

## Chapter 1

# Introduction to Toxicology

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## 1.1 INTRODUCTION

Toxicology is the fundamental science of poisons. A poison is generally considered to be any substance that can cause severe injury or death as a result of a physicochemical interaction with living tissue. However, all substances are potential poisons since all of them can cause injury or death following excessive exposure. On the other hand, all chemicals can be used safely if exposure of people or susceptible organisms to chemicals is kept below defined tolerable limits, *i.e.* if handled with appropriate precautions. If no tolerable limit can be defined, zero exposure methods must be used.

Exposure is a function of the amount (or concentration) of the chemical involved, and the time and frequency of its interaction with people or other organisms at risk. For very highly toxic substances, the tolerable exposure may be close to zero. In deciding what constitutes a tolerable exposure, it is essential to have data relating exposure to the production of injury or adverse effect. A problem often arises in deciding what constitutes an injury or adverse effect.

An adverse effect is defined as an abnormal, undesirable or harmful change following exposure to the potentially toxic substance. The ultimate adverse effect is death but less severe adverse effects may include altered food consumption, altered body and organ weights, visible pathological changes or simply altered enzyme levels. A statistically significant change from the normal state of the person at risk is not necessarily an adverse effect. The extent of the difference from normal, the consistency of the altered property and the relation of the altered property to the total well-being of the person affected have to be considered.

An effect may be considered harmful if it causes functional or anatomical damage, irreversible change in homeostasis or increased susceptibility to other chemical or biological stress, including infectious disease. The degree of harm of the effect can be influenced by the state of health of the organism. Reversible changes may also be harmful, but often they are essentially harmless. An effect which is not harmful is usually reversed when exposure to the potentially toxic chemical ceases. Adaptation of the exposed organism may occur so that it can live normally in spite of an irreversible effect.

In immune reactions leading to hypersensitivity or allergic effects, the first exposure to the causative agent may produce no adverse effect, although it sensitizes the organism to respond adversely to future exposures, often at a very low level.

The amount of exposure to a chemical required to produce injury varies over a very wide range depending on the chemical and the form in which it occurs. The extent of possible variation in harmful exposure levels is indicated in Table 1.1, which compares median lethal dose ( $LD_{50}$ ) values for a number of potentially toxic chemicals. The  $LD_{50}$  value is more descriptively called the median lethal dose and is defined below.

The  $LD_{50}$  is the statistically derived single dose of a chemical that can be expected to cause death in 50% of a given population of organisms under a defined set of experimental conditions. Where  $LD_{50}$  values are quoted for human beings, they are derived by extrapolation from studies with mammals or from observations following accidental or suicidal exposures.

The  $LD_{50}$  has often been used to classify and compare toxicity among chemicals but its value for this purpose is limited. A commonly used classification of this kind is shown in Table 1.2. Such a classification is entirely arbitrary and has some intrinsic weaknesses. For example, it is difficult to see why a substance with an  $LD_{50}$  of 200  $mg\ kg^{-1}$  body weight should be regarded only as harmful while one with an  $LD_{50}$  of 199  $mg\ kg^{-1}$  body weight is said to be toxic, when the difference in values is minimal. Further, there is no simple relationship between lethality and sublethal toxic effects. In particular, there is no simple relationship between lethality and effects of great concern, such as cancer or abnormal development of the human

**Table 1.1** *Approximate acute  $LD_{50}$  values for some potentially hazardous substances*

| <i>Substance</i> | <i><math>LD_{50}</math> male rat (<math>mg\ kg^{-1}</math> body weight) oral administration</i> |
|------------------|---|
| Ethanol          | 7000  |
| Sodium chloride  | 3000  |
| Cupric sulphate  | 1500  |
| DDT              | 100   |
| Nicotine         | 60  |
| Tetrodotoxin     | 0.02  |
| Dioxin (TCDD)    | 0.02  |

*Notes:* Values obtained from the Merck Index, Sigma-Aldrich Material Safety Data Sheets (Sigma-Aldrich Library of Chemical Safety Data), and Casarett and Doull's Toxicology. DDT, (1,1,1-trichloro-2,2-bis-(4-chlorophenyl) ethane); TCDD, 2,3,7,8-tetrachloro-dibenzo-p-dioxin.

