Proceedings 8th British Insecticide and Fungicide Conference (1975)

BIOCHEMICAL DESIGN OF PESTICIDES

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Summary The biochemical mode of action of existing pesticides is outlined, giving both the primary and presumed secondary actions, and the types of molecular binding at the primary sites are analysed. It is concluded that enzymes are not common primary sites of action for compounds derived from a random synthetic approach. New candidate pesticides should probably exert their toxic effect *in vitro* at micromolar levels or below, in order to compete with existing products, and it is suggested that it may prove very difficult to produce new pesticides with dramatically lower application rates than present products.

The basic criteria for selecting a target site are examined with the conclusion that enzymes, despite some disadvantages, do offer attractive sites for design, especially if active site directed irreversible inhibitors are synthesized. Fisons' experience with four different enzymes is reviewed, and work on growth regulators, selectophores and design rules from existing pesticides, referred to.

THE PRESENT SITUATION

There are approximately 500 different chemical structures, arranged in perhaps 50 families, now available as pesticides, yet it seems certain that none of the original members of any of the families was discovered rationally in the sense that there was any successful prediction of the type of activity expected from the novel structure. Rational design only appears to have been useful when making analogues of existing active materials, whether produced by man or occurring naturally; in some studies on the relation between structure and activity; and in a few cases where it has been possible to use selective metabolism to confer differential toxicity.

Given the enormity of the task, these successes of rational design are no small achievement, but the fact remains that no one has yet viewed a pest as a biochemical machine, selected a vital part of the mechanism, and designed a novel chemical structure that kills the pest, is sufficiently selective in its action to allow general use, and fulfills the normal criteria of potential profitability that encourage commercialisation. What has actually occurred since the war is that superbly active and often selective pesticides have been discovered by a trial and error process of random synthesis and screening in which perhaps one to two million compounds have been tested in the laboratories throughout the world, mainly belonging to industrial companies.

This success of empiricism over rationality is intellectually humiliating, especially to pesticide biochemists, and constitutes a challenge, which, if successfully overcome, would allow the discovery and development of pesticides without the necessity to synthesise and screen some ten to fifteen thousand new structures to find one new commercialisable compound. It is also possible that compounds designed from the start with a fundamental understanding of their biochemical mode of action might possess fewer toxicological and environmental disadvantages than compounds which have been selected without this fundamental background.

It is the purpose of this paper to outline the possibilities for rational design in the pesticide industry, but it should be stated at the outset that there is no easy way to find new pesticides. All that one can do is to use past methods coupled with the rational techniques that are now available, and which will be described below.

MODE OF ACTION OF EXISTING PESTICIDES

As a background to the problems of designing new pesticides it is useful to consider the biochemical mode of action of existing compounds. At least an outline knowledge exists of how perhaps 400 of the 500 compounds work, but, and this is an important proviso, only up to the state of biochemical knowledge in the area. Thus our knowledge of the mode of action of compounds affecting respiration is much better than of those affecting cell division, reflecting our relatively poor understanding of the mechanism of the latter process.

Pesticides with a known specific mode of action are listed in Figures 1 through 6, which show that the ultimate cause of death is by interference with nervous co-ordination, structural organisation, the supply of energy, or growth and reproduction, resulting from action at a variety of primary sites of action. It is worth noting that although there is often excellent information on the primary action of a pesticide, the causal chain resulting from this and ending in death is usually not known. Figure 7 shows that plant growth regulators interfere with the levels of naturally occurring plant hormones. Figure 8 lists pesticides, mainly fungicides, with a non-specific mode of action, i.e., compounds that react with a variety of relatively reactive groups within the organism.

What lessons can be learnt from this survey of existing modes of action? A chemist will wish to know how the compounds combine with their site of action, and this is summarised in Table 1.

Such an analysis excludes uncouplers of oxidative phosphoxylation and the bipyridylium herbicides since there is no evidence that they bind to a site, though this has not been specifically ruled out. One conclusion from Table 1 is that, perhaps surprisingly, enzymes do not seem to be very important as targets. With the well known exception of acetylcholinesterase, the target of the organophosphorus and carbamate insecticides, enzymes do not appear to have been "selected" very often by the process of random synthesis and screening. Possible reasons for this will be discussed later. A second conclusion is that "structural" molecules per se, as distinct from the enzymes that synthesise them, do not often constitute targets. Perhaps this is because too much pesticide is required to interfere with, for instance, the function of chitin or cellulose by actually becoming incorporated into the structural polymer as a false building block, so producing enough faulty structural material to cause death.

