Chapter 1

Assessment of herbicide effects

by Jens C. Streibig

Prerequisite knowledge Before starting this topic you should:

1. Understand basic plant physiology
2. Understand basic chemistry

Learning objectives At the end of this topic you should be able to:

1. Unambiguously define and quantify herbicide selectivity
2. Define and use selectivity in weed management
3. Understand the basic causes of selectivity in plants
4. Understand the use of adjuvants
5. Understand the most common way of assessing joint action of herbicide mixtures

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1.1 Introduction

Herbicides are unique in that they are designed to kill plants. Sufficiently high doses will kill both crop and weed, while small doses have no effect upon crop and weed.

The action of a herbicide is usually determined by its chemical and physical properties, its effect on plant metabolism, the plant and the environment.

The purpose of this chapter is to give an overview of the basic principles of how to assess herbicide selectivity and the causes for selectivity.

1.2 Short history of chemical control

For more than 7 millennia, human beings have changed the original vegetation by growing crops in monoculture. During this period the prevailing weed control methods have been relatively static. Even at present, hand weeding is still the most important means of control in many parts of the world, where agriculture depends on an army of professional hand weoders.

Real changes in control methods have only taken place over the last 200 to 300 years; for example agricultural implements to lessen the burden of hand weeding. Radical changes in the weed control methods in the developed world, however, only happened during the last 40 to 50 years when more than 200 chemical compounds for weed control have been made commercially available to agriculture.

In 1896 a French wine grower sprayed CuSO$_4$+CaO to control diseases on his vine. He also observed a beneficial side effect in that a weed, *Sinapis arvensis* L., exposed to the spray died. One year later another French grower discovered the herbicidal activity of H$_2$SO$_4$ that could selectively control some weeds without injuring the crops. These discoveries were in fact some of the first successful chemical weed control methods. Until recently, H$_2$SO$_4$ was used as a herbicide in many parts of Europe. Several other inorganic compounds were introduced for a shorter period. The first organic herbicide, DNOC, was patented by George Truffaut and K. Pastac in 1932 for use as a selective herbicide in cereals.

The rapid development of modern herbicides, experienced over the last 50 years, was stimulated by the need for increasing food production during World War II. The shortage of food in the UK made agricultural researchers take advantage of previous discoveries of auxins. Auxins are growth substances that increase plant growth, primarily by promoting cell elongation. Unknown to each other, workers at Jealott’s Hill Research Station and Rothamstead Experimental Station studied the possibilities to boost crop production with putative synthetic auxins, chlorinated phenoxyacetic acids. The researchers were capable of realizing when novel results were serendipitous. Both teams reported remarkable herbicidal activity. The substances appeared to act like an over-dose of auxin on the weeds, but did not harm the cereal crop.

With the return of peace the original findings, together with experiences of independent work from the USA, were published (Hance & Holly, 1990). The phenoxyacetic acids were fundamentally different from the other organic compounds, for example DNOC; they were
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systemic in that they had to be translocated in the plant to exert their action. Soon after the release of MCPA and 2,4-D, 2,4,5-T was also released and a new era of weed control began.

Within a few years, screening tests of literally thousands of putative herbicidal substances were set up by chemical companies and formed the basis for rapid development of new herbicides to control specific weed problems in specific crops. New important herbicide groups were developed. The s-triazines were reported to have unique effects compared with existing classes of compounds in 1955 (Esser et al., 1975), and three years before some substituted ureas were found to be potent inhibitors of photosynthesis (Geissbühler et al., 1975). For many years about half the commercialized herbicides were photosynthetic inhibitors.

But the era of the photosynthetic inhibitors has come to a close, Even though they are still important, the herbicides inhibiting the synthesis of various amino acids are now on a world wide basis the most important group of compounds.

In 1971 glyphosate, a competitive inhibitor of an enzyme of the shikimic acid pathway, was developed as a herbicide, an this compound has played a pivotal role in changing the market of for herbicides.

Perhaps at no other time in the history of herbicide research has so much knowledge been accumulated about a new group of herbicides in such a short time as that for the sulfonylureas (Beyer et al., 1988). Only a few years after their discovery, the site of action was found to be inhibition of the enzyme acetolactate synthase that forms a part of the combined pathway responsible for the biosynthesis of valine, leucine and isoleucine. The absence of this enzyme in man and other animals helps explain the low toxicity of the sulfonylureas. Because of their specific site of action and variety in molecular structures, sulfonylureas are potential candidates for tailoring chemistry to fit the crops. This specificity has also resulted in the unintentional development of sulfonylurea resistant biotypes of weeds after only a few years of use.

In principle, screening systems for new herbicide molecules have not changed much since the end of World War II. The chemical companies still screen thousands of molecules, in a laborious random empirical screening process, before a commercially viable compound is marketed. Today, random screening is still used but in conjunction with other strategies, for example analogue synthesis by linking together molecules of known herbicidal activity.

With the advent of the automation and small-scale technology, it is now possible with high throughput synthesis and screening to discover active chemicals for weed control. A common genomic technique for discovery of new target sites is to use gene inactivation to identify sites whose absence will be lethal or make a plant uncompetitive. Protein targets arising from these inactivated genes can be identified and the gene is cloned in a surrogate organism (e.g. bacterium), where the gene products can be detected. This work is not easy as a chemical inhibitor of the protein now has to be identified, which requires screening with chemical libraries that may contain 10,000 to a million compounds.

There are, however, some severe limitations to this technique. Too often there is a low correlation between the ability to inhibit a target and the ability to kill a plant. This is caused by the many impediments herbicides meet along the way, e.g., absorption through cuticle or roots, translocation to the growing tissues and penetration of organelles in which
it exerts its action. Several herbicides are pro-herbicides that are more or less biologically inert when taken up by the plant, but when inside the plant they are metabolized to active compounds. It is difficult to identify a pro-herbicide in an enzyme based high throughput screening. Since little has been done on characterization of the enzymes converting pro-herbicides to active compounds within the plant there seems to be a lot of hard research to be done to design high throughput screenings which will identify pro-herbicides (Gressel, 2002).

After a compound has been identified as being biologically active, be it by classical random or by modern high throughput screening, extensive bioassay protocols in the greenhouse and field or on tissue culture in the laboratory are used to explore its phytotoxicity and selectivity to several crop and weed species. There are many existing herbicides which would be useful in a given crop but for the minor problem that they also kill the crop.

Over the years the costs of developing new herbicides have increased dramatically and are now approximately 200,000,000 EURO of which 30% are spent on toxicological and ecotoxicological research. It takes about eight years from discovery to commercialization (Lindemark, 2003, personal communication).

The rational design of novel herbicides with desired physical, chemical and biological properties has long been the dream of many agrochemical companies. Until recently, this task could only be achieved by screening literally thousands of chemicals for the very few that would have the desired properties. As the years go by, larger and larger numbers of chemicals have to be screened to discover a more efficacious herbicide than obtained previously. The screening efforts today remind us of the infamous monkey who, given a computer with a word processor and sufficient time, could reproduce the works of H.C. Andersen, along with mountains of gibberish.

Incidentally, the discovery of herbicide resistant biotypes of formerly susceptible species has challenged many chemical companies to use this discovery for their own benefit. Concomitantly, the amount of information on mode of action for many groups of herbicides has increased rapidly in the past few years. As many herbicides only have one primary site of action, and for some, the herbicide-resistance gene determinants are single dominant traits, it makes them amenable to gene transfer techniques. This fact may open up a new productive area of research. The interest in genetically modified crops that can tolerate herbicides with satisfactory ecotoxicological properties has increased considerably over the past few years.

On a world scale Herbicide Resistant Crops constituted 85% of the total area of 52.5 million ha grown with Genetic Modified crops in 2001. While the Herbicide Resistant Crops grown in 2001 only provided benefits in terms of reduced herbicide costs, it is anticipated that some future Herbicide Resistant Crops will also increase yields because weed control will become more effective (Kudsk & Streibig, 2003).

