being the more usual. Possibly the failure of a number of herbicides such as CMU to act through the leaves may be due to their low solubility in the spray solution. Work done at Camp Detrick has shown that leaves will absorb from solution a wider range of substances than will roots. Nevertheless research done by the Shell Company has shown that within a group of related substances, certain ones will penetrate leaves better than others.

Although some proof has been obtained that aqueous solutions can penetrate leaves through the stomata, the general evidence shows that such solutions penetrate through the cuticle. On the other hand the Shell Company has found that oils penetrate via the stomata.

The cuticular penetration of aqueous solutions of 2,4-D and related substances can be increased fourteen fold by the inclusion of a wetting agent such as Tween 20 (Weintraub *et al.*, 1954). In general anionic and non-ionic wetters have more effect than cationic wetters. Triethanolamine and its salts act as cationic wetting agents. However, the use of wetting agents does not always increase the efficiency of a spray application as much as might be expected from the foregoing. The inclusion of a wetting agent can reduce the volume of spray retained on easily wetted vertical leaves, and can also reduce the specific

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action of a spray application.

The fact that the mesophyll cells have lipophilic surfaces, i.e. are not easily wetted by water, can help explain the improved absorption resulting from the use of a wetting agent (Bingham and Lewis, 1937).

On account of the waxy nature of the cuticle Crafts (1948) suggested that for ready entry through the cuticle, 2,4-D and other herbicides should be in a non-polar form. McNew and Hoffman (1950) found that toxicity to tomato and bean plants increases through the following series of 2,4-D compounds applied to leaves: sodium salt < ammonium salt < amine salts < acid < esters. Polarity decreases and penetration presumably increases in the same order.

Restricted absorption has often been suggested as a factor responsible for the failure of some herbicides on grasses. However, the investigations of Weintraub et al. (1954) have shown that the rates of entry of 2,4-D into millet and bean leaves were quite similar.

The pH of the spray solution can have a marked effect on the absorption (also translocation and toxicity) of herbicides, though conventional spray solutions seldom have any influence on the pH of cells more than a short distance from the area of application. Certainly there is evidence that many substances penetrate cells better as molecules than as ions; for that reason esters (which do not ionise) may be preferred for good penetration. Lowering the pH of the solution of a weak acid such as 2,4-D increases the concentration of the molecules of that acid and decreases the concentration of its ions. Hamner et al. showed in bean plants that lowering the pH of the 2,4-D to values of 3 or 2 under certain circumstances increased the effect of the 2,4-D at a distance. At lower pH values local injury resulted, with a decrease in the distant effect of the 2,4-D. Simon (1950) has extensively examined the action of pH on weak acids and weak bases, and concluded that such substances are most toxic in the molecular form. However, his work generally overlooks the effect of pH on cellular constituents. The work of Brian and Rideal (1952) shows that pH can affect the behaviour of proteins, lipoids and lipoproteins. These workers further claim that Methoxone is toxic in the ionic form.

Translocation of the absorbed herbicide can take place over short or long distances and may be upwards (usually from the roots and in the xylem) or downwards.

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Downward translocation may be of three types, viz. induced, passive, and active (or metabolic). Induced translocation can take place only when there is a water deficit, and it requires the use of a rapidly killing penetrating agent. On account of the erratic behaviour of herbicides depending on induced translocation, the more recently developed herbicides depend on other types of translocation.

Passive translocation occurs when non-polar substances such as 2,4-D esters are applied in hydrocarbons such as kerosene (Rice and Rohrbaugh, 1953). The movement appears to be due to capillarity and to take place mainly in the phloem parenchyma.

Metabolic or active translocation results from the metabolic activity of living cells, and is the most important form of translocation for herbicides applied to the leaves. Although a far wider range of substances can be translocated upwards or downwards in the xylem stream than can be translocated metabolically in the phloem, metabolic translocation is the most important type for foliar applications.