**Table 1.2** *An example of a classification of toxicity based on acute  $LD_{50}$  values (used in EC directives on classification, packaging and labelling of chemicals)*

| <i>Category</i> | <i><math>LD_{50}</math> orally to rat (<math>mg\ kg^{-1}</math> body weight)</i> |
|-----------------|--|
| Very toxic      | Less than 25   |
| Toxic           | From 25 to 200   |
| Harmful         | From 200 to 2000   |

embryo. Even in relation to lethality, it is not helpful because it gives no measure of the minimum dose that can be lethal and thus no guide to what might be a 'safe' exposure level.

In decisions relating to chemical safety, the toxicity (hazard) of a substance is less important than the risk associated with its use. Risk is the predicted or actual frequency (probability) of a chemical causing unacceptable harm or effects as a result of exposure of susceptible organisms or ecosystems. Assessment of risk is often assessment of the probability and likely degree of exposure.

By comparison with risk, safety is the practical certainty that injury will not result from exposure to a hazard under defined conditions; in other words, the high probability that injury will *not* result. Practical certainty is defined as a numerically specified low risk or socially acceptable risk applied in decision making for risk management.

In assessing permissible exposure conditions for chemicals, uncertainty factors are applied. A threshold of exposure above which an adverse effect can occur (and below which no such effect is observed) is defined from the available data, and this is divided by an uncertainty factor to lower it to a value that regulatory toxicologists can regard as safe beyond doubt. An uncertainty factor may be defined as a mathematical expression of uncertainty that is used to protect populations from hazards that cannot be assessed with high precision. For example, the 1977 report of the US National Academy of Sciences Safe Drinking Water Committee proposed the following guidelines for selecting uncertainty (safety) factors to be used in conjunction with no observed effect level (NOEL) data. The NOEL should be divided by the following uncertainty factors:

1. An uncertainty factor of 10 should be used when valid human data based on chronic exposure are available.
2. An uncertainty factor of 100 should be used when human data are inconclusive, *e.g.* limited to acute exposure histories, or absent, but when reliable animal data are available for one or more species.
3. An uncertainty factor of 1000 should be used when no long-term, or acute human data are available and experimental animal data are scanty.

This approach is subjective and is being continually updated.

Safety control often involves the assessment of 'acceptable' risk since total elimination of risk is often impossible. 'Acceptable' risk is the probability of suffering disease or injury that will be tolerated by an individual, group, or society. Assessment of risk depends on scientific data but its 'acceptability' is influenced by social, economic and political factors, and by the perceived benefits arising from a chemical or process.

## 1.2 EXPOSURE TO POTENTIALLY TOXIC SUBSTANCES

Injury can be caused by chemicals only if they reach sensitive parts of a person or other living organism at a sufficiently high concentration and for a sufficient length of time. Thus, injury depends upon the physicochemical properties of the potentially toxic substances, the exact nature of the exposure circumstances, and the health and developmental state of the person or organism at risk.

Major routes of exposure are through the skin (topical), through the lungs (inhalation) or through the gastrointestinal tract (ingestion). In general, for exposure to any given concentration of a substance for a given time, inhalation is likely to cause more harm than ingestion, which, in turn, will be more harmful than topical exposure. Exposure of the eye can have serious consequences and must also be given due consideration.

### 1.2.1 Skin (Dermal or Percutaneous) Absorption

Many people do not realize that chemicals can penetrate healthy intact skin and so this fact must be emphasized. Among the chemicals that are absorbed through the skin are aniline, hydrogen cyanide, some steroid hormones, organic mercury compounds, nitrobenzene, organophosphate compounds and phenol. Some chemicals, such as phenol or methylmercury chloride, can be lethal if absorbed from a fairly small area (a few square centimetres) of skin. If protective clothing is being worn, it must be remembered that absorption through the skin of any chemical that gets inside the clothing will be even faster than through unprotected skin because the chemical cannot escape and contact is maintained over a longer time.