The array of new herbicides has given the farmer a safety factor in growing crops and has to a certain extend also made him less dependent of earlier times crop rotation and fallow systems, partly implemented to cope with noxious weeds. Today, the farmer can ‘weed’ unwanted plants chemically and within certain limits be able to grow crops demanded by consumers without too many crop rotation restrictions. This development, however, has produced some unintentional side-effects, agriculturally and ecotoxicologically, that were
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not thought of during the earlier years of herbicide use.

1.3 The herbicides

More than 200 compounds with herbicidal properties are commercially available worldwide. They can be classified in multifarious ways, such as by chemical structure, method of use, mode of action etc.

Classification on the basis of common chemical functional groups can give an overview of the chemical and physical properties of related compounds such as pK\textsubscript{a}, water solubility and vapour pressure. These characteristics are important both from an agronomic and ecotoxicological point of view. They can give us some indication of the behaviour of compounds in plants and the environment. Water solubility and vapour pressure tell us how the compounds should be formulated most efficiently, and whether there is any risk of vaporization after application. The pK\textsubscript{a} and chemical structure are particularly important when we want to assess the behaviour of a herbicide in the soil.

Table 1.1 clearly shows great variation in water solubility, vapour pressure and pK\textsubscript{a} and biological activity of some selected herbicides. Even within the same chemical group, the physical and chemical properties may vary substantially. For some compounds, for example the sulfonylureas and triazines, pK\textsubscript{a} is essential when we wish to understand their behaviour in the soil. In some cases, a compound is classified differently by various authors, because the common functional group is not the most senior in IUPAC (International Union of Pure and Applied Chemists) terms. According to IUPAC, sulfonylureas with carboxyl groups are classified as benzoic acids, while those without are named as benzenesulfonylureas. On the basis of chemical and biological properties, e.g. similar mechanism of action, it is clearly advantageous to group them together. The indication in Table 1.1 about selectivity, use, etc. only gives an approximate guide to their main activity.

There are other important criteria for classification of herbicides with reference to their use and action in plants.

Table 1.2 gives some important criteria for classification of herbicides with reference to their use. These categories are by no means rigidly distinct; many foliage-applied herbicides have significant soil activity, and the balance between activities can often be shifted by the size of the dose, formulations and adjuvants. The term contact herbicide in Table 1.2 must not be taken literally, some transport in the plant following uptake is of course a prerequisite. For contact herbicides, however, transport is not directly related to long distance transport in xylem and/or phloem from the site of uptake. Some herbicides, for example paraquat and diquat, are easily translocated in the absence of light under controlled conditions and sometimes in the field. Such transport is of minor consequence for their phytotoxicity in the field, because they act rapidly in full sunlight with a consequent lack of translocation out of treated leaves (Fedtke, 1982).

The criteria of classification in Table 1.2 do not mean that these criteria themselves are independent. For example the degree of selectivity is very much dependent upon the time and method of application. This can be illustrated in Figure 1.1. If the selectivity is
Table 1.1: Herbicides grouped on the basis of common chemical functional groups. S=selective; NS=non-selective; F=foliar activity; So=soil activity; C=contact action and T=Translocated (Modified from Hance & Holly, 1990).

<table>
<thead>
<tr>
<th>Name</th>
<th>Mol. wt.</th>
<th>pKₐ</th>
<th>W. sol. ppm</th>
<th>vapour pres. µPa</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>2-(aryloxy)alkanoic acids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCPA</td>
<td>200.6</td>
<td>3.1</td>
<td>875</td>
<td>0.200</td>
<td>S,F,T</td>
</tr>
<tr>
<td>2,4-D</td>
<td>221</td>
<td>2.6</td>
<td>620</td>
<td>53·10⁶</td>
<td>S,F,T</td>
</tr>
<tr>
<td>MCPB</td>
<td>228.7</td>
<td>4.8</td>
<td>44</td>
<td></td>
<td>S,F,T</td>
</tr>
<tr>
<td><strong>Arylcarboxylic acids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dicamba</td>
<td>221.0</td>
<td></td>
<td>6500</td>
<td>45</td>
<td>S,F,So,T</td>
</tr>
<tr>
<td><strong>Esters of aryloxy-phenoxyalkanoic acids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diclofop-methyl</td>
<td>341.2</td>
<td>3</td>
<td>3</td>
<td></td>
<td>S,F,So,T</td>
</tr>
<tr>
<td>Fluazifop-butyl</td>
<td>383.4</td>
<td>1</td>
<td>55</td>
<td></td>
<td>S,F,So,T</td>
</tr>
<tr>
<td><strong>Nitriles</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ioxynil</td>
<td>370.9</td>
<td>4.0</td>
<td>50</td>
<td></td>
<td>S,F,C</td>
</tr>
<tr>
<td>Dichlobenil</td>
<td>172.0</td>
<td>18</td>
<td>67·10⁶</td>
<td></td>
<td>S,So,T</td>
</tr>
<tr>
<td><strong>Amides</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propyzamide</td>
<td>256.1</td>
<td>15</td>
<td>11·10⁶</td>
<td></td>
<td>S,So</td>
</tr>
<tr>
<td><strong>Anilides</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diflufenican</td>
<td>394.3</td>
<td>0.05</td>
<td></td>
<td></td>
<td>S,F,T</td>
</tr>
<tr>
<td>Propanil</td>
<td>218.1</td>
<td>225</td>
<td>12·10⁶</td>
<td></td>
<td>S,F,So,C</td>
</tr>
<tr>
<td><strong>Sulfonyleureas</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorsulfuron</td>
<td>357.8</td>
<td>3.6</td>
<td>60–7000</td>
<td>3.1·10⁻³</td>
<td>S,F,So,T</td>
</tr>
<tr>
<td>Metsulfuron-methyl</td>
<td>381.4</td>
<td>3.3</td>
<td>1100–9500</td>
<td>332·10⁻⁶</td>
<td>S,F,So,T</td>
</tr>
<tr>
<td>Thiameturon</td>
<td>387.4</td>
<td>4.0</td>
<td>260–2400</td>
<td>173·10⁻³</td>
<td>S,F,So,T</td>
</tr>
<tr>
<td><strong>Organophosphorus compounds</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glufosinate (Ammonium)</td>
<td>198.2</td>
<td>Very high</td>
<td>Low</td>
<td>NS,F,C</td>
<td></td>
</tr>
<tr>
<td>Glyphosate (Ammonium)</td>
<td>169.1</td>
<td>–</td>
<td>12·10⁶</td>
<td></td>
<td>NS,F,T</td>
</tr>
</tbody>
</table>

\(^a\) pH=5–7
Table 1.2: Possible criteria for classification of herbicides with reference to their use and action on plants.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degree of selectivity</td>
<td>Selective</td>
</tr>
<tr>
<td></td>
<td>Non-selective</td>
</tr>
<tr>
<td>Time of application</td>
<td>Pre-sowing</td>
</tr>
<tr>
<td></td>
<td>Pre-emergence</td>
</tr>
<tr>
<td></td>
<td>Post-emergence</td>
</tr>
<tr>
<td>Method of application</td>
<td>Foliage</td>
</tr>
<tr>
<td></td>
<td>Soil</td>
</tr>
<tr>
<td>Translocation in plants</td>
<td>Systemic</td>
</tr>
<tr>
<td></td>
<td>Contact</td>
</tr>
<tr>
<td>Mechanism of action</td>
<td>Photosynthesis</td>
</tr>
<tr>
<td></td>
<td>Auxin action</td>
</tr>
<tr>
<td></td>
<td>Amino acid metabolism</td>
</tr>
<tr>
<td></td>
<td>Microtubules</td>
</tr>
<tr>
<td></td>
<td>Lipid metabolism</td>
</tr>
</tbody>
</table>
Figure 1.1: How herbicides may be used for weed control in crops (Redrawn after Hance & Holly, 1990).
caused by physical separation between herbicide and crop (e.g. Directed and Overall Pre-emergence in Figure 1.1), we denote this apparent selectivity, whereas post-emergence soil and foliage acting herbicides, which hit both crop and weeds, exhibit genuine selectivity. Again categories are not rigidly distinct; many alleged foliage-applied herbicides have significant soil activity, and the balance between activities can often be shifted by dose rates, formulations and adjuvants.

