CHAPTER 1

Introduction

Chapter outline

In this chapter you will learn about:

- The history of toxicology
- Types of toxic substance
- Types of exposure
- Selective toxicity
- The basic principles of the dose–response relationship
- Receptors
- ED_{50} and TD_{50}
- The therapeutic index
- Synergy and potentiation
- Threshold dose and NOAEL

Toxicology is the study of the harmful interactions between chemicals and biological systems. Man, the other animals, and the plants in the modern world are increasingly being exposed to chemicals of an enormous variety. These chemicals range from metals and inorganic chemicals to large complex organic molecules, yet they are all potentially toxic. The study of the pathological, biochemical and physiological effects of such substances is the fascinating brief of the toxicologist. Toxicology, like medicine, is a multidisciplinary subject which encompasses many areas. This makes it an absorbing and challenging area of research. The challenge of toxicology is to apply basic biochemical, chemical, pathological and physiological knowledge along with experimental observation to gain an understanding of why certain substances cause the disruption in a biological system which may lead to toxic effects.

Approximately 65 000 chemicals are currently produced in the USA and 500–1000 new chemicals are added each year. Because of this escalation in the numbers of chemicals to which our environment may be exposed (Figure 1.1), it has become increasingly important to have some knowledge of the toxic effects they may have and to attempt to measure and assess these effects.

FIGURE 1.1 *Toxicology is concerned with the exposure of living systems in the environment to toxic substances from a variety of sources.*

In recent years, awareness of the problem of human and animal exposure to potentially toxic chemicals in our environment has grown. Perhaps one of the first to bring this to the attention of the public was Rachel Carson with her book ***Silent Spring***. This was a description of the devastating effects of pesticides on the flora and fauna of the North American environment. As discussed by Efron in her book ***The Apocalyptics, Cancer and the Big Lie*** (1984), Carson and certain later scientists probably exaggerated the dangers of chemicals, but her message was quite clear. Few would disagree that man should *be aware* of the synthetic chemicals to which the environment is exposed. Thus, toxicology has another dimension: the social, moral and legal aspects of exposure of populations to chemicals of unknown or uncertain hazard. Hazard and risk assessments and value judgements become important. The toxicologist is often asked to make such assessments and judgements. So toxicology has a very important role to play in modern society and consequently it is now growing rapidly as a new subject.

Historical aspects

Toxicology has been called the study of poisons, but this poses the question 'what is a poison?' Poisons can range from a naturally occurring plant alkaloid to a synthetic nerve gas. A poison is any substance which has a harmful effect on a living system; whether we regard a substance as a poison or not may depend on its use. For example, humans can protect themselves against the effects of harmful bacteria by killing them with antibiotics, such as **penicillin**; alternatively, humans can kill each other with the war gas **phosgene**. Both phosgene and penicillin, therefore, are poisons in the strictest sense of the word but we regard them entirely differently.

It is only recently that the study of poisons has become a truly scientific pursuit. In the past it was mainly a practical art utilized by murderers and assassins. Poison, as a subtle and silent weapon, has played an important part in human history.

Primitive man was aware of natural poisons from animals and plants and indeed used these on his weapons. The word toxicology is derived from ***toxicon*** – a poisonous substance into which arrow heads were dipped and ***toxikos*** – a bow. The study of poisons must have started by 1500 BC because the **Ebers Papyrus**, the earliest collection of medical records, contains many references and recipes for poisons. The ancient Egyptians were able to distil prussic acid from peach kernels, poisons such as arsenic, aconite and opium were also known to Hindu medicine as recorded in the **Vedas**, around 900 BC and the ancient Chinese used **aconite** as an arrow poison. **Hippocrates** in his writings (400 BC) showed that the ancient Greeks had a professional awareness of poisons and of the principles of toxicology, particularly with regard to the treatment of poisoning by influencing absorption. Poisoning was relatively common in ancient Greece so the study of poisons and the development of antidotes in particular was important. For example, **Nicander** of Colophon (185-135 BC), physician to Attalus, King of Bythnia, was allowed to experiment with poisons using condemned criminals as subjects. As a result of his studies he wrote a treatise on antidotes to poisonous reptiles and substances (Theriaca and Alexipharmica) and mentioned 22 specific poisons including **ceruse** (white lead), **litharge** (lead oxide), **aconite** (wolfsbane), **cantharides**, **conium** (hemlock), **hyoscyamus** (henbane) and **opium**. He recommended linseed tea to induce vomiting and sucking the venom from the bite of a venomous animal as treatments. Similarly, King **Mithridates** used criminals to search for antidotes to venom and poisonous substances and regularly protected himself with a mixture of 50 different antidotes (Mithridatum). Legend has it that he was unable to poison himself when suicide became necessary! The term **mithridatic** (meaning antidote) is derived from his name.

The first known law against poisoning was issued in Rome by Sulla in 82 BC to protect against careless dispensing. The Greek physician **Dioscorides** (AD 50) made a particularly significant contribution to toxicology by classifying poisons as animal, plant or mineral and recognizing the value of emetics in the treatment of poisoning. His treatise on Materia Medica was the major work on poisons for fifteen centuries.