### 1.2.2 Inhalation

Gases and vapours are easily inhaled but inhalation of particles depends upon their size and shape. The smaller the particle, the further into the respiratory tract it can go. Dusts with an effective aerodynamic diameter of between 0.5 and 7  $\mu\text{m}$  (the respirable fraction) can persist in the alveoli and respiratory bronchioles after deposition. Peak retention depends upon the aerodynamic shape but is mainly of those particles with an effective aerodynamic diameter of between 1 and 2  $\mu\text{m}$ . Particles of effective aerodynamic diameter less than 1  $\mu\text{m}$  tend to be breathed out again but a significant fraction may persist in the alveoli and cause harmful effects. Inhaled particles may also enter the gut (see below).

The effective aerodynamic diameter is defined as the diameter in micrometers of a spherical particle of unit density that falls at the same speed as the particle under consideration. Dusts of larger diameter than 10  $\mu\text{m}$  either do not penetrate the lungs or lodge further up in the bronchioles and bronchi where cilia create a flow of mucus, which returns them to the pharynx and from there they go to the oesophagus. This process is known as the mucociliary clearance mechanism.

From the oesophagus dusts pass through the gut in the normal way. Particles entering the gut in this way may cause poisoning just as though they had been ingested in the food. A large proportion of inhaled dust enters the gut and so its effects through this route must be assessed. A significant portion of inhaled dust consists of microorganisms. There is thus the possibility of bacterial infection. Presence of fungi and their spores may be associated with hypersensitivity responses or with mycotoxins, which may have a range of effects including cancer or even endocrine effects. As with any foodstuff, constituents of dust may affect the gut directly or be absorbed and cause systemic effects.

Physical irritation by dust particles or fibres can cause very serious adverse health effects but most effects depend upon the solids being dissolved. Special

consideration should be given to asbestos fibres which may lodge in the lung and cause fibrosis and cancer even though they are mostly insoluble and therefore do not act like classical toxicants: care should also be taken in assessing possible harm from manmade mineral fibres that have similar properties. The macrophage cells in the lung that normally remove invading bacteria and organic matter may take in insoluble particles. This is called phagocytosis.

Phagocytosis is the process whereby certain body cells, notably macrophages and neutrophils, engulf and destroy invading foreign particles. The cell membrane of the phagocytosing cell (phagocyte) invaginates to capture and engulf the particle. Hydrolytic enzymes are secreted round the particle to digest it and may leak from the phagocyte and cause local tissue destruction if the particle damages the phagocyte. If phagocytic cells are adversely affected by ingestion of insoluble particles, their ability to protect against infectious organisms may be reduced and infectious diseases may follow.

Some insoluble particles such as coal dust and silica dust will readily cause fibrosis of the lung. Others, such as asbestos, may also cause fibrosis depending on the exposure conditions. Fibrosis of the lung leads to breathing problems such as emphysema. It may follow from any chronic inflammation of the lungs and thus be caused even by soluble irritants such as certain metal salts.

Remember that tidal volume (the volume of air inspired and expired with each normal breath) increases with physical exertion; thus absorption of a chemical as a result of inhalation is directly related to the rate of physical work. This is why people living in cities subject to severe air pollution may sometimes be advised to avoid physical activity as far as possible.

### 1.2.3 Ingestion

As mentioned above, airborne particles breathed through the mouth or cleared by the cilia of the lungs are ingested and, outwith the workplace, we may have little control over this apart from avoiding heavily contaminated air, for example by avoiding active or passive smoking. We can keep our homes clean but air pollution in our immediate environment is otherwise beyond individual control. On the other hand, ingestion of potentially toxic substances in our food and drink, or as medication, is under individual control and, by using common sense, we can minimize any associated risks. The nature of the absorption processes following ingestion is discussed elsewhere.