For practical use in crops, we usually differentiate between selective and non-selective herbicides (Table 1.2). Selective herbicides are used in dose rates which adequately control the weeds without seriously affecting the crop. Thus the distinction between selective and non-selective herbicides is a question of dose rates in the field and the old axiom of Paracelsus applies:

"Was ist das nit Gifft ist:
alle Ding sind Gifft und nichts ohn Gifft.
Allein die Dosis macht das ein Ding kein Gifft ist." ¹

Paracelsus (1494–1541)

Consequently, basic studies of selectivity, all other things being equal, require knowledge of the relationship between dose and plant response from no effect at small doses, to complete kill at high doses, such as in Figure 1.2.

Dose-response curves are useful to describe herbicide action and selectivity in plants in relation to conditions at the time of application, climate etc.

With selective herbicides, two areas of the response curve are of interest. The upper limit at small doses illustrates crop tolerance, that is, the crop is only marginally affected by the herbicides; the lower limit at high doses illustrates the control of a weed (Figure 1.2). The middle steep part of the curve is, however, of scientific interest in that it illustrates the so-called intrinsic activity of the herbicides in the plant. An important way to illustrate differences of effects among herbicides is to use $ED_{50}$, which is the dose required to affect plant response 50% relative to the upper and lower limit.

1.4 The plant

When assessing the effect of a herbicide on plants it is important to know the stage of development, state of health, nutritional status and the genetic make-up of the plant as well as cultivation practices and the climate. Generally, we get the best effect of herbicides if they are applied when plants are either rapidly growing or are weakened by rapid growth, which temporarily depletes or exhausts their reserves. It means that plants in the germination and early-seedling stage are likely to be severely damaged by herbicides. This applies to both weeds and crops, and therefore the timing of application

¹Which substances are not poison: All substances are poisonous and none are without poison. It is the dose that makes a substance non-poisonous.
Figure 1.2: Top: logistic dose-response curve describing plant response, e.g. biomass, against the dose. The measurement of crop tolerance is in the area of the upper limit and the measurement of weed control is in the area of the lower limit. The dose required to affect the response 50%, often denoted $ED_{50}$, is commonly used to compare the general effect of herbicides on plants. Bottom: the log-logistic dose-response curve describing plant response, e.g. biomass, against logarithm of the dose (Streibig et al., 1993).
is crucial for the effect of many herbicides. For many perennial weeds, the most sensitive stage is when new shoots are still in a young stage and their development has depleted the reserves of nutrients of the root system.

Knowledge of the most sensitive stages of plant development to herbicides opens up for selectively controlling target weeds without serious injuring crop plants. For the majority of foliage-applied herbicides, the current advice is to spray when the weeds are small and susceptible, preferably between cotyledon stage and two to four true leaf stage. If the development of weeds is beyond these stages, we need more herbicide with a consequent drop in selectivity and increase in contamination of the environment.

1.5 Herbicide action and selectivity

Although selectivity does not necessarily associate with the mode of action of a herbicide or its use in the field, a brief outline of mode of action, mechanism of action and site of action is pertinent in order to understand the toxicity of herbicides. A more detailed description of this subject has been given by Cobb (1992) and Devine et al. (1993). In the present context, mode of action refers to the whole sequence of events in a plant, exposed to a herbicide, from contact with the herbicide and until the plant dies. The more specific term, mechanism of action refers to the physiological and biochemical changes obtained from in vitro experiments. The information of application, sensitive tissue and growth stage will usually be available at an early stage of herbicide development and allow herbicides to be classified as: germination inhibitors, contact herbicides, bleaching herbicides, desiccants, hormone killers, etc. (Table 1.3). The physiological answer to the mechanism of action aims at a particular pathway such as photosynthesis, respiration, carotenoid biosynthesis, mitosis, etc. The site of action refers to the enzyme or enzyme systems being inhibited. One of the reasons for studying the mechanism and site of action of herbicides is to understand why and how plants are damaged; but to answer this question on the molecular level has often proved difficult.

Table 1.3: Information on the effects of herbicides obtained from different types of test systems. (From Fedtke, 1982).

<table>
<thead>
<tr>
<th>Source of information</th>
<th>Type of information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application technique</td>
<td>Sensitive growth phase</td>
</tr>
<tr>
<td>Damaged tissue</td>
<td>Sensitive cell types</td>
</tr>
<tr>
<td>Cytological</td>
<td>Cellular and subcellular actions</td>
</tr>
<tr>
<td>Physiological</td>
<td>In vivo inhibited metabolic pathways</td>
</tr>
<tr>
<td>Biochemical</td>
<td>In vitro inhibited metabolic pathways, effect on biochemical composition</td>
</tr>
</tbody>
</table>

Based on all the events taking place in the plant, the action of a herbicide cannot be assessed on the basis of uptake, translocation and metabolism alone. Interactions make it difficult to evaluate the unambiguous cause and effect relationship. No doubt, however,
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metabolism of herbicides in plant species is an important factor. For example, herbicides inhibiting the electron flow from photosystem II to photosystem I are equally active on isolated chloroplasts from susceptible or tolerant species. The selectivity is usually based on metabolism of the herbicides to non-toxic compounds before they reach their site of action. The same applies to many other groups of herbicides. In some instances, the physical separation of the compound and the site of uptake in the plant may explain selectivity, as shown for the directed sprayings in Figure 1.1 (page 10). Well-known examples are the spraying of contact herbicide, for example paraquat, and glyphosate before crop emergence. Another example is the selectivity of soil applied diallate, triallate and propham against Avena fatua L.; the location of the site of uptake in the plant in relation to the placement of the herbicide controls selectivity.

In herbicide research we distinguish between various levels of complexity. The description of dose-response relationships for all system levels in Table 1.4 provides a better understanding of the intrinsic action and selectivity of a compound. In some instances, metabolic studies can explain most of its selective nature at dose ranges used in the field (e.g. s-triazines, sulfonylureas). In other instances there are too many unknown factors, which makes it virtually impossible to explain selectivity. The worst case is with herbicides having several sites of action and where absorption and translocation form an integral part of the whole picture. This is the case for the auxin herbicides. When we are able to quantify the delicate balance between dose, uptake, translocation and metabolism we will also be able to understand the real nature of selectivity.

Table 1.4: Classification of various systems to study herbicide action and selectivity (Fedtke, 1982).

<table>
<thead>
<tr>
<th>System</th>
<th>Data relevance</th>
<th>Type of experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Super complex</td>
<td>Growth related</td>
<td>Anatomy, morphology, dry matter production</td>
</tr>
<tr>
<td>Complex</td>
<td>Metabolism</td>
<td>Tracer studies, gas exchange, cell component studies, cell and tissue culture</td>
</tr>
<tr>
<td>Defined</td>
<td>Site related</td>
<td>In vitro studies with enzymes or organelles, binding studies</td>
</tr>
</tbody>
</table>

“In order to obtain simple systems, suitable for a more direct, ‘exact’ approach, the living organism has to be disintegrated. The further this disintegration goes, the more ‘exact’ will be our approach, but less life will remain in the biological system. As soon as we really approach exactitude, life will be gone.”