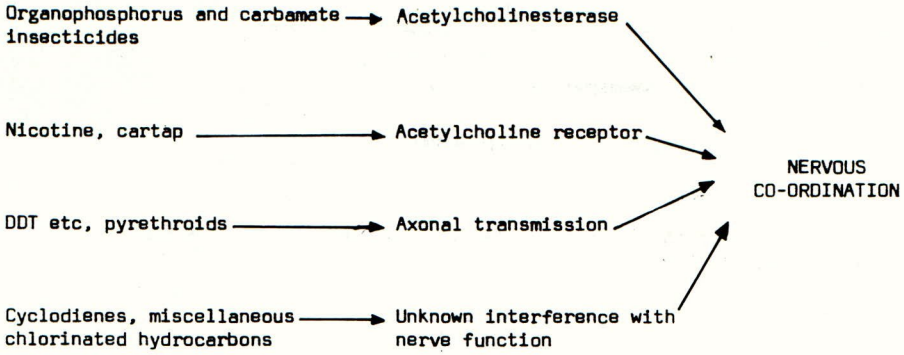


Fig. 1 Insecticides affecting the nervous system

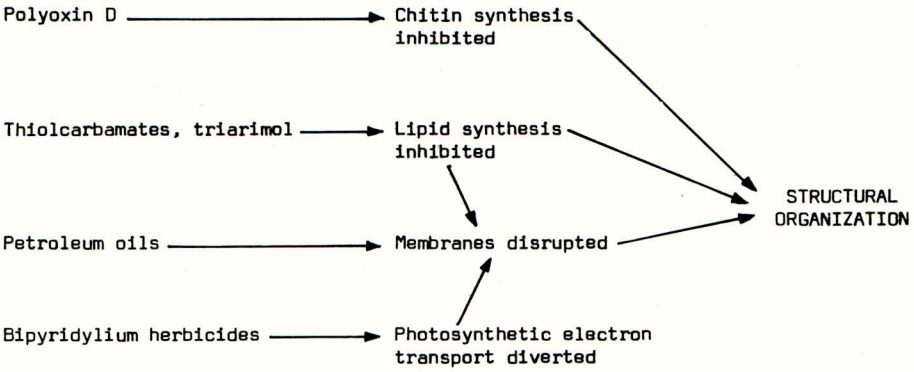


Fig. 2 Fungicides and herbicides affecting structural organisation

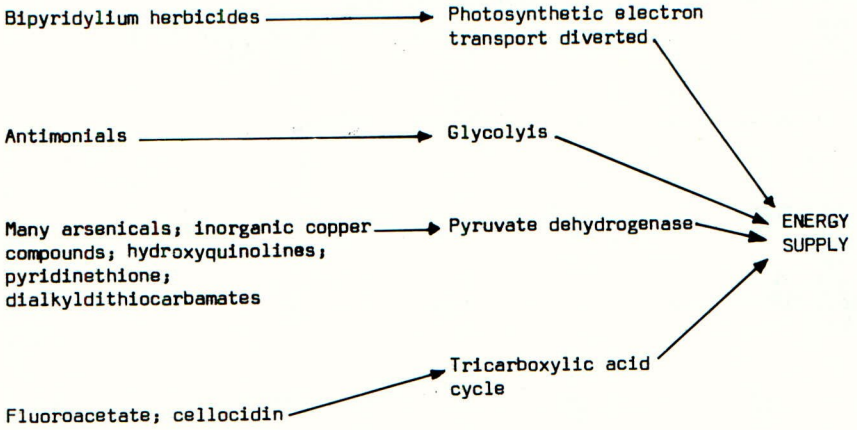


Fig. 3 Pesticides interfering with the energy supply

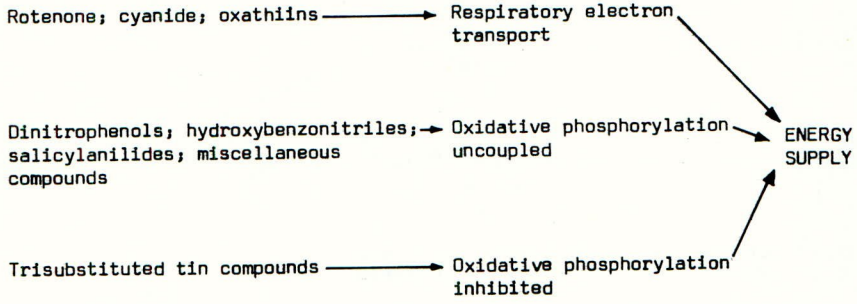


Fig. 4 Pesticides interfering with respiratory electron transport and oxidative phosphorylation

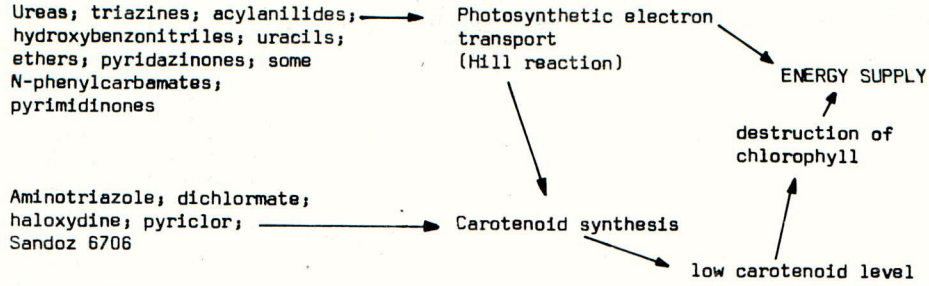


Fig. 5 Herbicides inhibiting photosynthetic electron transport and carotenoid synthesis

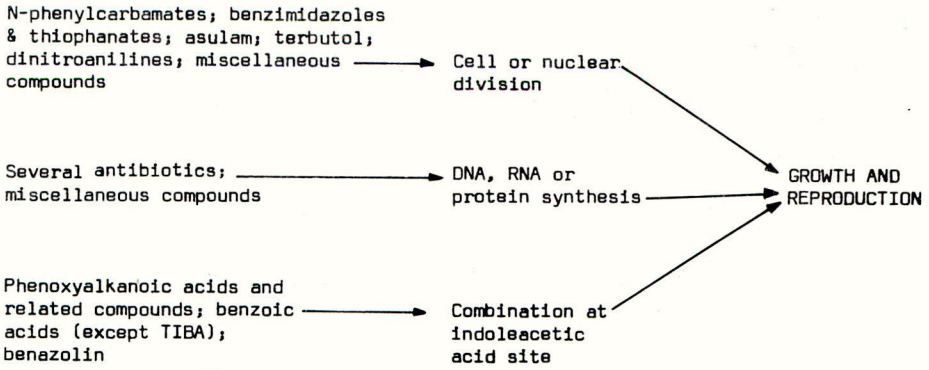


Fig. 6 Fungicides and herbicides interfering with growth and reproduction

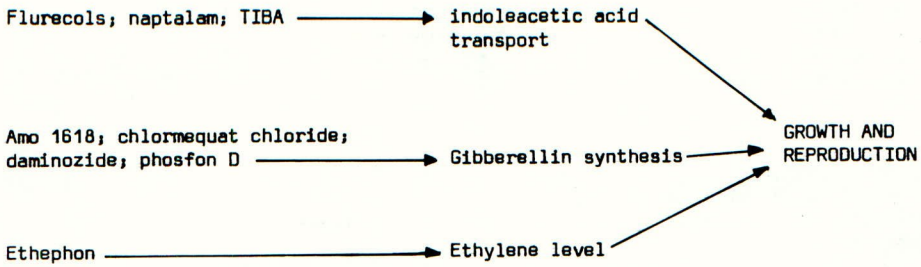


Fig. 7 Action of plant growth regulators

FLUORIDE

MERCURY CONTAINING PESTICIDES

FUNGICIDAL QUINONES

ALKYLENE BISDITHIOCARBAMATE FUNGICIDES

N-TRICHLOROMETHYLTHIO FUNGICIDES

MISCELLANEOUS COMPOUNDS

Fig. 8 Pesticides with a non-specific mode of action

Table 1

Summary of the types of binding at the primary sites of pesticide action

Type of site	Mechanism of Inhibition	Examples
Enzyme	Reversible	Antimonials; polyoxin; - cyanide
	Irreversible: ASDINs*	Organophosphorus and carbamate insecticides; fluorocitrate (from fluoroacetate);
	Others	Many arsenicals; inorganic copper compounds; many non-specific inhibitors that readily form covalent bonds with thiol and amino groups may kill by inhibiting enzymes
Natural receptor	Reversible	Nicotine; cartap; probably the phenoxyalkanoic acids and other indoleacetic acid mimics
Other sites	Reversible	DDT and related compounds; rotenone; oxathiins; trisubstituted tin compounds; ureas and other inhibitors of the Hill reaction.

A biochemist interested in pesticide design will wish to know the approximate concentration at which a candidate inhibitor must act on an in vitro system in order to compete with commercial compounds. In general most pesticides work at 0.1 to 10 μM on their presumed primary target in vitro. Unfortunately there have been very few measurements of the in vivo levels of pesticides necessary to cause a lethal effect, but synergised carbaryl works at approximately 15 μM (Hewlett and Wilkinson, 1967), picloram at 10 μM (Davis et al., 1972), while synergised NRDC 161 (a pyrethroid) operates at the extremely low level of 4 nM (Elliott et al., 1974).

Another way of assessing the concentration required is to calculate back from field considerations. This is done in Table 2 for a herbicide by making very broad assumptions concerning application rate, uptake, and volume of plant material, with the conclusion that it would not be unreasonable to find a concentration of perhaps 80 μM in the plant tissue. This correlates reasonably well with the above information on in vitro and in vivo levels.

This assumes a molecular weight of 250, no metabolism or other detoxification, and uniform distribution throughout the plant.

*See page for definition

Table 2

Likely concentrations of a typical herbicide in plant tissue

Variable	Limit Giving a High Final Concentration	Working Figure	Limit Giving A Low Final Concentration
Application rate (kg ha^{-1}	4	1	0.25
($mg\ m^{-2}$)	400	100	25
Uptake (%)	30	20	10
Plant material ($kg\ m^{-2}$)	0.1	1	10
Final Concentration in plant (μ moles kg^{-1})	4800	80	1

Exceptions to this could be sites in the nervous system, and also sites concerned with cell division, where one may imagine vital receptors present at very low concentrations in the tissue. Evidence that this is so for nerves is the high activity of recently discovered synthetic pyrethroids referred to above, plus the fact that materials that are extremely toxic to man are known to act on the nervous system.

The above reasoning has tacitly assumed a stoichiometric combination of pesticide with receptor before death results, but it could be argued that a poison which acted catalytically by generating a toxicant might prove lethal at extremely low levels. While this remains a theoretical possibility, it should be recalled that the bipyridylium herbicides, which do act catalytically, are still required at normal herbicidal rates in the field, and at micromolar levels in vitro.