The importance of concentration and time of exposure has already been pointed out. It should be remembered that exposure may be continuous or it may be repeated at intervals over a period of time; the consequences of different patterns of exposure to the same amount of a potentially toxic substance may vary considerably in their seriousness. In most cases, the consequences of continuous exposure to a given concentration of a chemical will be worse than those of intermittent exposures to the same concentration of the chemical at intervals separated by sufficient time to permit a degree of recovery. However, repeated or continuous exposure to very small amounts of potentially toxic chemicals may be a matter for serious concern if either the chemical or its effects, *e.g.* decreasing numbers of nerve cells, have a tendency to accumulate in the person or organism at risk.

A chemical may accumulate if absorption exceeds excretion; this is particularly likely with substances that combine a fairly high degree of lipid solubility with chemical stability. Such chemicals are found in the group of persistent organic pollutants (POPS), including several organochlorine pesticides, which are now largely, but not entirely, banned from use. Accumulation of water-soluble ions may also be a problem. Divalent lead ions accumulate in bone where they replace the chemically similar calcium ions. While in the bone, they cause little harm but when bone breaks down, during pregnancy or illness, the lead ions enter the blood and may poison the person who has accumulated them or, in the case of pregnancy, the unborn child. Fluoride ions also accumulate in bone and this is of concern in regard to schemes for water fluoridation.

### 1.3 ADVERSE EFFECTS

Adverse effects may be local or systemic. Local effects occur at the site of exposure of the organism to the potentially toxic substance. Corrosives always act locally. Irritants frequently act locally.

Most substances that are not highly reactive are absorbed and distributed around the affected organism causing systemic injury at a target organ or tissue distinct from the absorption site. The target organ is not necessarily the organ of greatest accumulation. For example, adipose (fatty) tissue accumulates organochlorine pesticides to very high levels but does not appear to be affected by them.

Some substances produce both local and systemic effects. For example, tetraethyl lead damages the skin on contact, and is then absorbed and transported to the central nervous system where it causes further damage.

Effects of a chemical can accumulate even if the chemical itself does not. There is some evidence that this is true of the effects of organophosphate pesticides and other neurotoxins on the nervous system. This may lead to poor functioning of the nervous system in humans in old age. Because of the time difference between exposure and effect, establishing the relationship between such delayed effects and the possible cause, no longer present in the body, is often difficult.

A particularly harmful effect that may accumulate is death of nerve cells, since nerve cells cannot be replaced, though damaged nerve fibres can be regenerated.

It will be clear that the balances between absorption and excretion of a potentially toxic substance and between injury produced and repair are the key factors in determining whether any injury follows exposure. All of the possible adverse effects cannot be discussed here but some aspects should be mentioned specifically.

Production of mutations, tumours and cancer and defects of embryonic and fetal development are of particular concern.

Adverse effects related to allergies appear to be increasing. Allergy (hypersensitivity) is the name given to disease symptoms following exposure to a previously encountered substance (allergen) that would otherwise be classified as harmless. Essentially, an allergy is an adverse reaction of the altered immune system. The process, which leads to the disease response on subsequent exposure to the allergen, is called sensitization. Allergic reactions may be very severe and fatal.

To produce an allergic reaction, most chemicals must act as haptens, *i.e.* combine with proteins to form antigens. Antigens entering the human body or produced within it cause the production of antibodies. Usually at least a week is needed before appreciable amounts of antibodies can be detected and further exposure to the allergen can produce disease symptoms. The most common symptoms are skin ailments such as dermatitis and urticaria, or eye problems such as conjunctivitis. The worst may be death resulting from anaphylactic shock.