So, the origins of toxicology lie in the use of poisons for murder, suicide and political assassination. It is well known for example that **Socrates** committed suicide by taking hemlock (Figure 1.2). There are many examples of poisons being used for nefarious purposes such as the poisoning of Claudius and his son Britannicus with **arsenic**. In the latter case, Nero employed a professional poisoner who put the arsenic into the water used to cool the soup and so avoided the taster. The prolific use of poisons in this way made it necessary for treatments to be devised and **Maimmonides** (1135–1204) wrote *Poisons and Their Antidotes* which detailed some of the treatments thought to be effective.

In the Middle Ages, especially in Italy, the art of poisoning for political ends developed into a cult. The **Borgias** were infamous during the fifteenth and sixteenth centuries. In seventeenth-century Italy, a woman by the name of Toffana prepared cosmetics containing arsenic (**Aqua Toffana**) which were used to remove unwanted rivals, husbands and enemies! Similarly **Catherine de Medici** prepared poisons and tested them on the poor and sick of France, noting all the clinical signs and symptoms.

One of the most important concepts in toxicology was espoused in the sixteenth century by a scientist by the name of **Paracelsus**. He was born Philippus Theophrastus Aureolus Bombastus von Hohenheim near Zurich in 1493 and was the son of a physician who was interested in chemistry and biology and was an

FIGURE 1.2 *Socrates drinking hemlock, the Athenian state poison. Reproduced with permission from the Mary Evans Picture Library, London.*

expert in occupational medicine. Paracelsus was a free thinker who disagreed with the dogma current at the time and espoused by Galen. Paracelsus thought observation was crucial and understood the importance of chemistry in medicine. He believed that 'like cures like', contrary to Galen who taught that diseases of a particular intensity would be cured by a medicine of opposite intensity. Consequently in the view of Paracelsus 'a poison in the body would be cured by a similar poison – but the dosage is very important'.

He advocated inorganic chemicals, such as salts, as treatments. These were believed to be too poisonous but he emphasized that the dose was very important. Paracelsus summarized this concept in the following famous phrase: 'All substances are poisons; there is none that is not a poison. The right dose differentiates a poison from a remedy.'

This concept is especially crucial to the safe use of drugs but also important for the safe handling of other chemicals (see below this chapter). It underlies the risk assessment of chemicals because from this relationship follows assessment of threshold doses and safe and non-toxic levels (see below Chapter 12). Even seemingly innocuous substances such as common salt could become poisonous under certain conditions. Paracelsus also believed that diseases were localized to particular organs and also that poisons would damage particular organs (target organs) something we now also know to be generally true.

His contribution to medicine and toxicology was enormous although not recognized until after his death in 1541.

Another significant figure in toxicology was **Orfila**, a Spanish physician (1787–1853) who recognized it as a separate discipline and contributed to forensic toxicology by devising means of detecting poisonous substances and therefore proving that poisoning had taken place. From then on toxicology began to develop in a more scientific manner and began to include the study of the mechanism of action of poisons. Indeed **Claude Bernard** (1813–1878) believed that the study of the effects of substances on biological systems could enhance the understanding of those systems. He identified the site of action of **curare** as either the nerve ending or the neuromuscular junction.

More recently, in 1945, Sir Rudolph **Peters** studied the mechanism of action of arsenical war gases and so was able to devise an effective antidote known as British Anti-Lewisite for the treatment of soldiers exposed to these gases. Other examples of toxic chemicals which have been studied at the mechanistic level with benefits for our understanding of basic biochemistry are cyanide and fluorocitrate. Cyanide inhibits the mitochondrial electron transport chain and fluorocitrate inhibits aconitase, one of the enzymes of the Krebs cycle.

Toxicology has now become much more than the use of poisons for nefarious purposes and the production of antidotes to them. The enormous and ever increasing number (65 000+) of man-made chemicals in the environment to which we may potentially be exposed has thrust toxicology into the limelight. It has also created the need for the organized study of toxic substances by the industries manufacturing them and for legislation to control them. This has in turn resulted in the establishment of government regulatory agencies to implement the resulting legislation.

Some of the industrial disasters which have occurred in recent times have highlighted the need for knowledge of the toxicity of compounds used in industry as well as for drugs and food additives. This knowledge is essential for the development of effective and rapid treatment of the toxic effects, just as it is essential for the treatment of overdoses and accidental poisonings. For example, one of the worst industrial disasters occurred at **Bhopal** in India in 1984 where a factory manufacturing the insecticide carbaryl leaked a large amount of the extremely noxious compound **methyl isocyanate** (Figure 1.3). Little was known of the toxicity of this compound and consequently treatment of the victims was uncertain and possibly inadequate.