DESIGN

Basic Criteria for selecting target sites

With this background knowledge on the mode of action of existing pesticides that were developed by trial and error methods, what are the desirable criteria for a target site for which a novel pesticide could be rationally designed? Firstly, the site should be present at a low concentration in the organism, thus tending to rule out incorporation into structural molecules as a target. Secondly, the site should be of short term importance to the life of the pest. There is little point in designing an inhibitor of a process which the organism can happily do without for a period of more than a few days, since in many cases it would be desirable for the pesticide to have been destroyed in that period to prevent the accumulation of residues. Thirdly, it is essential that a lead for chemical synthesis be available, otherwise there is no prospect of immediate progress towards testing the site. Fourthly, it is highly desirable, but not absolutely essential to have an in vitro assay. Such a test can separate the factors affecting activity at the target site from those concerned with uptake, transport and metabolism of the compound before it reaches the target.

Enzymes

Suitably selected enzymes offer targets that readily fulfill the four criteria given. However, as we have already seen, it appears that not many existing pesticide classes act directly on enzymes. Since some one or two million compounds have

probably been screened as pesticides since the war, a wide range of molecular properties must have been available from which to select the existing commercial pesticides, though there was doubtless some bias towards the synthesis of analogues of compounds already known to be active. Consequently, it is probably not just chance that enzymes are not common targets. It is possible to identify at least three factors that may account for this.

Firstly, the enzyme substrate will build up and compete with the inhibitor for unoccupied active sites. This is also true for non-enzymatic receptors such as that for acetylcholine in the nervous system, and the putative indoleacetic acid receptor in plants.

Secondly, enzymes, especially when arranged in enzyme systems, are subject to metabolic regulation. They have feed back mechanisms which, if inhibition is effective, may divert a substrate via another route to the same end point or which may prevent its increase in concentration past a certain level. Thus enzymes can have a flexibility of response denied to non-enzymatic receptors lacking these regulatory mechanisms.

Thirdly, anything less than total inhibition of an enzyme might have little or no effect on the organism since most enzymes have "spare capacity".

Finally, it may be that inhibition of certain non-enzymatic receptors tends to be more lethal than inhibition of enzymes themselves. In the case of inhibitors of photosynthesis acting on the Hill reaction it is probable that inhibition of the light trapping reaction causes the build-up of reactive species, which is probably more damaging than build up of a conventional enzyme substrate. Where non-enzymatic receptors are involved in the transmission of information, whether in the nervous system or cell division, the possibility exists that an inhibitor may not only reduce the information flow, but may also render it inaccurate.

Despite these factors adverse to enzymes, they do in general offer attractive sites for rational design of pesticides since the function of most enzymes is quite well understood so that their importance can be assessed, and the structure of the substrate offers an immediate chemical lead for synthesis of inhibitors, which are usually easy to assay in vitro.

Once an enzyme has been selected one can design an inhibitor for the active site that will bind either non-covalently or covalently. Inhibitors that bind non-covalently are usually close analogues of the substrate that compete with it for the site, with which they are in reversible equilibrium. Consequently they suffer from the disadvantage that, by inhibiting the enzyme they cause a build-up of substrate which tends to displace the inhibitor. It is likely that very tight binding, probably with a K_i <0.1μM, is required to achieve adequate inhibition by this somewhat self-defeating reversible type of binding.

A more promising approach is to synthesise active-site-directed irreversible enzyme inhibitors (ASDINs), which are molecules resembling the substrate, to which is attached a reactive anchoring group which can form a covalent bond with an amino acid residue near the enzyme active site. The ASDIN is therefore guided to a specific enzyme by virtue of its similarity to the substrate, and, once there, inhibits further catalytic activity by forming an essentially irreversible complex with the enzyme (Baker, 1967). The ASDIN approach presumes the presence of a reactive amino acid residue, conveniently located near the active site.

As part of a programme of rational design of pesticides at Fisons we have attempted to make ASDINs against four enzymes. The first, shikimate dehydrogenase, is important in aromatic amino acid synthesis in plants but does not occur in animals, and therefore offered an attractive target for a new herbicide. ASDINs were synthesised, but were rather weak on the *in vitro* assay, and none proved herbicidal (Baillie et al, 1972). Glycollate oxidase, the key enzyme of photorespiration, was our next target. The enzyme does not occur in mammals and we hoped that an active inhibitor would prove to be either a herbicide or a growth regulator (depending on one's view of the function of the enzyme). Compounds causing *in vitro* inhibition at low concentrations were prepared, but they acted by degrading to simpler compounds, and we were unable to synthesise an ASDIN (Corbett and Wright, 1971). For our third attempt we selected indoleacetic acid oxidase, which is responsible for regulating the level of indoleacetic acid, the naturally occurring plant growth hormone. Active inhibitors were made but were broken down by the enzyme, and no herbicidal activity resulted (Wright et al., 1973). Our fourth enzyme was choline acetyltransferase, which reverses the effect of acetylcholinesterase. A reversible inhibitor with the extremely low K_i of 0.06 μM was synthesised (Baillie et al. 1975) but, being charged, would be unable to penetrate the insect nerve cord. An uncharged analogue was much less inhibitory, but did kill insects. However, physiological studies indicated that this was probably due to combination with the acetylcholine receptor, and not with choline acetyltransferase.

The most disappointing feature of our work has been our inability to synthesise an ASDIN that was really active on the *in vitro* assay. Until this is done we feel unable to judge the merits of the ASDIN approach to rational pesticide design, and we are continuing with a programme of designing inhibitors of key enzymes.

Growth Regulators

Any compound that interferes with the synthesis or breakdown of a plant growth regulator (cf the indoleacetic acid oxidase site above), that releases or combines with a regulator, that interferes with its transport, or that interferes with binding at its presumed site of action, is likely to modify plant growth. At Fisons we made ethylene releasing compounds that were biologically active but proved to be of no commercial interest.

Selectophore Concept

A group conveying selective toxicity has been named a "selectophore" (see O'Brien, 1967). Since much selective toxicity of pesticides is based on metabolism, rather than on inherent differences at the site of action, the selectophore concept is useful, provided that one has an active molecule to start with. Figure 9 shows an active uncoupler (I) derived from a conventional synthesis programme at Fisons. However, it had undesirably high mammalian toxicity. Compound III, containing the diethysulphamoyl group designed as a selectophore was therefore synthesised with the hope that it would (i) be at least as good an uncoupler as (I) (by virtue of the lipid soluble ethyl groups) and (ii) would show much lower mammalian toxicity (due to degradation to the less toxic II, probably via IV) The figure shows that the toxicity of III is indeed much less than that of I, while the uncoupling activity is actually increased.

Design rules from existing pesticides

It is possible to use biochemical insight to design compounds that are active at sites on which existing pesticides act. Naturally, one can synthesise analogues of any natural or synthetic active compound in the hope that activity found in the original will also be present in the mimic, but there are some

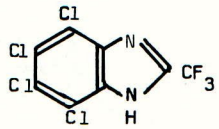
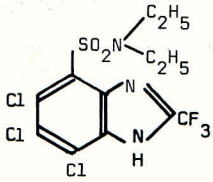
		RAT ORAL LD_{50} (mg/kg)	MINIMUM UNCOUPLING CONCENTRATION (µM)
I		2	0.6
II		600	22.0
III		80	0.25
IV		100	0.6

Fig. 9 Selectophore on an uncoupler

sites of action of existing compounds for which there appears to be enough biochemical information available to possibly allow the synthesis of an inhibitor of a new structural class.