Of particular importance in considering the safety of individuals is the possibility of idiosyncratic reactions. An idiosyncratic reaction is an excessive reactivity of an individual to a chemical, for example, an extreme sensitivity to low doses as compared with the average member of the population. There is also the possibility of an abnormally low reactivity to high doses. An example of a group of people with an idiosyncrasy is the group that has a deficiency in the enzyme required to convert methaemoglobin (which cannot carry oxygen) back into haemoglobin; this group is exceptionally sensitive to chemicals like nitrites, which produce methaemoglobin. Idiosyncratic reactions may occur to foodstuffs and to prescription drugs, giving harmful effects, which may be wrongly ascribed to chemicals in the environment or in the workplace.

Another factor to be considered is whether the adverse effects produced by a potentially toxic chemical are likely to be immediate or delayed. Immediate effects appear rapidly after exposure to a chemical while delayed effects appear only after a considerable lapse of time.

Among the most serious delayed effects are cancers; carcinogenesis may take 20 or more years before tumours are seen in humans.

Perhaps the most difficult adverse effects to detect are those that follow years after exposure in the womb: a well-established example of such an effect is the vaginal cancer produced in young women whose mothers were prescribed diethylstilbestrol during pregnancy in order to prevent a miscarriage.

Another important aspect of adverse effects to be considered is whether they are reversible or irreversible. For the liver, which has a great capacity for regeneration, many adverse effects are reversible, and complete recovery can occur. For the central nervous system, in which regeneration of tissue is severely limited, most adverse effects leading to morphological changes are irreversible and recovery is, at best, limited. Carcinogenic and teratogenic effects are also irreversible, but suitable treatment may reduce the severity of such effects.

#### 1.4 CHEMICAL INTERACTIONS

A major problem in assessing the likely effect of exposure to a chemical is that of assessing possible interactions. The simplest interaction is an additive effect: this is an effect, which is the result of two or more chemicals acting together and is the simple sum of their effects when acting independently. In mathematical terms,

$$1 + 1 = 2, \quad 1 + 5 = 6, \text{ etc.}$$

The effects of organochlorine pesticides are usually additive.

More complex is a synergistic (multiplicative) effect: this is an effect of two chemicals acting together, which is greater than the simple sum of their effects when acting alone; it may be called synergism. In mathematical terms,

$$1 + 1 = 4, \quad 1 + 5 = 10, \text{ etc.}$$

Asbestos fibres and cigarette smoking act together to increase the risk of lung cancer by a factor of 40, taking it well beyond the risk associated with independent exposure to either of these agents.

Another possible form of interaction is potentiation. In potentiation, a substance that on its own causes no harm makes the effects of another chemical much worse. This may be considered to be a form of synergism. In mathematical terms,

$$0 + 1 = 5, \quad 0 + 5 = 20, \text{ etc.}$$

For example, isopropanol, at concentrations that are not harmful to the liver, increases (potentiates) the liver damage caused by a given concentration of carbon tetrachloride.

The opposite of synergism is antagonism: an antagonistic effect is the result of a chemical counteracting the adverse effect of another; in other words, the situation where exposure to two chemicals together has less effect than the simple sum of their independent effects. Such chemicals are said to show antagonism. In mathematical terms,

$$1 + 1 = 0, \quad 1 + 5 = 2, \text{ etc.}$$

## 1.5 TOLERANCE AND RESISTANCE

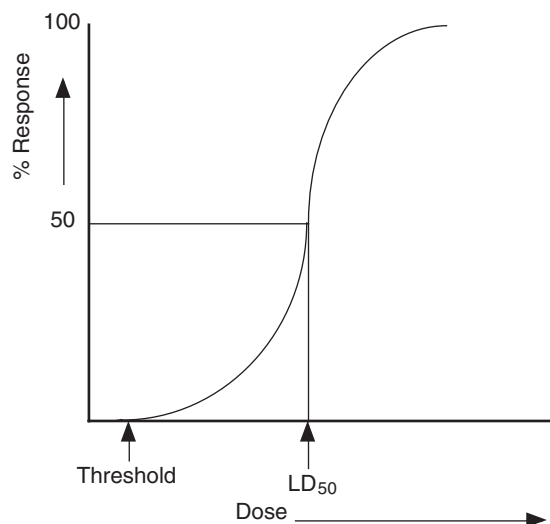
Tolerance is a decrease in sensitivity to a chemical following exposure to it or to a structurally related substance. For example, cadmium causes tolerance to itself in some tissues by inducing the synthesis of the metal-binding protein, metallothionein. However, it should be noted that cadmium–metallothionein accumulates in the kidney where it causes nephrotoxicity.