Another major reason for testing chemicals in toxicity and other studies is so that they may be classified according to hazard such as toxic, explosive or flammable. This will then enable decisions to be made about marketing and labelling. So we are exposed to toxic or potentially toxic compounds in many ways in our daily lives and toxicology is clearly a subject of great importance in society. This becomes apparent when we look at the types of poisons and the ways in which we are exposed to them. Indeed, the categories cover virtually all the chemicals one might expect to encounter in the environment. After consideration of this one might well ask '*are all chemicals toxic?*' The following phrase perhaps provides an answer: *'there are no safe chemicals, only safe ways of using them.*'

Types of toxic substance

Toxic substances fall into several classes in relation to the way man is exposed to them: drugs, food additives, pesticides, industrial chemicals,

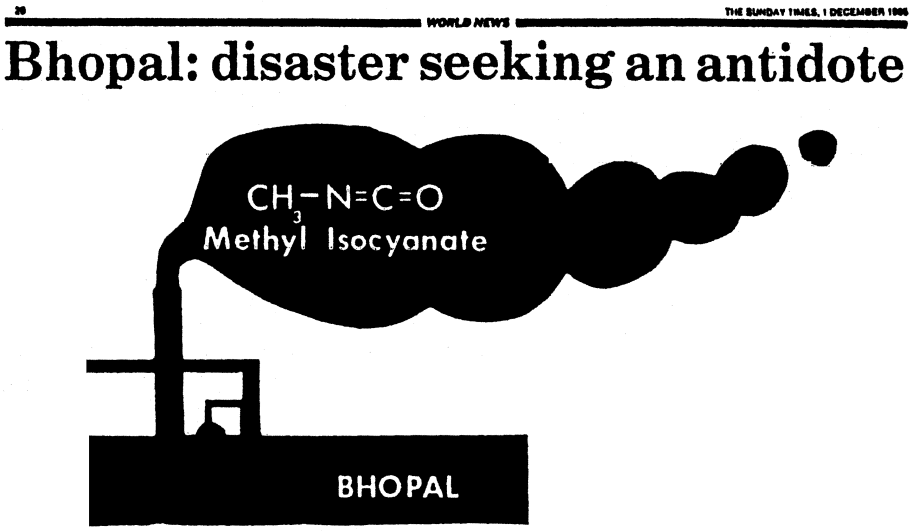

FIGURE 1.3 *A headline reminds us that a year after the disaster in Bhopal, India, in which thousands were killed and injured by the toxic chemical methyl isocyanate accidentally released from a chemical plant, there is no cure or antidote. Headline from* **The Sunday Times**, *1 December 1985, with permission.*

environmental pollutants, natural toxins and household poisons. Each of these categories will be discussed in more detail in later chapters but they will be briefly introduced here.

DRUGS

Most people in the Western world consume drugs of one sort or another throughout their lives. Drugs, however, have usually been designed to be highly potent in biological systems and consequently many are potentially toxic. Drug toxicity may be due either to an overdose or it may be a rare and unusual adverse effect, and examples of both of these will be considered in detail in Chapter 5.

Drugs vary enormously in chemical structure and possess a wide variety of biological activities. They are probably the only foreign substances of known biological activity that man ingests intentionally. Included in this category are alcohol and the active principles in cigarettes, both of which are used because of their biological activity and both, of course, have toxic properties. Drugs used in veterinary practice must also be considered here (and in the next section) as humans may consume meat from or other food derived from animals treated with these drugs.

FOOD ADDITIVES

This is the second category of foreign substances which are directly ingested. However, food additives are usually of low biological activity. Many different additives are now added to food to alter the flavour or colour, prevent spoilage, or in some other way change the nature of the foodstuff. There are also many potentially toxic substances which may be regarded as contaminants occurring naturally in food, resulting from cooking, or from other contamination, and specific examples will be discussed in a later chapter. Veterinary drugs and their breakdown products may also be found in foodstuffs as indicated above. Most

of these substances, both natural and artificial, may be present in food in very small amounts but for the majority little is known of their long-term toxicity. In many cases they are ingested daily for perhaps a lifetime and the numbers of people exposed is very large. Although reliable data are still scarce, there certainly seems to be evidence that at least some additives may be associated with adverse effects. Public awareness of this has now begun to influence the preparation and manufacture of food such that additive free foods are appearing on supermarket shelves.

INDUSTRIAL CHEMICALS

Industrial chemicals may contribute to environmental pollution (considered next), and they may be a direct hazard in the workplace where they are used, formulated or manufactured. There is a huge range of chemical types and many different industries may involve the use or manufacture of hazardous chemicals. In the broadest sense industrial exposure might include exposure to the solvents used in photocopiers and typists' correction fluid. Although in general exposure is controlled by law, often by the setting of control limits, realistic levels may still prove to be hazardous in the long term and acute exposure due to accidents will always occur. The long development time of diseases such as cancer often makes it difficult to determine the cause until sufficient of the workforce have presented with the disease for the association with the toxic compound to be made.

ENVIRONMENTAL POLLUTANTS

The main sources of pollution are industrial processes and the deliberate release into the environment of substances such as pesticides. The most visible pollutant, but perhaps not the most significant, is smoke from power stations and factories. Factories may also produce and emit more potent substances in smaller quantities although the level of these is generally controlled. Environmental pollutants may be released into the air, river or sea water or dumped onto land. Car exhaust fumes with several known toxic constituents constitute a major source of pollution.

Pesticides are deliberately sprayed onto crops or agricultural land with the potential for exposure either via the crop itself or through contamination of drinking water or air. With pesticides a major problem is persistence in the environment and an increase in concentration during passage through the food chain.