Ariëns, Simonis & Rossum (1964).
1.6 Assessment of effects with dose-response curves

As shown in Figure 1.2 (page 12) the difference between tolerance and control of a plant is determined by the size of the dose. The term ‘size of a dose’, however, is rather vague in that for some herbicides, only few g ha$^{-1}$ are needed to control weeds (e.g., many sulfonylureas) whereas for others we must apply several kg to obtain the same level of control (e.g., phenoxy acids). The consequence is that if we want to determine the potency or selectivity of a herbicide it is not enough only to look at one dose-response curve, as the proper assessment of selectivity should be stated in relative terms depending on the herbicide, crops and weeds in question. In order to avoid ambiguity in assessment of selectivity the dose-response curves are a good starting point (Streibig, 1992).

If we want to compare the phytotoxicity of two or more herbicides on the same plant species or maybe the selectivity of a herbicide upon a crop and a weed species, then we must compare several dose-response curves simultaneously and quantify our findings in relative terms. We must define a standard herbicide or a standard species. In principle there are two ways of assessing the action of herbicides and both can be illustrated by comparing dose-response curves. To make things simple we assume in the next two subsections that we compare the action of two herbicides on the same plant species.

1.6.1 Vertical assessment

Vertical assessment compares plant response at some preset dose levels (Figure 1.3, top). This is the most common method for evaluating herbicides in the field. If doses were chosen close to the upper or lower limit of the curves, differences between treatments would be less than if they were chosen in the middle part of the curve. If we are only working at dose-ranges in the middle part of the curves then, in this particular instance, differences would be almost independent of dose-levels. Consequently, the middle region is obviously the optimal part of the curve to obtain information about differences of effects.

The principle of vertical assessment is a common method to evaluate herbicides in the field. Usually herbicides are tested in two to three doses and their efficacy is compared with either the untreated control or with some standard herbicide treatment. Very rarely do we have so many doses in the field that we actually are able to draw the entire dose-response curve.

The nonlinear relationships in Figure 1.3, top, show that if a herbicide is tested with or without an adjuvant in a factorial experiment, we may get significant interactions, because the differences of effects are not constant. As this interaction is dose dependent due to the $S$-shaped curves, it may be considered trivial and of little biological significance when we appraise the action of the herbicide. If we choose only dose-ranges in the middle part of the curves, then differences are constant and independent of dose-levels and the interaction would disappear, i.e. the effects are additive.
Figure 1.3: Vertical (top, a) and horizontal (bottom, b) comparison of dose-response curves for two herbicides on a plant species.
1.6.2 Horizontal assessment

Doses of two herbicides giving the same response can also be compared (Figure 1.3, bottom). In this case the difference in horizontal displacement of the two curves is important. As the dose rates are on a logarithmic scale, the horizontal displacement expresses the ratio between doses yielding the same response. This ratio is also called the relative potency or the relative strength, \( R \), and can be defined as

\[
R = \frac{z_1}{z_2}
\]

(1.1)

where \( z_1 \) and \( z_2 \) denote the dose of two herbicides (\( H_1 \) and \( H_2 \)) having the same effect (equipotent doses). The relative potency tells us how much more or less test herbicide must be used to obtain the same effect as for dose \( z_1 \), for a standard herbicide. If \( R=1 \) then the two herbicides have the same potency; if \( R > 1 \) then the test herbicide, \( H_2 \), is more potent than the standard herbicide, \( H_1 \), and if \( R < 1 \) then the standard herbicide is more potent than the test herbicide.

We could consider the relative potency as being a measure of the biological exchange rate between herbicides, analogous to the more common practice of exchanging currencies from say Euro to Danish Kroner (DKK). With herbicides, for example, we know that to get adequate control of a common weed flora we need 4 g a.i. (Active Ingredients) of the test herbicide metsulfuron-methyl or 1000 g a.i. of the standard herbicide 2,4-D; then the relative potency, \( r \), between the two herbicides is 250 (1000/4). That means that if we want to substitute 2,4-D with metsulfuron-methyl we need 250 times less herbicides to get the same effect.

From Figure 1.3 it is obvious that this relative displacement of curves seems rather constant in the middle part of the curves. Furthermore, if the dose-response curves are having the same upper and lower limit and the same slope (see Section 1.10) they are said to be similar or parallel, which, from the point of view of ease of interpretation, implies that the relative potency is independent of the response levels considered.

This way of assessing the biological exchange rate between herbicides can also be used when we wish to find out how formulations or adjuvants affect the action of a herbicide. Adjuvants may either enhance or detract the action of a given herbicide. By assessing how an adjuvant affects a herbicide in this way, answers the question many farmers ask: “If I add an adjuvant to my spray, how much herbicide can I save without losing efficacy in relation to the recommended dose rate of the herbicides without adjuvant?”

A straightforward way of determining the relative strength is to compare doses giving a 50% control, the so-called \( ED_{50} \) (Figure 1.4).

1.7 Herbicide selectivity

Proper studies of selectivity of herbicides use horizontal assessment and are often done in two steps. The principles of selectivity is best illustrated by using the methods which chemical companies use to quantify selectivity in their research and development of new herbicides. The first step finds the range of doses that do not affect the crop growing
Assessment of herbicide effects

Figure 1.4: Comparison of two dose-response curves ([1] and [2]) at the $ED_{50}$ value, which is the dose required to affect the response, e.g. biomass, by 50%.

without weeds, and the second one finds out how well the herbicides control the target weeds growing without a crop. Thus we need dose-response curves for both the crops and the weeds. Since herbicides may have an effect on any plant, be it crop or weeds, we sometimes have to accept a small decrease in the yield of the crop growing in a weed free environment. For example a 10% decrease might be tolerated, that is, the $ED_{10}$ in Figure 1.5, top, while a 90% control of the weed, $ED_{90}$, is considered a reasonable control level of the weed. As seen from the figures the upper limits for the crop and the weed are different, but for the sake of clarity in interpretation Figure 1.5, bottom, shows the relative response being scaled so that the upper limits are both 1.00 for crop and weeds respectively (see Section 1.10).

In the development of herbicides the companies screen virtually 10,000’s of compounds, only a small fraction of which may have some activity. In order to handle this immense amount of information we must unambiguously define the selectivity of herbicides in the development phase. By accepting a 10% yield loss of the crop growing in weed free environment and by being satisfied with a 90% effect on the weeds growing in crop free environment, we get an Index of Selectivity, $IS$:

$$IS = \frac{[ED_{10}]_{crop}}{[ED_{90}]_{weed}}.$$  \hspace{1cm} (1.2)

To determine the sensitivity of a crop in the field together with weeds, the response curve, at least for the crop, would look a little bit different, in that the upper part of the crop response curve clearly shows that the control of the weeds by the herbicide, all other things
Figure 1.5: Estimation of selectivity for a herbicide use in a monoculture crop and monoculture weed (top, a). Bottom, b, shows the response curves where the responses have been scaled to 1.00 for the untreated control.
Assessment of herbicide effects

being equal, gives a higher yield of the crops until a certain point. Beyond this maximum point the harmful effect of the herbicide on the crop exceeds that of the beneficial effect of weed control.

Figure 1.6: Comparison of two dose-response curves for crop and weeds growing together.

Figure 1.6 shows the actual dose-response curves in a field situation with crop and weed growing together. Up to a certain dose, the herbicide has a yield increasing effect because the weed is controlled. The stimulation effect at small doses in Figure 1.6, however, is sometimes also found with dose-response curves for a species growing in monoculture. The nature of this stimulation is unknown and is always found for any herbicide we have tested in our laboratory when we use very small doses (Pestemer & Günther, 1993). Because of this stimulating effect of herbicides on plant growth in very small doses, it is often difficult to separate the effect of a herbicide on the crop and the weeds. This is the reason why we want to work with clean crops in basic studies of selectivity. The effect of a herbicide can be divided into:

1. Positive effects due to efficient weed control.
2. Positive effects due to the action of the herbicides in the crop itself.
3. Negative effects due to crop damage.