Resistance is almost complete insensitivity to a chemical. It usually reflects the metabolic capacity to inactivate and eliminate the chemical and its metabolites rapidly.

## 1.6 TOXICITY TESTING

### 1.6.1 Dose–Response and Concentration–Response

A dose–response (concentration–response) relationship is defined as the association between dose (concentration) and the incidence of a defined biological effect in an exposed population, usually expressed as percentage. Historically the defined effect was death. The classic dose–response or concentration–response relationship is shown in Figure 1.1. This is a theoretical curve and in practice such a Gaussian curve is rarely found. Curves of this kind form the basis for the determination of the  $LD_{50}$  or the  $LC_{50}$  (the median lethal concentration). The  $LD_{50}$  and  $LC_{50}$  are specific cases of the generalized values  $LD_n$  and  $LC_n$ . The  $LD_n$  is the dose of a toxicant lethal to  $n\%$  of a test population. The  $LC_n$  is the exposure concentration of a toxicant lethal to  $n\%$  of a test population. Thus, the  $LD_{50}$  is the statistically derived single dose of

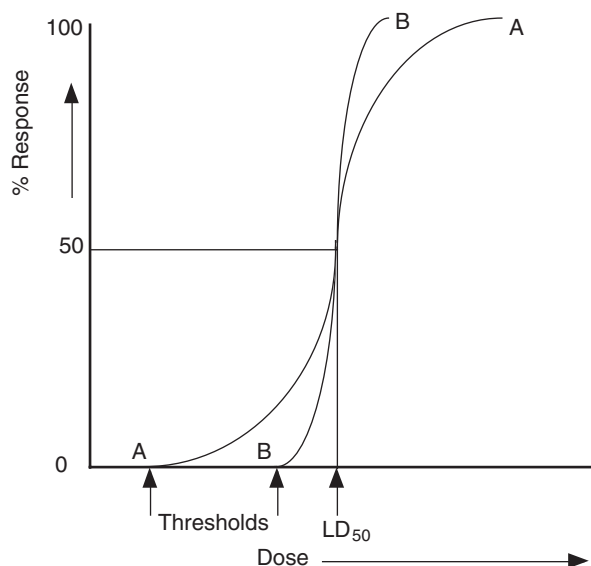


**Figure 1.1** *The classic dose–response curve*

a chemical that can be expected to cause death in 50% of a given population of organisms under a defined set of experimental conditions. Similarly, the  $LC_{50}$  is the statistically derived exposure concentration of a chemical that can be expected to cause death in 50% of a given population of organisms under a defined set of experimental conditions.

Another important value that may be derived from the relationship shown is the threshold dose or concentration, the minimum dose or concentration required to produce a detectable response in the test population. The threshold value can never be derived with absolute certainty and therefore the lowest observed effect level (LOEL) or the NOEL have normally been used instead of the threshold value in deriving regulatory standards. There is a move to replace these values by the benchmark dose (BMD). This is defined as the statistical lower confidence limit on the dose that produces a defined response (called the benchmark response or BMR, usually 5 or 10%) in a given population under defined conditions for an adverse effect compared to background, defined as 0%.

The use of the  $LD_{50}$  in the classification of potentially toxic chemicals has been described; it must be emphasized that such a classification is only a very rough guide to relative toxicity. The  $LD_{50}$  tells us nothing about sublethal toxicity. Any classification based on the  $LD_{50}$  is strictly valid only for the test population and conditions on which it is based and on the related route of exposure. The  $LD_{50}$  tells us nothing about the shape of the dose–response curve on which it is based. Thus, two chemicals may appear to be equally toxic since they have the same  $LD_{50}$ , but one may have a much lower lethal threshold and kill members of an exposed population at concentrations where the other has no effect (Figure 1.2). Remember, these are theoretical curves and in practice Gaussian curves of this sort are rarely, if ever, found.