NATURAL TOXINS

Many plants and animals produce toxic substances for both defensive and offensive purposes. Natural toxins of animal, plant and bacterial origin comprise a wide variety of chemical types, cause a variety of toxic effects and are a significant cause of human poisonings. The concept currently expounded by some individuals that '*natural is safe*' is in many cases very far from the truth and some of the most toxic substances known to man are of natural origin. Natural toxins may feature in poisoning via contamination in food, by accidental ingestion of poisonous plants or animals, and by stinging and biting.

HOUSEHOLD POISONS

These may include some of the substances in the other categories such as pesticides, drugs and solvents. Exposure to these types of compounds is usually acute rather than chronic. Many of the household substances used for cleaning are irritants and some are corrosive.

Consequently, they may cause severe skin and eye lesions to humans if they are exposed. If swallowed in significant quantities or if highly concentrated solutions are ingested, some household materials such as bleach and caustic soda can cause severe tissue damage to the oesophagus and stomach. Some of the drugs and pesticides which are widely available and consequently often found in the home are also very toxic. For example, the herbicide paraquat and the drug paracetamol are both toxic and have both contributed significantly to human poisoning deaths.

Types of exposure

In some cases the means of exposure is determined by the nature of the toxic substance. For example, gases and vapours lead to inhalation exposure whereas liquids give rise to problems associated with skin contact. Many industrial chemicals are often associated with chronic effects due to long-term exposure whereas household substances are usually involved in acute poisoning following a single episode of accidental exposure.

The types of exposure will be briefly discussed at this introductory stage but will be discussed again more fully in later chapters.

INTENTIONAL INGESTION

Drugs and food additives are taken in by many millions of people every day, in some cases for long periods of time. The exposure to these compounds, especially repeated or chronic exposure, may be associated with adverse responses such as allergic reactions. Alcohol and cigarettes are used by many people, often on a long-term basis, and these may lead to chronic toxic effects.

OCCUPATIONAL EXPOSURE

Occupational exposure to toxic compounds is mainly chronic, continual exposure. The route of exposure is either via inhalation or skin contact. Consequently lung disease and dermatitis are common industrial diseases. Acute exposure may occur in the event of an accident such as an explosion, spillage or leakage or because of bad working practices. Cleaning out reactor vessels which have contained solvents may lead to acute toxicity due to excessive exposure for example.

ENVIRONMENTAL EXPOSURE

Effluents from factories, either gaseous or liquid may sometimes briefly, or more often continuously, contaminate our immediate environment and also more distant environments such as the seas and oceans or the atmosphere in other countries. This form of exposure is usually chronic but there have been isolated accidents at factories where acute exposure of humans outside the factory occurs such as at Bhopal and Seveso. Chronic exposure to gases such as sulphur dioxide, nitrogen oxides and carbon monoxide occurs in industrial areas and regions of heavy traffic and may cause acute irritation but the chronic toxic effects are largely unknown.

Environmental exposure is also important in relation to pesticides contaminating air, water and food. Large-scale spraying means that most people are exposed to pesticides or their residues both within their food and directly via the air.

ACCIDENTAL POISONING

This type of exposure is usually acute rather than chronic. Drugs, pesticides, household

products and natural poisons may all be involved in this type of exposure, and children and the elderly are most commonly involved. Mistaken ingestion of a poisonous plant, cleaning fluid or drug falls into this category as does accidental ingestion of an excessive dose of a drug. Inhalation of fumes from fires and stoves is also an important cause of accidental poisoning.

INTENTIONAL POISONING

Fortunately homicide by poisoning is now relatively rare but suicide by poisoning is regrettably all too common. Drugs are commonly used but household products occasionally feature; both types are usually taken by mouth in these circumstances.

SELECTIVE TOXICITY

This is a very important concept in toxicology. It encompasses the differences in susceptibility to toxic effects between different species of animal or plant or between different cells, such as between tumour cells and normal cells.

It is in many cases a useful attribute which is utilized in the design of antibacterial drugs, pesticides or anti-cancer drugs. It is also of relevance to the prediction of toxicity in humans based on studies in another species.

The reasons for selective toxicity are various but can be divided into those due to differences in the absorption, distribution, metabolism and excretion of a chemical (toxicokinetics) or those due to biochemical differences affecting the presence of a receptor or target molecule (toxicodynamics).

For example the insect is more susceptible to the toxicity of **DDT** than mammalian organisms for two reasons. Firstly, the insect cuticle allows DDT to penetrate more readily than the mammalian skin. Secondly, the insect has a greater surface area to volume ratio and therefore absorbs relatively more DDT. Insects are more susceptible to some organophosphorus insecticides because the compound is metabolized by oxidative desulphuration to a compound that inhibits acetylcholinesterase, whereas in mammals enzymatic hydrolysis produces a metabolite that is more readily excreted but is not an inhibitor of acetylcholinesterase.

The rodenticide **norbormide** is active against rats because they possess a receptor in smooth muscle whereas humans, cats and dogs do not. Other **rodenticides** are based more simply on the fact that the rat does not have a vomit reflex, unlike many other mammals. Therefore after the oral ingestion of a poisonous chemical the rat is unable to rid itself of the substance by simply vomiting.