In the general biocidal area such sites would include uncoupling of oxidative phosphorylation, where lipid soluble acids of any structure would seem worth testing. In the herbicidal area there is quite good knowledge of the molecular characteristics necessary to bind to the putative indoleacetic acid site in the manner of 2,4-D, etc. Also, the molecular characteristics of compounds that will divert photosynthetic electron flow are quite well understood, though there would seem to be great difficulty in finding structures other than the bipyridyliums that are able to do it effectively.

In the insecticide field any ASDIN resembling acetylcholine would be worth testing on acetylcholinesterase to attempt to break the monopoly of the organophosphorus and carbamate insecticides on this site. Again, compounds structurally related to acetylcholine might bind to the acetylcholine receptor and prove insecticidal in the same way as nicotine and cartap. There is also considerable information on the type of structure required to interfere with the passage of ions in and out of the nerve axon in the manner of DDT.

There seem to be no leads of this type to the synthesis of fungicides.

CONCLUSION

As stated at the beginning there is no easy way to find new pesticides. However, despite the difficulties with biochemical design, it seems worthwhile to persevere with the approach. It would be an unwise company that ignored the information pertinent to rational design, or that worked solely with it.

Acknowledgements

I would like to thank my colleagues from Fisons Agrochemical Division for their help, and especially Dr. A.C. Baillie for assistance in preparing this paper.

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Proceedings 8th British Insecticide and Fungicide Conference (1975)

THE OPTIMIZATION OF PHYSICAL AND BIOPHYSICAL PARAMETERS IN PESTICIDE DESIGN

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Summary The parameters involved in pesticide design can apply in research and development and manufacturing as well as to the end-user. In attempting to place realistic boundary conditions upon the four performance features a certain amount of conflict occurs which are exemplified. These four performance features are (i) Technological, (ii) Biological, (iii) Economic, and (iv) Social/environmental.

The physical and biophysical parameters of concern are the basic physical properties, stabilities and mass transfer processes. Each performance factor in conjunction with a particular parameter must be judged in the light of the end use situation as an optimum in one situation maybe inappropriate in another.

How these parameters may be controlled in a designed manner is considered together with the prediction of particular physical and biophysical characteristics.

It is worthwhile spending a few minutes examining some of the implications in the title; the prominent two words are 'design' and optimization'. It is almost impossible for any group of persons to say they are going to design a particular pesticide and then do what they intend; the present state of knowledge is not yet that advanced.

Optimization is somewhat more feasible in an academic sense, however it is seldom realized in the manner generally envisaged or by processes we would desire to be true. External pressures, usually unknown at the planning stage, so influence predictive aspirations that the optimization is often disrupted before it can be realized in the field but since one man's optimization is another man's sub-optimization this may not be so critical.

Nevertheless it is an interesting exercise to investigate what we would desire in a pesticide if we could design and optimize ab initio. In this we must always state the desirable features in terms of the persons desiring such features. It is no doubt true that what is desirable to the legislative authority may not be desirable to the farmer and similarly between manufacturer and farmer etc. In fact, within an industry, what is optimal or desirable to the marketing function may be neither to the research or the manufacturing function.

Having exposed some of these factors without resolving them, it is worthwhile examining 'Physical and Biophysical Parameters', in order to provide a more substantial core to this paper. If we examine Tables 1 and 2 it is apparent that

numerous parameters are important to different degrees to different people. In this it will be noted that chemicals only are discussed while other options for pest control are available, although many of these are still at the 'drawing-board' stage. Chemicals currently remain the major pest-control mechanism and will continue to do so into the mid-future. However, speculation as to the features desired in other pest control processes can be considered in the light of Tables 1 and 2.

Table 1

Economic Features of the Product	Biological Features of the Product
Cost effectiveness	Movement (transport processes)
Production costs and offtakes	Persistence
Market lifetime	Activity to crop and pest
Crop and pest spectrum	Application rate and timing
Pre-commercialization costs	Application frequency
Promotion sales and advertising costs	Application process
Gross proceeds and turnover time	Reliability and dose response curve
Added value to farmer	Speed of control
	Spectrum of use
Technical Features of the Product	Joint action
	Selectivity
Manufacturing process	
Formulation processes	**Environmental Features**
Storage	
Transport	Interactions with earth, air energy, and water
Application regime	Interactions with useful biomass

Table 2

1. Physical State
   (a) Boiling point
   (b) Melting point
2. Solubilities
   (a) Hydrophilic solvents
   (b) Lipophilic solvents
   (c) Partition characteristics
3. Size and Shape of Molecule
   (a) Parachor
   (b) Topology/topography
   (c) Molecular weight
4. Vapour Pressure and Volatility
5. Stability/Reactivity
   (a) Photolytic
   (b) Thermal
   (c) Hydrolytic
   (d) Redox
   (e) Acid-base properties
6. Diffusion Characteristics
7. Surface activity and Adsorption properties
8. Special features
   (a) Polarity
   (b) Spectroscopic properties
   (c) Polymorphs
   (d) Isomers

Of the features listed in Table 1 we will, in the main, ignore the economic because they are not relevant to the talk although they remain of prime importance to all concerned. The technical will be touched upon but not elaborated because, by and large, they are under the direct control of the pesticide industry and are optimized according to the philosophies of individual firms. That is the boundary conditions for many of these features are defined by the term and under the control of particular individuals.

The biological and environmental features, however, have less well defined

boundaries and are outside of most control processes once the chemical is applied. Thus it is in this area that challenges occur to (a) examine and attempt to define the system and (b) to effect some measure of control.

In these we call upon physical-chemical and mathematical techniques applied to biology and the environment under the gross title of biophysics. Re-assessing the views presented earlier they can be presented as studies (i) in the industry, (ii) in the pest* and (iii) in the environment. The areas (ii) and (iii) each involve transport processes, loss processes and pesticide activity. Pesticide activity can be either in the intrinsic sense of applied dose response or in the field sense of application rate and infestation control. In the field the statistical evaluation must be considered in conjunction with the chemical, physical and biological evaluation.

While a major feature of a pesticide must be in the molecular constitution, that is a particular arrangement of a particular set of atoms, we are not primarily concerned with organic synthetic processes in this paper. However, once the molecular arrangement is decided upon then this confers all of the properties on the molecule and if we develop an optimum physico-chemical and biophysical specification for a molecule the mechanism to produce such a molecule may not exist.

Thus we reach a sub-optimization before we start.

Each property of a molecule may be manipulated to some extent for a certain time span; however, ultimately, kinetic processes will allow thermodynamic control to be exercised. That is, the particular arrangement of atoms will move to their most stable state at a speed which can sometimes be influenced. A trivial example might be keeping a photosensitive chemical in a dark area.

One way or another all of the important words have now been manipulated into the text and it remains to point out that considerations can be on a micro or macro scale. Thus an individual pest may be studied as part of a population but it will never be wholely representative of the population and a population in the natural environment will respond differently from an isolated population.

In the research and development area alone we can distinguish several stages of research and development each of which can be optimized, Table 3.

Table 3

Hierarchy of Investigation

I.	Molecular interaction studies, e.g. enzyme inhibition studies
II.	Organelle studies, e.g. nervous system investigations
III.	Whole pest studies, e.g. laboratory dose-response data
IV.	End user simulations, e.g. field trials

As the factors controlling optimization at each stage are likely to be different, the choice of a compound at one stage for progression to the next is fraught with difficulties.

Consider first that the 'best' inhibitor from stage one is progressed, this will

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*Pest implies all non-useful biomass detracting from the development of useful biomass. The term is dynamic and will mean different things at different times.

not have optimum properties for stage two etc except by an improbable accident. Thus an optimum at any stage sub-optimizes the whole. Alternatively if we wish to optimize the whole picture, which we do, then we must sub-optimize at each stage. Such an approach requires an extensive amount of faith to be applied by researchers at each stage and in particular by management in general. The pursual of these sub-optimizations may relate to the data presented here.