1.7.1 Time-dose-response relationships

The effect of a dose was already stated by Paracelsus 400 years ago. To modify his axiom to our purpose — a sufficiently low herbicide dose is not phytotoxic, whereas a sufficiently
high dose kills any plant. Another important factor that influences the effect of a dose is the duration of exposure. High doses may be required to produce a response that would otherwise result only after much longer exposure with a much lower doses. This means that the effect of a dose must be assessed in relation to the duration of exposure (Figure 1.7).

Figure 1.7: Hypothetical logistic dose-response growth surface. Log(dose) axis increasing to the right and the time axis increasing inwards and the response on vertical axis (Streibig et al. 1993).

The exposure time for soil acting herbicides can be defined as the time the root system is in contact with the dissolved herbicide in the soil solution. It resembles the way duration of exposure is defined in toxicology (Hartung, 1987). For foliar-applied herbicides the situation is somewhat different. The exposure time is usually quite short, but the principle of duration of exposure still applies; the exposure time can be defined as the period from application and until the herbicide has disappeared. The functional relationships may change in Figure 1.7 but not the principle that may help improve our understanding of the factors influencing plant response to dose rate and time of exposure. The common dose-response curve is sectioned by a plane parallel to the dose and response axes and perpendicular to the duration of the exposure axis. If we wish to look at the duration-response curve at any one dose, the response surface in Figure 1.7 is sectioned parallel to duration of exposure and response axes and perpendicular to the dose axis.

To our knowledge generalized response surface models, such as the one shown in Figure 1.7, have not been published for herbicides, probably because of lack of data and the rather large experimental design which is necessary. Günther & Pestemer (1990) have briefly discussed the importance of such response surfaces.
1.8 Adjuvants and herbicides

Only few herbicides are efficient weed control agents when applied as technical grade materials. Therefore, research in and development of herbicides tries to improve performance of a compound by mixing it with various formulation substances and adjuvants. Formulation of herbicides is done by the producer, and the end user may try to increase efficacy by adding various adjuvants such as surfactants, sticking agents, mineral and crop oils. Formulation research is a trendy area, because we want to get rid of older organic solvent formulations and adjuvants that often are more hazardous to the health and the environment than are the herbicides themselves.

Often adjuvants are being considered biologically inactive by themselves, and therefore we can assume that it is only the herbicides that act in the plant.

The reasons for using adjuvants could be to save active ingredient of an expensive herbicide (save money), to lower the environmental adverse effects of herbicides, avoid spray drift, etc. However the reason, it is obvious that the selectivity of a herbicide may change when we use adjuvants, and consequently it is important that the end user is aware of this fact, otherwise it may be a costly experience.

A typical way of analysing the effect of an adjuvant is shown in Figure 1.8a. Only the herbicide is biologically active within the dose range. In this case the mixture of adjuvant, in a fixed concentration, and the herbicide synergized the action of the herbicide (Figure 1.8b).

If several dose-response curves with different fixed concentration of an adjuvant are available, then we can illustrate the change of herbicide dose, which gives the same response level, by using the so-called isobole method (Figure 1.9). An isobole is essentially a contour plot at a given response level (say $ED_{50}$). According to Figure 1.9, we can classify the action of adjuvants as: additive when the adjuvant does not affect the efficacy of the herbicide; synergistic when the adjuvant makes it possible to lower the dose of the herbicide without loss of efficacy and finally antagonistic when the adjuvant makes it necessary to increase the dose to obtain the same effect.

Synergism and antagonism are equally important in herbicide research. A synergizer may increase the efficacy by enhancing retention and/or absorption of a herbicide, or more seldom it may block degradation of the herbicide in the plant. A synergizing effect makes us able to: reduce the dose of the herbicide and/or compensate for the erratic effect caused by environmental fluctuation (climate etc). For example, it could improve rainfastness of herbicides so that rain, immediately after spraying, will not influence efficacy. Also synergizers, which affect the metabolism in the species we want to control, are important in that we can broaden the spectrum of weed control for a herbicide that otherwise is ineffective.

An antagonizer may decrease herbicidal activity by reducing absorption or facilitate rapid degradation in the plant or block the site of action. Compounds, affecting the metabolism of the herbicides, are called safeners or antidotes. Search for safeners to protect crops but not the weeds from the effect of a herbicide is an important part of herbicide research in that the selective use of a herbicide in crops, otherwise sensitive to this herbicide, may be extended.
Figure 1.8: Dose-response curves for a herbicide and a biologically inert adjuvant (top, a), and the response-curves for the herbicide applied alone and with a fixed concentration of the adjuvant. In this case the adjuvant enhances the effect of the herbicide (bottom, b).
1.9 Herbicide mixtures

In Section 1.8 we looked at mixtures of a herbicide and a putative biologically inert adjuvant and saw how we could summarize our findings with isoboles. In the literature, definition of synergism, antagonism and additivity in this instance is rather consistent. While, for ease of interpretation, the effects of adjuvants implied that only the herbicide was biologically active, we now turn to mixtures where both components are biologically active. In weed control we often use mixtures to: save costs of application, expand the control of a diverse weed flora, slow down the selection pressure to delay development of herbicide resistant weeds and exploit mixtures that are more efficacious than expected from the effect of the single herbicides.

When we will look at mixtures of herbicides, however, this has given rise to a Babylonian confusion of definitions of synergism, antagonism and additivity in the weed science literature. When two herbicides are active in the test plant the situation becomes slightly more complicated in that we have to consider the conjoint effect of both compounds. As was the case for mixtures with only one of the two herbicides being active, the basis is the response curves of the two herbicides applied separately. But now there are different reference models to choose from, and there is no consensus about which reference model should be used in different situations.

Defining how compounds act in mixtures is essential for the scientific understanding in weed control, toxicology, ecotoxicology and pharmacology. The mechanisms of joint action of herbicides can be biochemical, by changing how much of a compound reaches its
Assessment of herbicide effects

site(s) of action; competitive, by changing the binding of each other at the active site; physiological, by changing the biological effect; or chemical, when the herbicides react chemically with each other inside or outside the organism.

The assessment of the effect of mixtures could be based on various concepts whether we work within toxicology, pharmacology or weed control.

Here we will concentrate on two reference models. the *additivity of doses*, that is horizontal assessment and the reference models are called Additive Dose model, ADM. The Multiplicative survival model, MSM does not require horizontal assessment of dose-response curves. Although extensively discussed, the scientific literature does not always differentiate between different concepts even though the choice of concept is founded on assumption about site(s) and mode(s) of actions of the herbicides.

The ADM implicitly assumes that the herbicides have the same mode of action and do not interfere with each other’s action at the binding site. If the compounds have the same mode of action but compete for the same binding site we cannot expect the mixtures to follow ADM. The MSM, however, implicitly assumes that the herbicides elicit their effect independently of each other. Consequently, the choice of reference model could be based on the known mode of action of the mixed compounds. The choice between MSM and ADM, the two biologically reasonable models, could be based on the investigator’s perception of how the herbicides act in the plants. The following subsections are more comprehensive dealt with by Streibig & Jensen (2000).

### 1.9.1 Multiplicative Survival Model (MSM)

This reference model assumes that the two herbicides exert their action independently of each other and that the responses be expressed in terms of percentage or proportions of some hypothetical maximum response. MSM was developed for qualitative responses (dead or alive; affected not affected, etc.) that have a maximum response of 1, or in percentage terms 100%. For quantitative responses, such as biomass, the maximum usually has to be estimated from experimental data and thus the proportion or percentage does not have quite the same meaning. With herbicides the maximum response, such as percentage control or percentage of untreated control, is based upon a mean response; while with qualitative responses the percentage refers to the proportion of individuals that is killed. The problem of finding a maximum response with herbicide bioassay can partly be overcome by using dose-response curves and express the responses as in Eq. 1.13.

Most work on mixtures in weed science, however, does not take advantage of the dose-response curves but re-scales the mean responses in relation to the biomass of the untreated control.