Penicillin is active against certain bacteria because it interferes with synthesis of the cell wall in multiplying bacteria but mammalian cells do not have a cell wall and therefore are not affected.

Dose–response relationship

> 'All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy' **Paracelsus** (1493–1541).

Paracelsus was probably the first to recognize the concept that toxicity is a relative phenomenon and that it depends not only on the toxic properties but on the dose of the compound administered. This relationship between the dose of a compound and the response it elicits is a fundamental concept in toxicology. However, first we must consider the nature of the response itself. The toxic response that is simplest to observe is death but this is a crude parameter to measure. Another indicator of a

toxic response is the presence of a pathological lesion such as liver cell necrosis. A more precisely measured response is a biochemical, pharmacological or chemical change.

We can distinguish between so-called '**all or none**' responses, such as death, and **graded** responses, such as the inhibition of an enzyme or the level of a marker of pathological damage. Both 'all or none' responses and graded responses can show a typical dose–response relation. In both cases there will be a dose at which there is no measurable effect and an upper dose where there is a maximal response. Very often in a toxicity study, either in whole animals or in isolated cells, lethality will be the first parameter of toxicity utilized but this gives little if any information about the underlying mechanism of toxicity. However, it is often important to know the limits of dosing in practical terms. Although it is not always necessary to know the lethal dose, it is important to know whether toxicity occurs at the dose or a multiple of the dose likely to be encountered by man or animals. However in certain situations it is extremely difficult or impossible to quantify the likely human dose and may be similarly difficult to extrapolate the likely effects in man from the available data (see also Chapter 12).

It should be noted that strictly speaking the word dose means the total amount of a substance administered to an organism whereas the term dosage includes a characteristic of the organism, typically body weight or surface area. Dosage is more precise, therefore, and can be related to other organisms, for example as mg substance/kg body weight. We can therefore talk about dosage–response relationships.

With 'all or none' responses (lethality for example) the normal way to determine and represent the dose–response relation is to determine the percentage of the animals or cells in a particular dosage or concentration group which show the response. This response is then plotted against the dosage or concentration resulting in a typical sigmoid curve as illustrated in Figure 1.4. By using probit analysis the data can be plotted as a straight line.

When the response is a graded one the actual values measured are plotted against the dosage or concentration giving the same type of curve (Figure 1.4).

RECEPTORS

Although receptors are known to play an important part in pharmacological responses, with toxic effects at present the role of receptors seems more limited. There are, however, a few well understood receptor toxicant interactions such as that between the aryl hydrocarbon hydrolyase (Ah) receptor and a number of aromatic compounds such as dioxin (TCDD). It is possible that many toxic responses do not involve direct interactions with receptors in the pharmacological sense. It is more likely

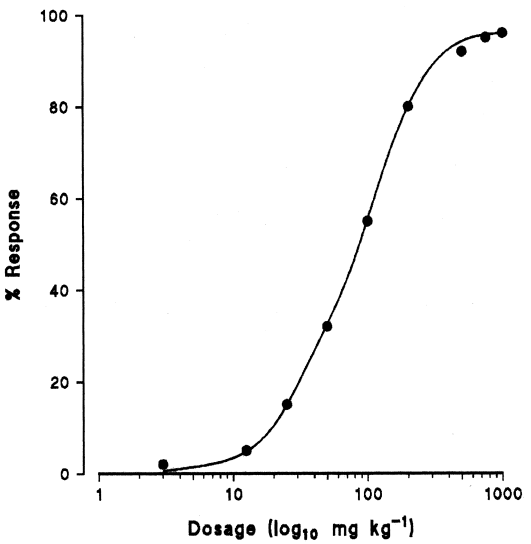

FIGURE 1.4 *A typical dose–response curve where the percentage response is plotted against the log of the dosage.*

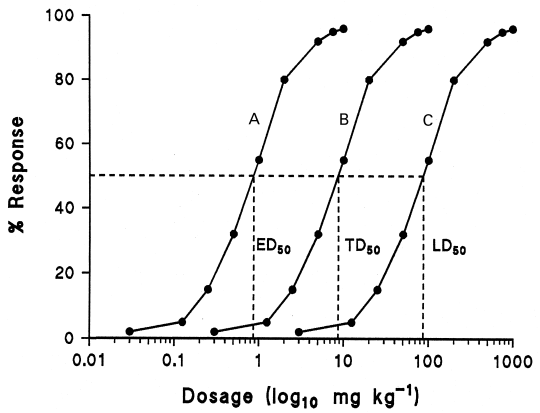

FIGURE 1.5 *Comparison of dose–response curves for efficacy (A), toxicity (B) and lethality (C). The effective, toxic or lethal dosage for 50% of the animals in the group can be estimated as shown. This graph shows the relationship between these parameters. The proximity of the ED_{50} and TD_{50} indicates the margin of safety of the compound.*

that toxic effects result from disturbances in enzyme function and metabolic pathways or damage to structures such as membranes and structural proteins. For some toxicants interaction with a particular protein or enzyme target does underlie the toxic effects. For example, the toxicity of **cyanide** and **carbon monoxide** involve interaction with and disturbance of the function of important proteins (cytochrome aa_3 and haemoglobin, respectively, see Chapter 11). The toxic effects of these two compounds are a direct result of these interactions and the magnitude of the effects, it is assumed, depend on the number of molecules of toxicant bound to the protein. Thus, the more molecules of protein occupied by the chemical, the greater will be the toxic effect. There will be a concentration of the toxic compound at which all of the molecules of the receptor are occupied, however, and hence there will be no further increase in the toxic effect. This relationship gives rise to the classical dose–response curve (Figure 1.4). It is beyond the scope of this book to discuss this in more detail but several of the references in the bibliography may be consulted for more information.