The factors to look for can be developed from several directions; (a) imposed limitations, e.g. legislation (b) by empirical recognition of the properties of good commercial products (c) from fundamental scientific principles. For the latter the current state of knowledge is still underdeveloped; however, considerable strides are being made here and it can certainly provide part of the picture. It needs to be borne in mind, however, that whatever scientific picture emerges the variable environment will not conform to what we would wish to be the case. Thus the influence of statistical analysis is high and the researchers need to be aware of this.

Approaching the problem by providing a series of imposed limitations guided by empirical relations and scientific principles, we can develop the boundary conditions within which optimization can be attempted. Thus even if we are not optimal we are in the correct area and should finish up with a material that will do the job.

Let us take the view that what is desired is a product which controls the pest at the right time, to the right extent and in the right place. More than this, it should not act before it is required and once it is no longer required it should be converted into innocuous material.

As an overrider to this, no matter what the farmer desires the general public feel better if the lifetime of the chemical is short compared with the lifetime of man. Let us arbitrarily say that less than 1% of man's life expectancy is acceptable to public opinion; thus 7-8 months for, say, 5 half-lives of the chemical in the use situation. This means a half-life of about 50 days or a gross disappearance rate constant under practical user conditions of 1 to 2 x 10^{-2} day^{-1} at the most persistent end. The least persistent end will be a half-life of say 12 hours (k = 1.4 day^{-1}). Let us look at other parameters for the former case in this light.

First let us approach mass transfer. Once a chemical has been applied in the field it can move in the solid, liquid, or gas phase. To do this it must first enter the appropriate place and leave its initial resting phase. These can be considered as two distinct processes.

Mass transfer is a desirable process in that we seldom wish pesticides to remain at the exact positions taken up at application. On the other hand mass transfer provides one of the major loss processes for pesticides because we have little control of the transfer processes in this field.

Diffusion in the gas phase is fast thus a high vapour pressure can produce quite high rates of transfer of material to sites remote from the desired sphere of activity. A good example of this is water, which has a vapour pressure of about 15 mm Hg at ambient temperatures, and an average of 20 tons are lost from each hectare of soil per day in the UK.

If we examine the rates of loss of pesticides from the soil surface and how these change with vapour pressure a picture emerges of the type shown in Fig 1.

This assumes that the pesticide behaves like an ideal material and the ideal gas laws are employed (Adams,1952) in conjunction with the concept of permeability of a still air layer (Hartley,1969) to derive the equation (1).

$$\text{Flux} = 5.87 \times 10^{-8} . \frac{M.D}{l} . P \qquad \ldots\ldots (1)$$

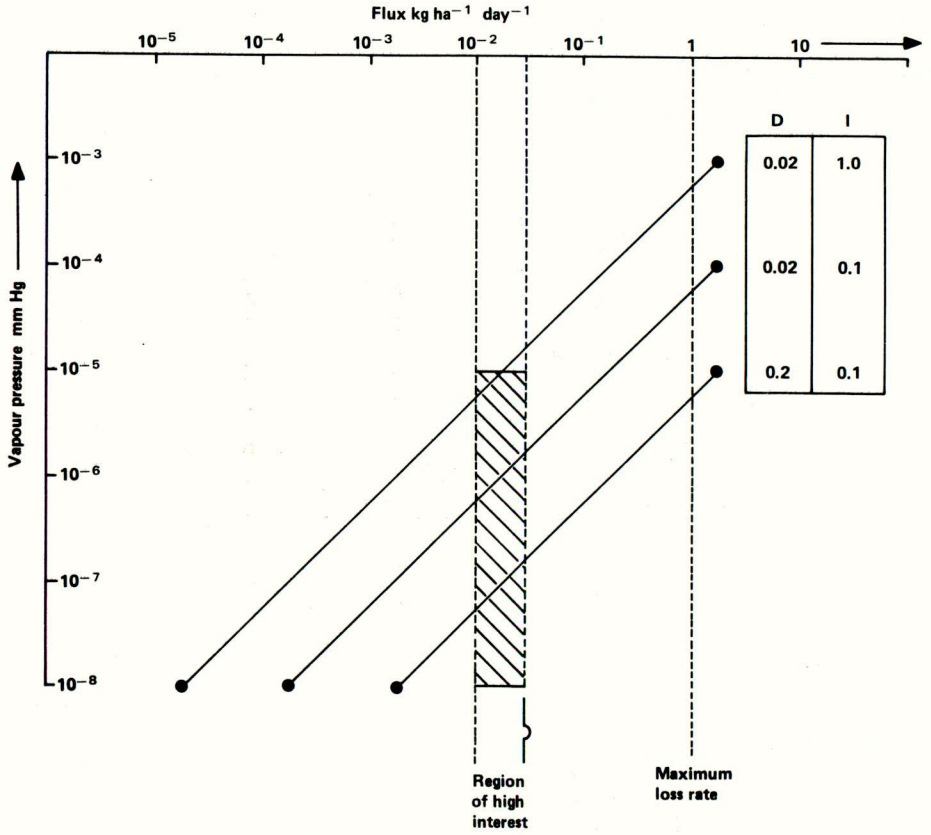

Fig 1 Pesticide loss by evaporation (m.wt. = 250)

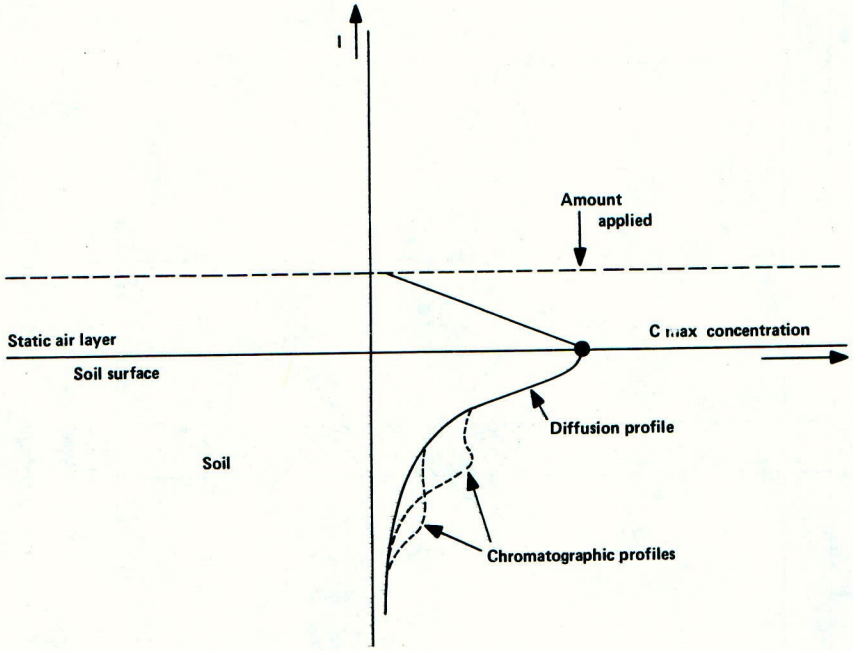

Fig 2 Mass transfer from soil surface

In this flux is measured in grammes per square centimetre per second when M is the molecular weight, D is the diffusion coefficient for the gas in $cm^2 s^{-1}$, P is the vapour pressure in mm Hg and l is the thickness of the static air layer in centimetres. The diffusion coefficient for small molecules in the gas phase (Jost,1960) is in the range 10^{-1} to 10^{-2} cm^2 s^{-1} while the vapour pressures of pesticides are around 10^{-3} to 10^{-8} mm Hg at 25°C. The static air layer will depend upon many features but particularly wind speed, and a thickness of about 0.1 cm is often quoted for ambient conditions (Monteith,1973).

