# DEVELOPING STRATEGIES FOR HERBICIDE DESIGN

J.L. Huppatz
CSIRO Division of Plant Industry, GPO Box 1600
Canberra A.C.T. 2601

*Summary.* The concepts used in the design of herbicides are changing with the elucidation of particular molecular targets for herbicide action. The traditional empirical approach to herbicide discovery, the screening of large numbers of new chemical compounds, will gradually give way to a more directed approach based on a biochemical knowledge of target enzymes of proteins. Two metabolic pathways unique to the plant kingdom are of particular interest, viz., photosynthesis and the biosynthesis of essential amino acids. Modern techniques of molecular biology, X-ray crystallography and computer modelling are being utilized to study target sites in these pathways to ultimately provide a more rational basis for herbicide design.

## INTRODUCTION

The use of herbicides has become an integral and increasing part of modern agricultural practice. Despite significant recent additions to the range of available herbicides and increasing sophistication in their use, the discovery of new weed control agents combining high potency, particular selectivity, and environmental acceptability remains an attractive goal for the agrochemical industry.

In the past, the discovery of herbicides has arisen empirically from innovative synthetic chemistry coupled to biological screens designed to detect appropriate activity and selectivity. The increasing costs involved in the synthesis and evaluation of tens of thousands of compounds and the extensive toxicological and regulatory requirements for registration of a new product have forced the agrochemical industry to examine alternative approaches to herbicide design.

Ideally, a herbicide should control weeds with minimal toxicity to other organisms. This implies the specific inhibition of metabolic pathways unique to plants; photosynthesis and essential amino acid biosynthesis are obvious examples. However, to effectively design herbicidal molecules using such a biochemical rationale requires a detailed knowledge of a particular biological site or process at the molecular level, and such information has only recently begun to emerge.

It is not surprising, then, that the majority of new products reaching the market-place are still the result of chance observation or, increasingly, the result of detailed focus on known herbicide classes to produce new compounds of superior activity and/or selectivity. Modern techniques, such as computer-assisted molecular design and QSAR (quantitative structure-activity relationships), have greatly assisted the latter approach to the discovery and optimization of herbicidal activity.

However, in certain instances, particular targets for herbicide action in key metabolic pathways have been identified and are currently the subject of intense investigation. This article will concentrate on developments in two such areas, photosynthesis and essential amino acid biosynthesis, with particular emphasis on their significance for future herbicide design.

## PHOTOSYNTHESIS

Herbicides can interfere with the photosynthetic process in plants by a number of different mechanisms, but by far the largest group of photosynthetic herbicides owe their toxicity to the ability to inhibit photosynthetic electron transport near photosystem II (PSII) (2). Such compounds include the economically important urea (diuron, linuron), triazine (atrazine, simazine), triazinone (metribuzin), and uracil (bromacil) classes of herbicides and a new class of highly potent PSII inhibitors, the cyanoacrylates, which have proved particularly useful in probing the architecture of the PSII herbicide binding site (4). Recent research has shown that these compounds, despite profound differences in their chemistry, all bind to a 32 kD protein (the $Q_B$ protein) in the PSII complex of chloroplasts (2). The study of mutants resistant to atrazine and the biochemical and biophysical characteristics of photosynthetic electron flow has led to a clearer understanding of the nature and function of the $Q_B$ protein. Recently, an X-ray crystallographic structural determination of the PSII reaction centre in photosynthetic bacteria has been carried out (1), enabling a detailed model of the corresponding system in higher plants to be proposed (11). A tentative picture of the three-dimensional architecture of the herbicide binding niche can be deduced from this model. A complementary study of the structural requirements for activity of the various classes of PSII inhibitors and their orientation on the binding site should enable the "lock and key" features of the model to be refined and, ultimately, lead to the design of new herbicides of this type.

## AMINO ACID BIOSYNTHESIS

Recently, the molecular targets of two important herbicide types, which interfere with essential amino acid biosynthesis, were established. Glyphosate inhibits a critical enzyme, 5-enolpyruvyl-shikimate-3-phosphoric acid (EPSP) synthase, required for the synthesis of aromatic amino acids, tryptophan, tyrosine and phenylalanine (10). The sulfonylurea herbicides (e.g. chlorsulfuron) also interfere with amino acid biosynthesis, though, in this case, the target is acetohydroxy acid synthase (AHAS), the first common enzyme in the biosynthetic pathways to the branched-chain amino acids, leucine, valine and isoleucine (6, 7). A second class of herbicides, the imidazolinones (e.g. imazaquin), also inhibit this enzyme (9) but are less potent, both *in vitro* and as herbicides in the field, than the sulfonylureas.

Both types of amino acid biosynthetic inhibitors represent major advances in weed control technology, and have profoundly influenced the structuring of research programs aimed at herbicide discovery. The identification of enzyme targets has allowed strategies directed towards the synthesis of specific enzyme inhibitors. Assays involving enzyme targets are being used for rapid optimization of activity *in vitro*. Alternative approaches to inhibitor design based on the biochemical properties of known target enzymes are being investigated. For example, new inhibitors of AHAS have been designed to specifically interfere with the feed-back mechanisms regulating enzyme function (3).

Furthermore, the study of enzyme targets in amino acid biosynthesis has been greatly aided by the use of readily manipulatable microbial systems and by recombinant DNA and gene cloning technology (5). Purified AHAS, obtainable in gram quantities by these techniques (8), should provide the starting point for full biochemical characterization and determination of a complete three-dimensional structure by X-ray crystallography. As with the PSII herbicide binding site, such information may provide a basis for the design of novel and useful herbicides.

## CONCLUSION

Although most herbicides in current use are the product of the empirical synthesis-screen approach, the strategies being used to develop new weed control agents are rapidly evolving. The discovery of high potency herbicides giving effective control at a few grams per hectare and the identification and understanding of their site of action at the molecular level in plant biochemical systems opens the way for more efficient design and evaluation of potential crop protection chemicals. Although the prospect of tailoring a specific chemical for a particular biochemical target site is still well into the future, the very fact that useful targets have been identified makes possible a much more directed approach. Aided by computer modelling and QSAR techniques, the chemist is able to concentrate effort on a known target, thereby enhancing the prospects of new herbicidal discovery.

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