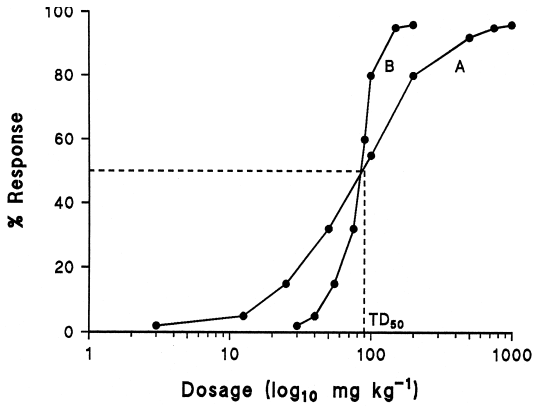

FIGURE 1.6 *Comparison of the toxicity of two compounds A and B. Although they both have the same TD_{50} compound A is more* potent *than compound B.*

Therefore the interpretation of the dose–response relationship is based on certain assumptions:

1 the response is proportional to the concentration at the target site;

2 the concentration at the target site is related to the dose;

3 the response is causally related to the compound administered.

The target site might be a receptor in which case the dose–response relationship may be similar to those observed with pharmacological effects. That is the receptor must be occupied by the toxic compound in order for there to be a response and there will be a point at which all the receptors are occupied, giving the maximum response.

However, with some toxic effects such as the liver necrosis caused by **paracetamol** or **carbon tetrachloride** for instance, although a dose-response relation can be demonstrated there

may be no simple toxicant–receptor interaction which underlies the response.

Thus carbon tetrachloride is probably toxic as a result of a variety of effects including damage to membranes and inhibition of enzymes.

The later events or sequelae may indeed involve receptors which lead to genes being switched on or off or physiological events such as changes in blood pressure.

However, there are some well researched areas where receptors are crucially involved. One already alluded to are the biological effects of **dioxin** and related compounds. Here interaction with the Ah receptor directly leads to increased synthesis of cytochrome P450 (see below) and other effects. The toxicity of dioxin also seems to be related to the receptor interaction (see Chapter 9). The second well documented example of a receptor interaction is the peroxisome proliferators which interact with the **peroxisome proliferator activated receptor** (PPAR). (See Chapter 4.)

Although a toxic response may be observed after exposure to a substance at one particular dose, it is usual to demonstrate responses at several doses of the compound in question and that there is a relationship between the dose and the magnitude of the response.

The shape of the dose–response curve depends on the type of toxic effect measured and the mechanism underlying it. For example, when **cyanide** reacts with cytochrome a_3 it binds irreversibly and curtails the function of the electron transport chain in the mitochondria. As this is a function vital to the life of the cell the dose–response curve for lethality is very steep for cyanide. The more precise the measurement made and the greater the number of determinations the more precise will be the curve and parameters derived from it.

Once a dose–response relationship has been demonstrated there are several parameters which can be derived from it. When lethality has been used as an endpoint, the LD_{50} can be determined (Figure 1.5). This is defined as the dosage of a substance which kills 50 per cent of the animals in a particular group, usually determined in an acute, single exposure study. It is not an exact value and in recent years there has been much discussion as to its usefulness and necessity in toxicology (see Chapter 12). The LD_{50} value may vary for the same compound between different groups of the same species of animal. The value itself is only of real use in a comparative sense, giving the toxicologist an idea of how toxic a compound is relative to other substances (Table 1.1, Figure 1.6) or enabling toxicity to be compared using various routes of administration (Table 1.2) or in different species for example (Table 1.3). It is also widely used for classification purposes,

TABLE 1.1 *Approximate LD_{50} values for a variety of chemical substances*

Compound	LD_{50} mg kg^{-1}
Ethanol	10,000
DDT	100
Nicotine	1
Tetrodotoxin	0.1
Dioxin	0.001
Botulinus toxin	0.00001

Source: T. A. Loomis (1974), *Essentials of Toxicology,* 2nd ed. (Philadelphia: Lea & Febiger).