Using this information Fig 1 can be constructed. This describes the variation in flux from the soil in kg ha^{-1} day^{-1} as a function of vapour pressure for several values of diffusion constant and static air thickness.

A check on this curve can be made by considering water, for which the answer is known. The model predicts that 90 tons of water will be lost per hectare per day if the static air layer is 0.2 cm. However, this assumes the loss is from a continuous surface of water in its normal condition not water evaporating from soil.

Thus we can obtain the practical result by justifying a reduction in loss to a quarter of this level. This can be done by stating that the soil/water interaction reduces the vapour concentration of water and/or that the diffusion path should be greater. How this concept relates to compounds with very much lower vapour pressures is not known. Fig 1 is still valuable however in that it provides a frame of reference and allows an optimal region to be identified (see cross hatching). The process does, of course, assume that the saturated vapour pressure is achieved, which will not be so if the compound is strongly sorbed onto soil (Spencer and Cliath,1972). Such sorption could reduce the vapour pressure from its defined saturated vapour pressure by a factor of 10 (Spencer and Cliath,1969). While this example refers to soil the process on a leaf can be similarly conceived.

Further than this the material will be disappearing into the soil or plant via a combination of diffusion and other processes which give rise to an overall picture of asymmetric diffusion upon which a chromatographic type process may be superimposed (Freed and Hague,1973; Graham-Bryce,1969; Hamaker,1966). Fig 2 gives an idealized picture of this. Thus the length of time for which the vapour flux model of Fig 1 applies may be quite short with the total distribution picture taking control within the first hour. A chemical in an environment composed of a binding solid, say soil or plant material (Bridges and Farrington,1974), the air and water will be distributed amongst all three as a dynamic system which may achieve equilibrium. The analysis of the dynamic system is difficult but some model systems at equilibrium are instructive.

Fig 3 gives the conceptual picture of this distribution with the cross-hatched area as that usually associated with pesticides other than fumigants. In Figs 3 and 4 the values C_G, C_S and C_W refer to concentrations in the gaseous state, in the adsorbing solid, and in water respectively. If the pesticide has the following properties, (a) vapour pressure = 10^{-5} mm Hg (b) water solubility of 10 ppm and (c) a soil/plant solids solubility of 1000 ppm, it will tend to set up the equilibrium system given in Fig 4. In this

$$K_1 = \frac{C_S}{C_L} \; ; \quad K_2 = \frac{C_L}{C_G} \; ; \text{ and } K_3 = \frac{C_S}{C_G}$$

Thus $K_1 . K_2 = K_3$

If the molecular weight is 250

$$K_1 = 100; \quad K_2 = 7.5 \times 10^4; \quad K_3 = 7.5 \times 10^6$$

Even for compounds with quite low saturated vapour pressures the redistribution

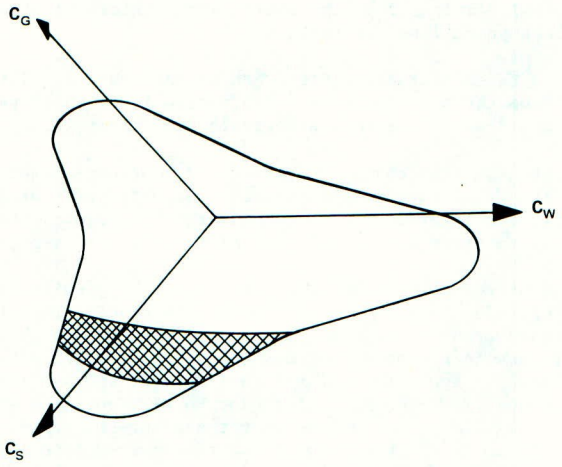

Fig 3 The conceptual distribution of a pesticide

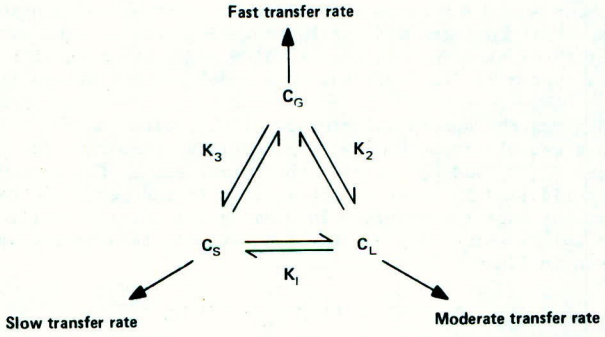

Fig 4 Mass transfer from the Equilibrium System

of molecules on the soil/plant surface and in the water will take place very quickly via the gas phase. Thus at a vapour pressure of 10^{-8} mm Hg this redistribution will be accomplished in a few minutes (Seary and Beruto, 1974) with the establishment of a system approaching a monolayer of pesticide on the surface.

Consideration of the diffusion coefficients in conjunction with the thickness of wax layers on plants and insects suggests that the concept of diffusion through the wax as a major transport process for entry to the biosystem may not be correct, see Table 4. The range of values for the diffusion coefficients in the three phases gives some idea of the relative merits of transfer via each process. Thus in gases diffusion coefficients are in the range 10^{-1} to 10^{-2} cm^2 s^{-1} while in liquids small molecules have diffusion coefficients of about 10^{-5} cm^2 s^{-1}. The movement in the solid phase is much slower and is very dependent upon the solid's structure; however, 10^{-10} to 10^{-12} cm^2 s^{-1} are reasonable values for non-crystalline materials. These values are for ambient temperatures. Rather than attempt to define an optimization process for such a complex system we will move to the better defined area of hydrolytic stability.

The hydrolysis of pesticides is a major route for breakdown, although it may not be the controlling process. Fig 5 gives the idealized picture and shows how the half-life can vary with the pH of the solution. Thus partition factors are not involved. As is usual, compounds most susceptible to acid hydrolysis (Anderson and Capon, 1969) are less susceptible to base hydrolysis, curve (a). However, compounds most susceptible to base hydrolysis (Johnson, 1967) are often more stable in acid, curve (b). The compounds hydrolysed 'evenly', that is equally susceptible to acid and base, are depicted in curve (e).

In the light of the earlier remarks regarding optimal field lifetimes these have been indicated on the figure. Thus if it was considered that a molecule would be exposed to a region of pH 10, as the most severe regime, then we have delineated a region of high interest and this is marked with the cross-hatching. Naturally this will only be critical if hydrolysis is the major breakdown mechanism. Many of the organophosphate (Cox and Ramsey, 1964) and carbamate (Bender and Homer, 1965) insecticides fall within this region and some are even more stable than considered optimal from this viewpoint. However, these compounds are usually oxidized (Welling et al, 1974), or photolysed (Comelisse et al, 1975)at rates which make this analysis redundant. Similar diagrams to Fig 5 would be valuable for oxidation and photolysis but so few data exist regarding these processes for pesticides that it is difficult to assess any prediction and virtually impossible to 'optimize and design' for these parameters.

For hydrolysis, however, linear free energy relationships of the Hammett type facilitate both prediction and design. Such approaches have been employed extensively in the last decade (Ginjaar and Hooidonk, 1967; Metcalf, 1971).

As stated earlier, oxidative and photolytic processes are too little understood for pesticides. It is of course appropriate to say that photolysis can be avoided by ensuring that the compound does not absorb light below about 300 nm. For some compounds photolysis is the major destructive mechanism and for compounds which are otherwise very stable it is a valuable mechanism to prevent long-term accumulation.

The other stability factor, that of thermal decomposition, is seldom operational in the field and is much more relevant to the manufacture of the compound.

Microbiological metabolism and destruction of the pesticide is another major loss mechanism, particularly in soil. It is again difficult to assess any approach to optimization in this area but it appears to be generally accepted that the presence of halogen inhibits microbiological attack upon a pesticide. No doubt this is a major feature of the success of halogenated hydrocarbons in soil.