**Figure 1.2** Two substances with the same  $LD_{50}$  but different lower lethal thresholds

The determination and use of the  $LD_{50}$  are likely to decline in future as fixed dose testing becomes more widely used. In fixed dose testing, the test substance may be administered to rats or other test species at no more than three dose levels: the possible dose levels are preset legally to equate with a regulatory classification or ranking system. Dosing is followed by an observation period of 14 days. The dose at which toxic signs are detected is used to rank or classify the test materials.

A retrospective study of  $LD_{50}$  values showed that between 80 and 90% of those compounds which produced signs of toxicity but no deaths at dose levels of 5, 50 or 500  $mg\ kg^{-1}$  body weight oral administration had  $LD_{50}$  values from the same studies of more than 25, from 25 to 200, or from 200 to 2000  $mg\ kg^{-1}$  body weight, corresponding to the European Union classification for very toxic, toxic and harmful.

The initial test dose level is selected with a view to identifying toxicity without mortality occurring. Thus, if a group of five male and five female rats is tested with an oral dose of 500  $mg\ kg^{-1}$  body weight and no clear signs of toxicity appear, the substance should not be classified in any of the defined categories of toxicity.

If toxicity is seen but no mortality, the substance can be classed as 'harmful'. If mortality occurs, retesting with a dose of 50  $mg\ kg^{-1}$  body weight is required. If no mortality occurs at the lower dose but signs of toxicity are detected, the substance would be classified as 'toxic'. If mortality occurs at the lower dose, retesting at 5  $mg\ kg^{-1}$  body weight would be carried out and if signs of toxicity were detected and mortality occurred, the substance would be classified as 'very toxic'.

For a full risk assessment, testing at 2000  $mg\ g^{-1}$  body weight is also required if no signs of toxicity are seen at 500  $mg\ kg^{-1}$  body weight.

Fixed dose testing reduces the number of animals required and, because mortality need not occur, also greatly reduces possible animal suffering. Fixed dose testing can also identify substances that have high  $LD_{50}$  values but still cause acute toxic effects at relatively low doses or exposures.

In assessing the significance of  $LD_{50}$  or other toxicological values, it is necessary to note the units used in expressing dosage. Normally dosage is expressed in  $mg\ kg^{-1}$  body weight, but it may be expressed as  $mg\ cm^{-2}$  body surface area as this has been shown in a number of cases to permit more accurate extrapolation between animals of different sizes and from test mammalian species to humans.

For biocides, selective toxicity is the key property, since they are to be used to kill pests with minimal harm to other organisms. Selective toxicity depends upon differences in biological characteristics that may be either quantitative or qualitative. Minimizing the amount of pesticide used and targeting its application is crucial to avoid harm to non-target organisms.

Although now applied to many species, toxicity testing was originally aimed at establishing, by tests on laboratory animals, what effects chemicals are likely to have on human beings who may be exposed to them and the shape of the dose–response relationship. On a body weight basis, it is assumed for toxicity data extrapolation that humans are usually about 10 times more sensitive than rodents. On a body surface–area basis, humans usually show about the same sensitivity as test mammals, *i.e.* the same dose per unit of body surface area will give the same given defined effect, in about the same percentage of the population. Knowing the above relationships, it is possible to estimate the exposure to a chemical that humans should be able to tolerate.

In many countries there is now a defined set of tests that must be carried out on every new chemical that is to be used or produced in an appreciable quantity, usually above 1 tonne/year. Table 1.3 gives an example of test requirements applicable in a number of such countries.