In its simplest and most common form, MSM is called Abbot’s formula in entomology and Limpel’s formula or Colby’s equation in weed science. Abbot’s formula or Colby’s equation requires only a single dose of each herbicide applied alone and the mixture of both doses. For example, if a dose of 0.2 kg ha\(^{-1}\) of herbicide A gives a response of 90.91% of the untreated control and 5 kg ha\(^{-1}\) of herbicide B gives 8.21% of the untreated control, then a mixture of 0.2 kg of herbicide A and 5 kg of herbicide B will yield 7.46% of the untreated control. In other words by giving 9.09% control, herbicide A leaves 90.91% of...
the untreated control to be further affected by 5 kg of herbicide B, which on its own leaves 8.21% of the untreated control.

Suppose that a dose of a mixture contains the doses, $z_a$ plus $z_b$ and the responses $P$, $P_{a0}$ and $P_b$ range between 0 (no control) and 1.00 (complete control). The dose $z_a$ of herbicide A administered separately evokes a response $P_a$ and a dose $z_b$ of herbicide B evokes a response, $P_b$. The response, $P$, of a mixture consisting of $z_a$ plus $z_b$ would be

$$1 - P = (1 - P_a)(1 - P_b)$$

Rewritten, it becomes more familiar for weed scientists

$$P = P_a + P_b - P_aP_b$$

If the response $P$ is expressed as % control the product, $P_aP_b$ is of course divided by 100 and we get the familiar Colby equation.

If the response is expressed as proportion of untreated control ($Q$, $Q_a$, $Q_b$) then

$$Q = Q_aQ_b$$

Eq. 1.5 can easily be extended to any number of herbicides in a mixture:

$$Q = Q_aQ_b...Q_n$$

When the dose-response curves for the herbicides applied separately are known, we can predict the behaviour of mixtures satisfying MSM (Figure 1.10, Top). Note that the shape of the isoboles differs at the response levels considered. This is clearer when the isoboles are re-scaled, so that any response level of the two herbicides applied separately is 1.00 (Figure 1.10, Bottom).

### 1.9.2 Additive Dose Model (ADM)

The Additive Dose Model assumes that the herbicides can substitute for each other at equivalent biological rates, i.e. their relative potency, at any response levels. In the additive case, the relative potency between two herbicides is analogous to the exchange rate between currencies. For illustration, assume the exchange rate between Danish Kroner (DKK) and the Common European Currency, Euro (EUR) is 7.5 DKK to one EUR. If we have 750 DKK and 100 EUR, we have to convert to a common currency to express their total value. If we want to spend in another EU country, then we must exchange 750 DKK for 100 EUR and thus express the total value as 200 EUR. If we want to spend in Denmark, we must exchange the 100 EUR for 750 DKK and then have a total of 1,500 DKK. The value is the same in that the 7.5 to 1.0 exchange ratio corresponds to their relative potency. Exchanging money without changing its value is analogous to additivity. Applying ADM to herbicides means that if we can control a weed population by 90 % by applying 0.04 kg ha$^{-1}$ of metsulfuron-methyl and 1 kg ha$^{-1}$ of MCPA has the same
Figure 1.10: MSM isoboles from a mixture experiments. Top, isoboles at $ED_{20}$, $ED_{50}$ and $ED_{90}$. Bottom, The dose axes have been scaled so the three $ED$-levels for the separate herbicides are 1. Notice the form of the isoboles is not the same at different $ED$-levels.
effect, then the biological exchange rate is 250. It means that a mixture of 0.02 kg of metsulfuron-methyl and 0.5 kg of MCPA will control the weed population by 90%

An ADM isobole is a straight line as shown in Figure 1.11. If ADM is satisfied, the straight line represents all mixtures of the two herbicides that will give the same response.

Figure 1.11: ADM Isobole for two herbicides. Any mixture along the line satisfies ADM.

If the response curves for the herbicides applied separately are similar, then we obtain parallel isoboles irrespective of the response levels considered (e.g., $ED_{20}$, $ED_{50}$, $ED_{90}$) Figure 1.12, Top. If the response curves are not similar we still get straight lines, but now they are not parallel (Figure 1.12, Bottom). If the $ED_x$ levels investigated are re-scaled, so that any $ED_x$ level of the herbicides applied separately is 1.00, then the isoboles will all fall on the same line as shown in Figure 1.11, regardless of the slopes of the isoboles. This is in contrast to MSM where the shape of the isoboles differs from each other at various response-levels.

The principle of biological exchange rate, that is the relative potency, is the basis for our hypothesis that: the herbicides do not change their effect in the plant when they are applied in mixture. Mathematically it can be expressed as

$$\frac{z_a}{Z_a} + \frac{z_b}{Z_b} = 1 \quad ,$$

(1.7)

where $z_a$ and $z_b$ are doses of herbicide A and B in a mixture which yield the same response as the dose of $Z_a$ and $Z_b$ of herbicide A and B applied separately. The relative potency (biological exchange rate) between A and B is

$$r = \frac{Z_a}{Z_b} \quad .$$

(1.8)

By combining Eq. 1.7 and Eq. 1.8, we get

$$z_a + rz_b = Z_a = rZ_b \quad ,$$

(1.9)
Figure 1.12: ADM isoboles at different response levels. Top, when a suit of response curves for the herbicides applied separately and in mixtures are all mutually similar. Bottom, when the response curves are not similar.
If we know the relative potency between herbicide A and B applied separately, we can calculate any mixture of A and B giving the same response. Eq. 1.9 describes a straight line relationship between herbicide A and B in any mixture (Figure 1.11). This line is called an isobole which satisfies the ADM.

Eq. 1.9 shows that the additivity of doses is based upon the relative potency between the two compounds applied separately, and it expresses the isobole as a straight line with an intercept of $z_a$ and a slope $r$.

Whatever the mathematical expression, the equations infer that with ADM the herbicides in any mixtures can be substituted with each other on the basis of their relative potency without changing the conjoint effect.

Any departure from the straight line isobole indicates greater effect than expected from ADM or less than expected from ADM. ADM is equally valid for quantitative responses, such as growth inhibition and qualitative responses such as the proportion of surviving organisms.

While it is straightforward to evaluate biologically equivalent dosages using ADM and by drawing isoboles, it is more complicated to calculate the effect of ADM mixtures. This is in contrast to the MSM, where it is easy to calculate the response. Calculating the response of an actual mixture is equivalent to finding the ADM $ED_x$ isobole on which the mixture is located.

### 1.9.3 Antagonism and synergism

MSM is by far the most popular reference model because of its simplicity and lack of any requirement for multiple doses to predict expected responses. ADM has rarely been used because of its requirement for multiple doses and interpolation on the response curves to find the desired level of control. The biological basis for choosing either MSM or ADM, however, requires knowledge of the mode of action of the herbicides. If the herbicides act independently of each other, MSM could be used, otherwise ADM is more appropriate.

MSM and ADM can be considered the extremes of a continuum of models. Hewlett and Plackett (1979) have developed a general theory for joint action of compounds, but the theory is complicated which has precluded its wide acceptance.

While the terms antagonism, synergism and additivity are well defined with only one herbicide being active in the mixtures (Figure 1.9) this is not the case when both herbicides are active because of the different shapes of the isoboles for MSM and ADM (Figure 1.10 to Figure 1.12). The ambiguity of the terms synergism, antagonism and additivity has caused confusion in the literature. In toxicology and pharmacology the term potentiation is sometimes used instead of synergism and is only related to the ADM (Hewlett and Plackett, 1979). We will, however, the term synergism, antagonism and multiplicativity/additivity according to the illustration in Figure 1.13.

If the isoboles of MSM are used and the mixtures follow this isobole within acceptable limits, we will use the term multiplicative action. If mixtures are placed below the isobole we will term it synergism, and if the mixtures are placed outside we will term it antagonism. The same applies to the ADM in Figure 1.13, except that mixtures following the
Figure 1.13: Isoboles for mixtures of herbicides at a given response level, e.g. $ED_{50}$. 