TABLE 1.2 *Effect of route of administration on the toxicity of various compounds*

	Pentobarbital[1]	Isoniazid[1]	Procaine[1]	DFP[2]
Route of administration	LD_{50} mg kg^{-1}	LD_{50} mg kg^{-1}	LD_{50} mg kg^{-1}	LD_{50} mg kg^{-1}
Oral	280	142	500	4.0
Subcutaneous	130	160	800	1.0
Intramuscular	124	140	630	0.9
Intraperitoneal	130	132	230	1.0
Intravenous	80	153	45	0.3

[1] Mouse toxicity data.
[2] Di-isopropylfluoro phosphate; Rabbit toxicity data.
Source: T. A. Loomis (1968), *Essentials of Toxicology* (Philadelphia: Lea & Febiger).

such as hazard warnings for example. Recently there has been a proposal by the British Toxicology Society for an alternative means of assessing the relative harmfulness of a compound which simply involves dosing a few animals with a range of doses and noting the responses. The chemical can then be classified as for example very toxic, toxic or not very toxic without the use of the LD_{50} test. (For a further discussion see Chapter 12.)

The ED_{50} (effective dosage for 50 per cent) and the TD_{50} (toxic dosage for 50 per cent) are similar parameters to the LD_{50} (Figure 1.5). They can be derived from the dose–response curve where the pharmacological effect or the toxic effect is plotted against dosage instead of lethality. The response can be either a quantal, all-or-none parameter such as death or the presence or absence of a tumour or a graded response such as the inhibition of an enzyme. So the response may be expressed as a proportion of the animals responding or the actual response, respectively.

TABLE 1.3 *Species differences in toxicity of ipomeanol*

		Location of tissue damage		
	LD_{50} mg kg^{-1}*	Liver	Kidney	Lung
Rabbit (New Zealand White)	40	–	–	+
Mouse (A/J Strain)	20	–	+	+
Rat (Fisher Strain)	12	–	–	+
Hamster (Syrian Golden)	140	+	–	+
Guinea Pig (Hartley)	30	–	–	+

*The ipomeanol was administered intraperitoneally in 25% aqueous propylene glycol to all species.
Source: J. S. Dutcher and M. R. Boyd (1979), *Biochem. Pharmacol.* **28**, 3367.

An important parameter in relation to drugs is the **Therapeutic Index**. This is determined from the ratio of either the LD_{50} or TD_{50} and the ED_{50}:

$$\frac{LD_{50}}{ED_{50}} \quad \text{or} \quad \frac{TD_{50}}{ED_{50}}$$

The larger the value the greater is the margin of safety between the dose of drug that is effective pharmacologically and the dose that is toxic. However, the therapeutic index does not give any indication of the shape of the dose–response curves and therefore possible overlap between the toxicity and therapeutic effect. Comparison of the dose–response curves will yield this information however (see Figure 1.5). Comparison of the dose–response curves for different compounds will indicate which is the more hazardous (see Figure 1.6).

SYNERGY AND POTENTIATION

In many cases exposure to chemicals occurs not to a single substance but to mixtures. This is especially so with drugs where a patient may be treated with several drugs at the same time. It may also be the case with exposure to environmental pollutants and industrial chemicals.

The effects of such mixtures may be different from the effects of each constituent separately and consequently may be unpredictable. The simplest situation is when each compound has similar effects and the overall toxicity of the mixture is the sum of the individual toxic effects. The effects are then described as additive. However, this may not necessarily be the case; for example, two substances may cause a greater response together than the sum of the individual responses. This is known as a **synergistic** effect. For example, **carbon tetrachloride** and **alcohol** together are more toxic to the liver than expected from the sum of the two individual toxic effects. Potentiation is a similar effect except that the two compounds in question may have different toxic effects or only one may be toxic. For example, the drug **disulphiram** (antabuse) at non-toxic doses potentiates the toxicity of alcohol and is used for the treatment of alcohol abuse. The drug inhibits the enzyme aldehyde dehydrogenase and so allows an accumulation of acetaldehyde (ethanal) which has unpleasant effects.

The converse effect sometimes observed is a decreased response from a mixture compared with the constituents. This is referred to as antagonism. After repeated exposure, the response may lessen despite similar dosage; tolerance has developed. This may be due to induction of enzymes (see Chapter 2) and hence increased metabolism or to a change in the response or number of receptors. Alternatively repeated exposure can result in accumulation and an exaggerated response.

Such effects must of course be considered when assessing risk from exposure to chemicals and attempting to predict effects.

THE THRESHOLD DOSE AND NO OBSERVED ADVERSE EFFECT LEVEL (NOAEL)

For some compounds and types of toxic effect there will clearly be a dose below which no effect or response is measurable. There is thus a threshold dose. This can be clearly demonstrated for quantal responses such as lethality, the presence or absence of a pathological lesion or a teratogenic effect for example. This means that there will be a dose at which the response does not occur in any individuals in the population (Figure 1.7). Alternatively the concept could apply to a variable response such as enzyme inhibition which can be measured with increasing concentrations of the compound in question.

The concept of a threshold dose for the toxic effect is an important one in toxicology because it implies that there is a 'no observed adverse effect level', or NOAEL. While this is generally accepted for most types of toxic effect, for chemical carcinogenesis mediated via a genotoxic mechanism this is a controversial issue. In the case of such carcinogens the dose–response curve when extrapolated seems to cross the x-axis at the origin rather than at some positive value or dosage level (Figure 1.7). This means that there is a response at all exposure levels tested and so within the limits of the analytical techniques available no safe exposure level can be set with confidence (see Chapter 12).