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Mitchell and Brown (1946) found that the metabolic translocation of 2,4-D from the leaves depends on the simultaneous movement of organic food materials. If such herbicides are not translocated within a few hours of application, they can become fixed to cells adjacent to the area of application, and will not be subsequently translocated under favourable conditions. Accordingly there can be a diurnal variation in the effectiveness of Methoxone (Greenham, 1950).

More recently it was found that the presence of boron markedly influences the translocation of sugars from leaves (Gauch and Dugger, 1953). It was further shown that boron applied with growth regulators will increase their translocation (Mitchell et al., 1953).

The general evidence is that when extensive translocation of 2,4-D and similar substances is required, the spray solution should not have a rapid action on the leaves. On the other hand, if only local toxicity is required, better results can be obtained by including adjuvants such as chlorinated benzenes or ammonium thiocyanate (Hitchcock and Zimmerman, 1948).

Nevertheless certain rapidly acting poisons such as arsenic acid can be translocated metabolically in

skeleton weed (Greenham and Wilkinson, 1942). Presumably the ready translocation of arsenic acid depends on its chemical analogy to phosphoric acid. However, attempts to use phosphate-tagged poisons such as 2,4-dichlorophenoxyethyl phosphate have not been promising on skeleton weed.

It is surprising how a slight change in chemical structure can affect the metabolic translocation of herbicides. Thus sodium trichloroacetate ($\text{C Cl}_3 \cdot \text{COO Na}$) is not translocated from grass leaves whereas Dalopon (sodium α -dichloropropionate- $\text{CH}_2 \cdot \text{CCl}_2 \cdot \text{COO Na}$) is readily translocated (Warren, 1954). In fact it is dangerous to postulate how a substance is translocated on the basis of the behaviour of an analogue.

Little is known of the chemical form in which substances are metabolically translocated in the phloem. Certainly there is no chemical combination between 2,4-D and sugars (Weintraub and Brown, 1950).

At one stage it was thought that the basis of selectivity between a grass and a broad-leaved plant lay in differing abilities to translocate the applied poison, but this is not necessarily so. Wood *et al.* (1947) showed the same concentration of 2-iodo-3-nitrobenzoic acid produced a far greater effect in young bean leaves than in young oat leaves, and they concluded that the different behaviour of the herbicide in the oat leaves was due to the different way in which it reacted with cell constituents. This conclusion is supported by the work of Brian and Rideal (1952) with monolayers; they concluded that the foundation of species resistance may be based on the extent of adsorption of the herbicide to sites other than that associated with the physiological response.

It must be noted that although the simultaneous movement of food material is necessary for the metabolic translocation of 2,4-D, this herbicide can be extremely toxic when applied to starved tissues (Davis and Smith, 1950).

Some substances are decomposed before they become toxic. Thus the action of 2,4-dichlorophenoxyethyl sulphate ("Crag No. 1"), which has a selective action on seedlings and is relatively harmless to established plants, depends on its conversion to 2,4-dichlorophenoxyethanol or to 2,4-D (Vlitos, 1953). Either the mature plants do not absorb the sulphate compound via their leaves, or they lack the necessary enzymes to decompose it. Certainly microflora in the upper layers of the soil decompose the sulphate

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compound, the end products being absorbed by and killing the seedlings.

Poisons may be structurally non-specific or structurally specific. The former act "physically", i.e. depend on some physical properties which make for accumulation at a vital interphase where they can upset respiration, etc.; the latter have certain groups which combine with chemical constituents. Illustrative of the structurally non-specific group are the oils, which are considered to accumulate at the cell membrane (Currier, 1951). Other non-specific poisons are the alcohols, the activity of which within limits depends on the surface tension of their aqueous solutions (i.e. on surface activity). However, as regards the growth-regulator group of herbicides there is no general correlation between surface activity and toxicity (Paley and Muir, 1952). In such herbicides, chemical reactivity plays an important part.