MSM and ADM Antagonism

MSM Isobole

MSM Synergism and ADM Antagonism

ADM Isobole

MSM and ADM Synergism

Dose of Herbicide A

Dose at Herbicide B
Assessment of herbicide effects

ADM isoboles are called additive. From the above, it is obvious that the reference model should always be clearly stated whenever statements about antagonism or synergism are made, because mixture effects might be deemed synergistic under MSM but antagonistic under ADM.

Both with MSM and ADM the response curves of any mixture will revert to one of the response curves of herbicides applied alone if the dose of the other herbicide in a mixture approaches zero. In contrast to ADM, a problem with MSM is highlighted if it is used to predict the response of a mixture of a herbicide with itself. In this purely hypothetical example, MSM may predict antagonism or synergism depending on the response level considered (Green et al., 1995).

When the dose-response curves for the herbicides applied alone are similar then the response curves for fixed mixture ratios are required to be mutually similar to satisfy ADM at any response level. With non-parallel response curves, however, a complication arises in that the dose-response curves of mixtures in fixed ratio and satisfying ADM do not follow the same form. This is exemplified by logistic curves of the dose-response curves of the herbicides applied separately (Jensen and Streibig, 1994; Streibig, 1983). Our experience is that these small deviations from the logistic curves are difficult to detect statistically.

If the dose-response curves for the herbicides applied alone are parallel and the mixtures follow MSM, then the response curve of the mixtures in fixed ratio will not have the same shape as do the response curves for the herbicides applied alone.

1.10 The logistic dose-response curve

Weed science is a rather new discipline, rooted in agronomy, plant physiology and botany. It became a science in its own right at the eve of World War II after the discovery of the selective properties of MCPA and 2,4-D that killed the weeds but left the cereal crop unharmed. The experimental traditions in weed science are chiefly based upon analysis of variance of field experiments with herbicides. Dose-response curves, commonly used in toxicology and pharmacology, have played a minor role except for screening programs used by industry in search for new herbicides.

In the early days of herbicide development, Woodford (1950) wrote that although statistical methods have been devised and accepted as standard practice in the assessment of toxicity of chemicals in many branches of biology, little attempt has been made to use them in the evaluation of herbicides. In consequence much of the work in this field has been empirical, and it has often been impossible to judge the precision, let alone the accuracy, of the results obtained. About the same time, Blackman (1952) and Blackman et al. (1951, 1958) published papers on the principles of phytotoxicity based on biological assay, the purpose of which was to measure the activity of a biologically active substance by the response of living material. The work of Blackman and colleagues thoroughly discussed the experimental designs, the statistical analysis and the assessment of relative potency. Unfortunately, it was almost forgotten or neglected.

Browsing through the original literature and reviews of herbicide experiments (often called bioassays) clearly showed how little impact these early works had. This probably stems
from the fact that nonlinear regression techniques needed to describe the sigmoid dose-response relationship is still a grey area for many weed scientists. The assessment of herbicide efficacy is in many instances confined to analysis of variance, whereas a more appropriate method would be regression analysis. The analysis of variance is central to most applications of statistical methods in the analysis of experiments. This is true for bioassay, but perhaps the fundamental importance of regressions and related concepts is particularly apparent in this context. As Finney (1979) puts it: “As a branch of applied statistics, biological assay appears to have somewhat specialized appeal. Although few statisticians have worked on it intensively, to the majority it appears as a topic that can be neglected, either because of its difficulties or because it is trivial... Despite this I am convinced that many features of bioassay are outstanding good for concentrating the mind on important parts of biometrics practice and statistical inference”.

When plants are treated with herbicides the observed effects can be of two different types, graded or quantal. The response is graded or quantitative, if the results are changes in plant biomass, height, content of metabolites, photosynthetic capture of CO₂ etc. Quantal or qualitative response, otherwise called ‘all-or-none’ response, is used if the individual plants can be classified killed, not germinated etc. With graded response each dose rate yields a response on a continuous scale and thus carries more information than the ‘all-or-none’ response. Consequently, we only concentrate on graded response in this section.

Experience has shown that in most instances a logistic dose-response curve describes reasonably well what happens in the crop and weeds in response to dose rates. The logistic curve can be expressed as follows (Streibig et al., 1993):

\[ y = C + \frac{D - C}{1 + \left( \frac{z}{ED_{50}} \right)^B} \]  

(1.10)

Or using the logarithm of the dose, also called the log-logistic curve

\[ y = C + \frac{D - C}{1 + \exp\left[ B (\log(z) - \log(ED_{50})) \right]} \]  

(1.11)

The parameters and variables in Eq. 1.10 and 1.11 are defined in Table 1.5. The shape of a logistic curve is shown in Figure 1.14. When comparing several dose-response curves from an experiment, such as in Figure 1.3 (page 16), we can extend Eq. 1.11 to:

\[ y = C + \frac{D - C}{1 + \exp\left[ B (\log(Rz) - \log(ED_{50})) \right]} \]  

(1.12)

where \( R \) in Eq. 1.12 is the relative potency between the two curves having the same \( D \), \( C \), and \( B \) parameters i.e. the curves are similar also called parallel.

The advantage of working with similar curves is that the relative potency, \( R \) (see Eq. 1.1) is constant at any one response level. The parallel line assumption between a standard and test herbicide is a necessary but not a sufficient condition for assuming a similar mechanism of action of the herbicides in the plant. Accordingly, a test for parallel lines
Table 1.5: Interpretation of variables and parameters in logistic dose-response curve (Eq. 1.10 and 1.11).

<table>
<thead>
<tr>
<th>Parameter/ variable</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$y$</td>
<td>Plant response (e.g. biomass)</td>
</tr>
<tr>
<td>$z$</td>
<td>Dose (e.g. gram active ingredients ha$^{-1}$)</td>
</tr>
<tr>
<td>$D$</td>
<td>Upper limit of plant response (upper asymptote)</td>
</tr>
<tr>
<td>$C$</td>
<td>Lower limit of plant response (lower asymptote)</td>
</tr>
<tr>
<td>$ED_{50}$</td>
<td>The dose required to half the plant response between the upper and lower limit</td>
</tr>
<tr>
<td>$B$</td>
<td>The proportional slope of the curve around $ED_{50}$ (the point of inflexion)</td>
</tr>
</tbody>
</table>

Figure 1.14: The log-logistic dose-response curve corresponding to Eq. 1.11. The parameters in this example are: $D=50$, $C = 2$, $ED_{50} = 1$, $B = 2$. 
could be the first crude indication of putative similar mechanism of action of a novel herbicide lead if assayed together with a standard herbicide with known mechanism of action. It is, however, important to stress that parallel lines also can be found for herbicides of contrasting mechanism of action.

![Figure 1.15: Logit-transformed responses plotted against log(dose).](image)

If one has access to regression programmes with non-linear regression procedures, the data can be used to estimate the parameters of the logistic curve. Sometimes access to non-linear regression programmes is difficult, and therefore we could try the second best solution by transforming the response to a straight line with the so-called logit transformation (Eq. 1.13). This requires that the lower limit, \( C \), and the upper limit, \( D \), are known from data. If the dose-range is properly distributed over the whole response range from response of untreated control \( (D) \) to high doses where the plants are almost dead \( (C) \), the response can be transformed to a straight line as in Figure 1.15. Note that logit\((y) = 0\) when the response is half way between \( D \) and \( C \), and logit\((y) \) is only defined for \( y < D \) and \( y > C \).

\[
\text{logit}(y) = \log \left( \frac{D - y}{y - C} \right).
\]  

(1.13)

Now we have an impression of a mathematical framework for analysing the effect of herbicides in plants.
1.11 Causes of selectivity

Until now we have only looked at the ways in which action or selectivity of herbicides can be measured. The next step is to unravel why species, e.g. the ones in Figure 1.6 (page 20), differ in sensitivity. The physiological and biochemical causes of selectivity are given elsewhere (Fedtke, 1982; Cobb, 1992; Devine et al., 1993), and here we will only give a brief outline of some of the more pertinent factors of general nature.