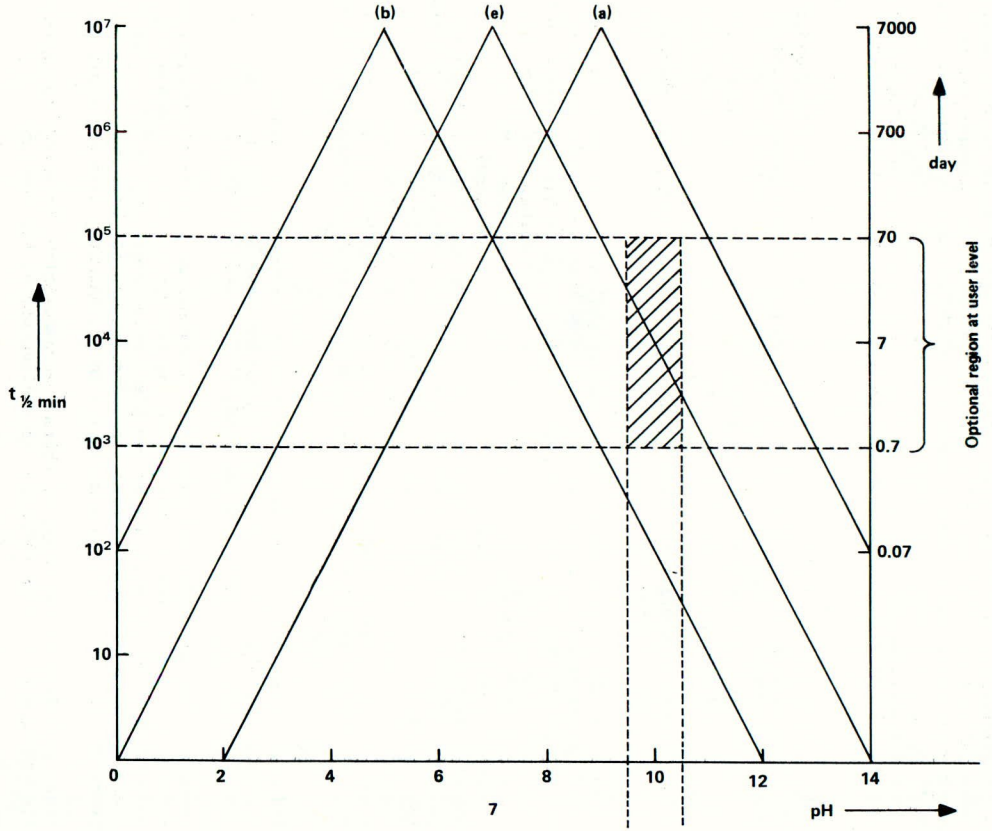

Fig 5 Acid and Base catalysed breakdown

In conclusion it is worth stating that any optimization process requires some model of the physical, chemical and biophysical features of a pesticide as a frame of reference (Sun, 1968). These however must be viewed in the context of the end user/field situation coupled with the particular crop/pest complex of the objective (Blau and Brock Neely, 1974). Considerable courage is required to pursue such aims during early research as the choice of sub-optimal compounds during the early studies will meet with considerable opposition and it takes several years to approach the ultimate justification.

Table 4

Relative Times for Movement of a Chemical by Diffusion Through Different Phases

Phase	Diffusion Coefficient $cm^2\ s^{-1}$	Time to move - Distance indicated	
		0.01 cm	0.1 cm
Gas	0.1	2.5×10^{-4}s	2.5×10^{-2}s
	0.01	2.5×10^{-3}s	2.5×10^{-1}s
Liquid	10^{-5}	2.5s	2.5×10^{2}s
	10^{-6}	25s	2.5×10^{3}s
Solid	10^{-10}	2.5×10^{5}s (3 days)	2.5×10^{7}s (300 days)
	10^{-12}	2.5×10^{7}s (300 days)	2.5×10^{9}s (30,000 days)

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Proceedings 8th British Insecticide and Fungicide Conference (1975)

APPROACHES TO THE RATIONAL DISCOVERY OF NEW PESTICIDES: THE ROLE OF BIOLOGICAL TESTS

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Summary The biologist contributes to the rational design of pesticides by identifying the properties they must possess to be commercially successful, and by devising appropriate tests. These tests must produce results quickly if the chemist is to use them to steer synthesis in an active series. They must sometimes give more precise data than a standard screen.

At present biochemical investigations and attempts to optimise known activity by correlating biological and physical properties are more likely to increase the cost of pesticide development than reduce it. They have not yet made pesticide research significantly less empirical, and the empirical approach is likely to remain predominant in the foreseeable future.

In this situation the cost of pesticide development can be reduced if the biologist targets his screens properly and eliminates unsuccessful chemicals at an early stage. This calls for a highly rational approach backed by considerable experience.

Resumé Le biologiste contribue au processus rationnel de la création des pesticides par l'identification des propriétés qu'ils doivent posséder pour être assurés d'un succès commercial, et par l'établissement de tests appropriés. Ces tests doivent produire des résultats rapides pour que le chimiste puisse les utiliser afin de conduire la synthèse vers une série active. Ils doivent quelquefois fournir des données plus précises que cells d'un triage standard.

Pour l'instant, les investigations bio-chimiques et les essais ayant pour but l'exploitation maximum de l'activité par corrélation des propriétés biologiques et physiques ont pour effet d'augmenter, plutôt que de diminuer, le coût du développement d'un pesticide. La recherche pour de nouveaux pesticides n'en est pas pour autant moins empirique, et cette approche empirique probablement prépondérante dans le futur immédiat.

Compte tenu de cette situation, le coût du développement d'un pesticide peut être réduit si le biologiste établit correctement l'objectif de ses triages et élimine au début des recherches les produits chimiques inadéquats. Ceci demande une approche éminemment rationnelle supportée par une experience considérable.

The Conference programme prints an introduction to this session that begins - "The ever increasing cost of pesticide development makes it essential to reduce as far as possible the degree of empiricism involved in the discovery of effective new pesticides." It is easy to agree that savings in expensive research and development programmes are highly desirable, but I do not believe that they alone supply the motive behind attempts to make pesticide research more rational. Scientists are offended if they are forced to rely on an empirical approach, and the desire to explain and so control events provides another strong motivating factor. It may even obscure the need to cut costs.

Indeed, the attempt to move away from empiricism towards the deliberate design of new pesticides has so far involved additional work and more, not less, expenditure on pesticide development. The rational approach has not yet proved itself sufficiently for there to have been a consequent cut back in established techniques of research or the number of chemists and biologists making and testing new chemicals. In the biochemical field studies of the metabolism of known pesticides have sometimes contributed to the design of new active molecules, though it is questionable how frequently they guide the chemist into areas he would not otherwise have entered relying only on informed guesswork. Few workers believe that the more basic work to understand the biochemistry of pests and plants will pay off consistently for a long time.

Techniques to optimise desirable properties in series of known activity by correlating physical and biochemical properties promise a shorter term pay-off, but it is undoubtedly costly to collect the data on which they rely. Data collection can also be time consuming, and the chemist may have covered much of the ground before guidance becomes available to him. Indeed it is arguable that optimisation procedures will prove to be of most use in reducing the risk of overlooking effective products, if they lie in difficult areas of chemistry, or for more trivial reasons, such as the non-availability of intermediates, that are quite likely to be significant in a practical situation.