The obvious task of a herbicide is to interrupt some vital process, but just which vital process is interrupted is not always clear. The toxicity of nitrophenols is due to their uncoupling of the phosphorylation process during the respiratory degradation of trioses. In this instance there is no chemical analogy between the herbicide and the process interrupted. In other instances the structure of a herbicide gives the clue to the mechanism of action. Thus the toxicity of chlorate is due to its antagonism of nitrate metabolism (Aberg, 1938). The absorbed chlorate is reduced to chlorite, which inactivates the system which would normally metabolise nitrite; moreover the toxicity of chlorate can be reduced by added nitrate. Similarly the action of arsenate is due to the inhibition of the formation of triose phosphates in respiration. Of the innumerable mechanisms offered to explain the action of 2,4-D, the most feasible are those which are based on its analogy with indole acetic acid.

For a long time it was puzzling why growth regulators could be stimulating at low concentrations and be toxic at high. The enzyme kinetic studies of Foster, McRae and Bonner (1953) not only give the answer, but also give the best explanation for the toxic action of 2,4-D. They find that growth depends on a two-point attachment of the growth regulator to its substrate. At higher concentrations of the regulator there is competition for points of attachment; some regulator molecules become attached by one point, and others by the other point. The normal growth system is interrupted, resulting in a toxic system. An extension of this theory will explain why 2,4-D and similar

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substances have most effect on young and actively growing systems: it is in such systems that the natural auxin (indole acetic acid) is most active and that the growth substrate can be most readily disturbed.

Much has been written about the activity of compounds in relation to chemical structure. Most of it is about growth regulators (e.g. Veldstra, 1953; Muir and Hansch, 1953). Various attempts have been made to generalise on the structure necessary for activity, though none are completely successful. Muir and Hansch point out that usually a free ortho position next to the oxygen linkage is required for activity, but comment that fluorine substitution is an exception.

The formula of 2,4-D, viz. $2,4\text{-Cl}_2\text{C}_6\text{H}_3\text{OCH}_2\text{COOH}$ is deceptively simple. Such compounds can be considered as having four sites of specificity: the ring, the ether oxygen, the methylene group, and the carboxyl group.

The ring system must always have some unsaturated bonds. Substitution of halogen atoms in the ring confers activity; halogen substitution is generally the most effective, chlorine and fluorine being the most efficient of the halogens. The most important positions are 4, 2-4, 2-4-5, 2-5, and 3-4. Which substitution is to be preferred depends on the plant to be killed. Replacement of the oxygen linkage by nitrogen or sulphur reduces activity. On the other hand, the oxygen linkage and methylene group can be eliminated, and the ring substitution altered, and active compounds such as 2,6-dichlorobenzoic acid result.

Replacement of one of the hydrogen atoms in the methylene group with a methyl group is possible, and the resulting compound is optically active but still toxic. Replacement of one hydrogen with ethyl or larger groups leads to diminished activity. Replacement of both hydrogen atoms by methyl groups results in almost complete inactivation, leading Osborne and Wain (1951) to postulate the necessity of an α -hydrogen for biological activity. If the methylene group is extended to a straight chain, the resulting compounds may or may not be active, depending on the number of carbon atoms in the chain and the test used. For certain growth responses there should be an odd number of carbon atoms, though an odd number is not necessary for toxicity.

Activity of the molecule is diminished or destroyed by substituting a phosphonic radical or sulphonic radical for the carboxyl. However, activity is not necessarily

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diminished, and indeed may be enhanced by conversion of the carboxyl group to certain derivatives such as ester, amide, anilide, ureide, hydrazide, nitrile, acid chloride, alcohol, or aldehyde.

An important feature so far given little attention is the effect of repeated application of herbicides. Work by R.M. Moore on hoary cress shows that treated plants are subsequently more susceptible to a second application. On the other hand, work with Lemna (Robertson-Cunningham and Blackman, 1952) or with Sinapis alba (Linden, 1953) shows that provided there is small but sufficient time interval, a second application of 2,4-D has far less effect. Possibly this developed resistance depends on the synthesis within the plant of new enzyme systems.

This brief account makes no pretence at covering the whole field but aims at introducing the subject. It is hoped that subsequent papers and discussion will fill in some of the gaps.

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