The NOAEL is important for setting exposure limits. For example, the **acceptable daily intake (ADI)** is based on the NOAEL. This is a factor used to determine the safe intake for food additives and contaminants such as pesticides and residues of veterinary drugs and, hence, to establish the safe level in food (see Chapter 12).

In the industrial setting, exposure is regulated in a similar way and the term used is the **Threshold Limit Value** (**TLV**; USA) or **Maximum Exposure Limit** (**MEL**; UK) which is usually based on exposure for an eight-hour working day (see also Chapters 6 and 12). The NOAEL is usually based on animal toxicity studies with the compound in question, using the most sensitive species and most discriminating test.

It is clear therefore that the dose–response relationship is a crucial concept in toxicology.

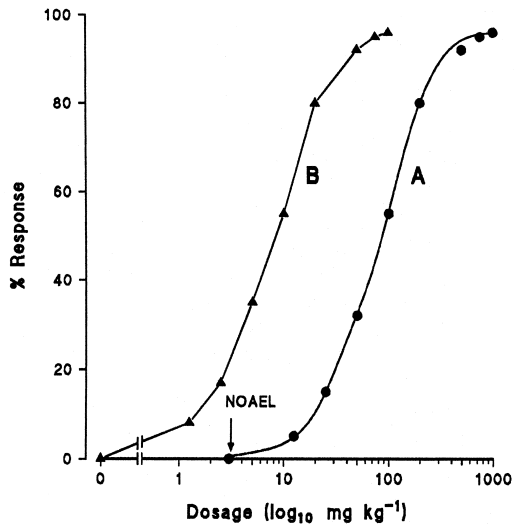

FIGURE 1.7 *Comparison of the dose–response relationships for two compounds A and B. For compound B there is a response at any dose with no threshold. For compound A there is a dose or threshold level below which there is a No Observed Adverse Effect (NOAEL). For compounds such as B there is no safe dose.*

Summary and learning objectives

In this chapter you will have learnt about the ***origins*** of toxicology in antiquity, mainly in relation to intentional poisoning. Some notable figures were mentioned especially Maimonides, Paracelsus, Orfila and Bernard. These individuals all helped toxicology develop from an art into a science. The breadth and scope of toxicology is illustrated by the variety of ***types of toxic substance*** to which we are exposed, ranging from drugs, food additives, industrial chemicals, environmental pollutants, household poisons to natural toxins.

This is also underlined by the ***types of exposure*** such as occupational, accidental or intentional. Toxicity may be ***selective***, affecting different cell types (tumour vs. normal) or species (mammals vs. microorganisms) differently. This concept is used for the design of anticancer drugs, antibiotics and pesticides.

One of the most important concepts for you to remember because it underlies toxicology, is the ***dose–response relationship***. It was first formulated by Paracelsus in his famous phrase 'All substances are poisons, there is none that is not;

the right dose distinguishes a poison from a remedy.' This relationship between the dose of the toxicant and the effect it produces or the toxic response is based on three premises: that the response is proportional to concentration of toxicant at the target site; that the concentration of toxicant is proportional to the dose; that the response is causally related to the toxicant. The target site may be a ***receptor*** with a specific function (e.g. Ah receptor) or an enzyme (e.g. cytochrome aa_3) or a protein (e.g. haemoglobin). However, receptors are not always involved in toxic reactions.

Exposure to chemicals may be to mixtures when ***synergy*** or ***potentiation*** may occur.

Several important parameters can be calculated from the dose–response curve. These are the No Observed Adverse Effect Level (***NOAEL***), which is determined from the bottom of the curve, the maximal effect, and the dose causing a 50 per cent effect or which affects 50 per cent of the animals dosed. This could be a biochemical or pharmacological effect (***ED_{50}***) a toxic effect (***TD_{50}***) or a lethal effect (***LD_{50}***). From these can be determined the ***therapeutic index*** (TD_{50}/ED_{50}) and the margin of safety (TD_1/ED_{99}).

From the NOAEL can be determined the Acceptable Daily Intake (ADI) or Tolerable Daily Intake (TDI) that is involved in the risk assessment of food additives or food contaminants, respectively.

Questions

Q1. Choose one answer which you think is the most appropriate.
A particular dose of a chemical A, is toxic to animals *in vivo*. Another chemical, B, is not toxic even when given at doses several orders of magnitude higher than the dose of A. When A and B are given together at the same dose, the toxic response is greater than that of the dose of A alone.
Is this an example of:

a antagonism
b synergism
c additivity
d potentiation
e none of the above.

Q2. Which information may be gained from an acute toxicity study?

a the No Effect Level
b the LD_{50}
c the therapeutic index
d the target organ
e all of the above.

Q3. The therapeutic index is usually defined as?

a TD_{50}/LD_{50}
b ED_{50}/LD_{50}
c LD_{50}/ED_{50}
d ED_{50}/TD_{50}
e LD_1/ED_{99}.

SHORT ANSWER QUESTIONS

Q4. Explain the following:

a TD_{50}
b dose–response relationship
c therapeutic index
d NOAEL.

Q5. Explain selective toxicity using examples.

Q6. Write notes on the following:

a ED_{50}
b ADI
c margin of safety

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