This statement of opinion will show that I start my consideration of the approach to the more rational discovery of pesticides with a forthright acceptance of empirical screening as the dominant factor for the foreseeable future. To some this may seem a gloomy paradox. However, the design of new active molecules is only one part of the discovery process. The selection from among active chemicals of those likely to be a commercial success is equally important, and the efficient operation of selection procedures demands as much thought as making the chemicals themselves. The biologist's essential contribution to pesticide work lies in this area. Fortunately if he is successful he does have a good chance of cutting the cost of research and development immediately as well as at the same time improving the success rate. Furthermore by thinking carefully about selection procedures, he cannot avoid generating essential information for his colleagues trying to design new products deliberately.

The cost problems faced by the pesticide industry are commonly put down to increasingly stringent registration requirements which call for ever greater expenditure on programmes to demonstrate that new products are safe. Certainly expenditure of this type is important, but at bottom the industry's problems stem largely from its enormous success over the last thirty years. There are not now many crop/pest situations for which there is no treatment at all. Thus many new products meet established competition, and as an increasing number of pesticides lose their patent protection, the difficulty of launching new chemicals increases because existing treatments become more widely available at lower prices.

However there are still good opportunities to launch new products because the market requirements change. Thus resistance may develop, or regulations governing the use of products are rewritten, or integrated control programmes requiring more

selective chemicals become a possibility. But to take advantage of these opportunities it is imperative to understand what is happening in detail. The biologist in particular has to interpret the information from the changing market in terms of technical properties of special value to be sought in new products, and then he must target his screens accordingly. This calls for lengthy experience and an ability to deal rationally with a large quantity of sometimes conflicting information. The difficulties are increased because, ideally, one should know the market needs not now but in about five years time, to allow for the lag in developing a new product.

To an audience of this kind the characteristics to be looked for in new pesticides will be well enough known in general terms. They must be such as to offer the farmer a recognisable benefit. Thus a new pesticide might help him modify his management systems in a beneficial way, or simply give better control or lower costs than he can achieve now. The possibilities are many. For particular products one might seek

(1) Greater selectivity to assist integrated control programmes

or

Toxicity to a wide range of pests to obviate the need to apply two chemicals.

(2) Long persistence to bring phosphate and carbamate insecticides closer to the performance of organochlorines, especially against soil pests.

or

Short persistence if residues in the crop are a problem.

(3) Increased ability to penetrate and move within the plant.

(4) A particular pattern of dispersal in the soil, to eliminate the need for incorporation by cultivating.

This list could be much extended, but the examples given illustrate one very important difficulty for the biologist when he comes to apply a knowledge of market requirements to an optimisation approach intended to steer synthesis towards chemicals with particular characteristics. Some at least of the desirable properties - long persistence and movement within the plant or soil for instance - will by their nature tend to be demonstrable only by time consuming experiments. The collection of biological data can then lag considerably behind the rate of synthesis of new chemicals. Ideally, perhaps, the chemist should make a few compounds, wait for the data, and then make more. In practice he is unable to switch inspiration and interest on and off in just this way. He is likely to carry on his work without waiting for guidance, and either he will make more examples in an unsatisfactory part of an active series than are necessary, or he will by serendipity arrive at the same conclusion as his colleagues correlating physical properties with biological effects, but sooner. There is, therefore, a considerable onus on the biologist to produce results as quickly as he can.

As an example of what can be done in this respect I shall quote some work carried out by a colleague, Dr Claire Shephard. Dr Shephard wished to find out whether it was possible to make members of the pyrimidine group of fungicides to control powdery mildew on vines after application via the roots. One of the group, dimethirimol, which gave a poor control of vine mildew, was observed to cause necrosis along the leaf veins if applied at abnormally high concentrations. This same chemical controlled cucumber powdery mildew well; and in the cucumber, high concentrations caused interveinal necrosis, leaving the veins healthy. The difference in phytotoxicity pattern suggested that one factor associated with poor control of the disease on vine was a failure of the chemical to move in sufficient quantity from the veins into the leaf lamina.

A common way of measuring translocation in plants is to use radiolabelled chemicals. However, the cost of making a rather large number of labelled examples of a series is high, both in terms of time and money. It would certainly have delayed, if not negated altogether, any attempt to correlate translocation in the pyrimidine group with physical and chemical parameters. Dr Shephard therefore decided, in the face of some opposition from purist colleagues, to see whether phytotoxicity could be used as an indicator of the movement of other members of the group. She accepted the obvious difficulties of interpreting the data, in the hope that they would provide by a short cut a sufficient indication of new structures to be made.

Fortunately the results were clear and consistent with the view that three factors influenced ability to translocate to the leaf lamina - pK, partition coefficient in water/octanol, and water solubility. It was also clear that no member of the series made so far combined the properties required for both a high degree of fungicidal activity and good translocation; nor could any be predicted. However an attempt was made to synthesise a chemical which should have translocated well and then broken down to an active fungicide, but the preparation did not succeed. Nevertheless the relatively rapid and non-quantitative biological assessment of translocation was at least useful in showing that a further extensive programme of speculative synthesis in the pyrimidine group was unlikely to be worthwhile.

It is worth emphasising that a kind of optimisation process based on acute observation and memory rather than quantitative measurements is also commonly associated with routine screening. It requires a high degree of experience and imagination in the biologist in charge, who must spot useful combinations of properties at far from their optimal level on very scanty evidence. If he can see trends in these properties, sometimes over a longish period of time, the chemist may be helped considerably. Screen data may be stored on magnetic tape for easy access, but the inventive connections must still be made in the minds of the chemist and biologist who look at results week by week.

Nevertheless quantitative measurements of biological properties of a kind not easily obtained during screening are frequently essential if desirable traits are to be optimised. An example from the herbicide field, supplied by Dr Alan Hawkins, will show how careful measurements revealed possibilities in a chemical series that the screens did not.

Dr Hawkins was working with a series of herbicides which were grass killers, but which failed to show any worthwhile degree of selectivity between barley and wild oat with the rates and application methods being used for screening. He therefore set up special additional tests. Plants were grown in nutrient solution in which the herbicides were dissolved at a number of concentrations. The dose response curves for weed and crop were nearly parallel, and therefore the ratio of LD50s gave a good measure of selectivity.

Activity against the wild oat was then plotted against this selectivity ratio, and three groups of chemicals became apparent -

Group 1. High activity, poor selectivity. Phytotoxic symptom - chlorosis.

Group 2. Low activity, poor selectivity. Phytotoxic symptom - stunt.

Group 3. Moderately low activity, good selectivity. Stunt and chlorosis.

Thus the change of technique and more precise observations provided a basis for an attempt to make wild oat herbicides by optimising activity in group 3 while retaining selectivity. Unfortunately in the event this failed because the necessary correlations with physical and chemical properties could not be established.

Having now shown how the biologist can contribute to the rational design of new pesticides, I shall end by re-emphasising my main point: this is not the only or even the most effective way to apply intelligence to pesticide research in the hope of making it more cost-effective. Success on this score is more likely to stem from improvements to the procedures by which new products are picked for development, and at least in ICI's case the scope for savings here is very worthwhile. If we consider just that part of the R&D budget devoted to discovering new products and bringing them to the market, very approximately

30% is spent on finding active molecules; that is on chemical synthesis and testing for pesticidal activity, and on associated biochemical and physico-chemical backup

30% is spent on selecting from the active molecules those with possible commercial potential

40% is spent on confirming this promise and in obtaining the information needed to sell the few real successes.

For practical purposes an effective new product cannot be said to have been discovered until part way through the third of these stages, so the value of making the selection process as efficient as possible is readily apparent. On the whole, although everyone recognises that it is becoming harder to launch a new product, the supply to the screens of chemicals with some degree of biological activity remains reasonably high. So providing he does not overlook the really novel, and therefore potentially very valuable type of activity, the biologist is giving the chemist good service simply by directing his attention away from unprofitable work in chemical series with the wrong characteristics to fit the market.