1.11.1 Foliar retention, cover and absorption

The retention of a spray primarily depends on the angle of leaves. When a droplet lands on a leaf, two things can happen, the droplet can be retained, or it may bounce and be redirected to other parts of the plant, or eventually end on the soil. The retention on the leaf is a function of the kinetic energy and surface tension of the droplet, and the nature of the leaf surface. Generally, droplets with high surface tension are more likely to bounce off than are droplets with low surface tension. In practice, droplets < 100 $\mu$m in diameter are unlikely to bounce off, but so small droplets are prone to drift and perhaps affect sensitive plants outside the field. When spraying, the kinetic energy can to a certain extent be controlled by the operator and the surface tension of the droplets can be manipulated with spray adjuvant such as surfactants or various formulation products.

In some instances, differential spray retention may contribute to herbicide selectivity between species, for example the difficult to wet leaves of peas, can partly explain the selectivity of some herbicides in this crop.

Surfactants often affect the leaf surface wetting and the contact angle of the droplets. As seen in Figure 1.16, the lower the contact angle, the bigger the area of the waxy leaf cuticle is covered by the droplet. One of the things which is beyond the operator’s control is the composition and structure of the cuticle of the plant itself. Generally, plants in an early stage of development have lesser cuticular wax than have older plants, and therefore more herbicide is likely to be retained and absorbed by younger leaves than by the older ones.

Table 1.6 shows differences in contact angles for water at the cuticle of some species. As a rule of thumb we say that if the contact angle is larger than 90°, then the leaf is difficult to wet.

The penetration of the spray through the cuticle is dependent on a variety of factors. Since plant cuticles are heterogeneous it is difficult to get good data that unambiguously unravel the cause-effect relationship between penetration of a given herbicide through a
Table 1.6: Contact angle between water and cuticle of some plant species. (From various sources).

<table>
<thead>
<tr>
<th>Species</th>
<th>Contact angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peas</td>
<td>169</td>
</tr>
<tr>
<td>Barley</td>
<td>166</td>
</tr>
<tr>
<td><em>Avena fatua</em></td>
<td>161</td>
</tr>
<tr>
<td><em>Sinapis arvensis</em></td>
<td>64</td>
</tr>
<tr>
<td><em>Stellaria media</em></td>
<td>78</td>
</tr>
<tr>
<td><em>Chenopodium album</em></td>
<td>157</td>
</tr>
</tbody>
</table>

given cuticle. A long living theory, however, claims that the route of herbicide through the cuticle is determined by the polarity of the herbicides (see Figure 1.17). This theory explains roughly the often observed phenomenon that the speed of absorbing polar herbicides is slower than is the speed of absorbing non-polar herbicides. In practical spraying this is very important in that rain shortly after spraying will wash off more polar herbicides not yet absorbed than the already absorbed non-polar herbicides. In other words the rainfastness of a herbicide is partly determined by its polarity.

### 1.11.2 Root absorption

Many herbicides are absorbed by the roots. Whilst the exposure to shoot absorption is short, within a few hours, the exposure of roots to soil acting herbicides can last for a very long period and is a dynamic interaction between available herbicide in the soil solution and the growth of root surface which can take up the herbicides.

The route of root entry of herbicides is rather different from that of foliage-applied herbicides. Active root tissue lacks the cuticle of the leaf, and furthermore root endodermis is lined with a suberized layer (The Casparian strip), which effectively separates the aqueous continuum of the epidermis and the cortex from the vascular tissue in the stele. Herbicide penetration of the root is typically characterized by rapid initial entry, followed by a prolonged period of slow entry. The initial phase is often independent of metabolic processes, whereas the latter prolonged period is probably associated with metabolic activity.

There is no agreement upon the role of transpiration in herbicide absorption by roots. For the time being it might be wise to assume that within certain limits the root absorption of herbicides is fairly independent of water movement into and through the plant. It is probably more related to the physico-chemical factors that govern the positioning of molecules into root tissue.

### 1.11.3 Translocation

Some herbicides are not dependent on translocation in plants, and some crude generalization of the relationship between site of uptake and translocation pattern is found in
Figure 1.17: A hypothetical picture of the penetration of polar and non-polar herbicides through the cuticle. (From Klingman & Ashton, 1975).

Table 1.7. Phenmedipham, used selectively in beet, ioxynil and bromoxynil, used in cereals and glufosinate belong to the group of contact herbicides. They exert their action immediately after uptake through the plant cuticle into the mesophyll tissue. In this case the selectivity is chiefly a subtle balance between uptake and metabolism. The majority of contact herbicides belong to the group of foliar applied compounds. Commonly used soil acting contact herbicides are: trifluralin, triallate, diallate and propham (Fedtke, 1982). The method of herbicide translocation does not in itself affect selectivity, but may affect toxicity. Very toxic compounds translocated in the phloem may block further translocation to the primary site(s) of action. Well-known examples are the auxin type herbicides, dicamba, picloram and the phenoxy acids (Cobb, 1992; Fedtke, 1982).

<table>
<thead>
<tr>
<th>Site of uptake</th>
<th>Contact</th>
<th>Systemic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td>(+)</td>
<td>Xylem (+) Phloem (+)</td>
</tr>
<tr>
<td>Foliage</td>
<td>+</td>
<td>(+)      +</td>
</tr>
</tbody>
</table>

Table 1.7: General classification of the relationship between site of herbicide uptake and translocation.
1.11.4 Metabolism

Detoxification of absorbed herbicides differs among plant species. For example, detoxification of atrazine is different in the two tolerant species, maize and sorghum (Esser et al., 1975). The sulfonylureas inhibit acetolactate synthase, an enzyme which is necessary for the production of the amino acids, valine and isoleucine (Beyer et al., 1988). Such inhibition takes place in most plant species tested so far, and the selectivity is primarily based upon rapid detoxification of the herbicides in tolerant species. The metabolism of sulfonylureas includes many of the known deactivation and activation mechanisms (Brown et al., 1991). Some 20 sulfonylureas are commercially available in several crops. Whatever mechanism controls the differential response to a herbicide, the dose is important in determining selectivity. As pointed out by Fedtke (1982), the primary site of herbicide action may also be associated with other secondary and/or tertiary sites of action depending on the dose administered. For most herbicides, selectivity is, therefore, a delicate balance between uptake, translocation and metabolism of the active compound. It is this balance which governs the action of herbicides in plants.

The role of metabolism in herbicide selectivity is probably the most important one and is dealt with in numerous textbooks (Cobb, 1992; Devine et al., 1993). Fedtke (1982) points out that for the xylem-mobile herbicides the selectivity of a compound can be described by

\[
\text{Selectivity} = \frac{\text{Detoxification}}{\text{Uptake} \times \text{Translocation}}.
\]  

As long as the detoxification keeps pace with the incoming uptake and translocation in the denominator, the treated plants will exert tolerance towards this compound. Thus, a low or high capacity of detoxification in itself is not sufficient for the determination of sensitivity or tolerance, but should be associated with the uptake and translocation.

1.12 Summary

Herbicides can be classified into multifarious ways, e.g. soil versus foliage applied, selective versus nonselective, their chemical structure or their mode of action. An important issue, however, is the measurement of herbicide selectivity. Here we based selectivity on dose response curves by looking at response levels relative to the untreated control. For example, selectivity of a herbicide among crop species could be defined as a response level of 50% of untreated control, whilst the selectivity of a herbicide among weeds and crops is the ratio between 10% reduction in relative untreated control for the crop and 90% reduction for the weeds. With the response curves you can go beyond sheer selectivity and look at how adjuvants, such as surfactant etc, affect the action of herbicides and develop biologically sound hypotheses about how the joint action of herbicide mixtures can be understood. The mathematics of a response curve is also included as is a brief outline of the causes of selectivity.
Assessment of herbicide effects

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