## 1.7 EPIDEMIOLOGY AND HUMAN TOXICOLOGY

Epidemiology is the analysis of the distribution and determinants of health-related states or events in human populations and the application of this study to the control of health problems. It is the only ethical way to obtain data about the effects of chemicals other than drugs on human beings and hence to establish beyond doubt that toxicity to humans exists. The following are the main approaches that have been used in epidemiology.

### 1.7.1 Cohort Study

A cohort is a component of the population born during a particular period and identified by the period of birth so that its characteristics (such as causes of death and numbers still living) can be ascertained as it enters successive time and age periods. The term ‘cohort’ has broadened to describe any designated group of persons followed or traced over a period of time.

In a cohort study, one identifies cohorts of people who are, have been, or in the future may be exposed or not exposed, or exposed in different degrees, to a factor or

**Table 1.3** Example of the information required in some countries for notification and hazard assessment of new chemicals

| <i>Base set information</i> |  |
|-----------------------------|--|
| 1                           | Identity of the substance  |
| 1.1                         | Name   |
| 1.1.1                       | Names in the IUPAC nomenclature  |
| 1.1.2                       | Other names (usual name, trade name, abbreviation)   |
| 1.1.3                       | CAS number and CAS name (if available)   |
| 1.2                         | Empirical and structural formula   |
| 1.3                         | Composition of the substance   |
| 1.3.1                       | Degree of purity (%)   |
| 1.3.2                       | Nature of impurities, including isomers and by-products  |
| 1.3.3                       | Percentage of (significant) main impurities  |
| 1.3.4                       | If the substance contains a stabilizing agent or an inhibitor or other additives, specify: nature, order of magnitude: ... ppm; ...%   |
| 1.3.5                       | Spectral data (UV, IR, NMR or mass spectrum)   |
| 1.3.6                       | Chromatographic data (HPLC, GC)  |
| 1.4                         | Methods of detection and determination<br>A full description of the methods used or the appropriate bibliographical references   |
| 2                           | Information on the substance   |
| 2.0                         | Production (process, quantity and estimate of resultant exposure)  |
| 2.1                         | Proposed uses  |
| 2.1.1                       | Types of use<br>Describe: the function of the substance and the desired effects (including processes, form marketed, quantity and exposure estimate)   |
| 2.1.2                       | Fields of application with approximate breakdown <ul style="list-style-type: none"> <li>– Industries</li> <li>– Farmers and skilled trades</li> <li>– Use by the public at large</li> </ul>                          |
| 2.1.3                       | Waste quantities and composition of waste  |
| 2.2                         | Estimated production and imports for each of the anticipated uses or fields of application   |
| 2.2.1                       | Overall production and/or imports in tonnes per year <ul style="list-style-type: none"> <li>– First 12 months</li> <li>– Thereafter</li> </ul>   |
| 2.2.2                       | Production and/or imports, broken down in accordance with 2.1.1 and 2.1.2, expressed as a percentage <ul style="list-style-type: none"> <li>– First calendar year</li> <li>– The following calendar years</li> </ul> |
| 2.3                         | Recommended methods and precautions concerning:  |
| 2.3.1                       | Handling   |
| 2.3.2                       | Storage  |
| 2.3.3                       | Transport  |
| 2.3.4                       | Fire (nature of combustion gases or pyrolysis, where proposed uses justify)  |
| 2.3.5                       | Other dangers, particularly chemical reaction with water or tendency to explode as a dust  |

**Table 1.3** (Continued)

| <i>Base set information</i> |  |
|-----------------------------|--|
| 2.4                         | Emergency measures in the case of accidental spillage  |
| 2.5                         | Emergency measures in the case of injury to persons ( <i>e.g.</i> poisoning)   |
| 3                           | Physicochemical properties of the substance  |
| 3.0                         | State of the substance at 20 °C and 101.3 kPa  |
| 3.1                         | Melting point  |
| 3.2                         | Boiling point °C at ... Pa   |
| 3.3                         | Relative density ( $D_4^{20}$ )  |
| 3.4                         | Vapour pressure Pa at ... °C   |
| 3.5                         | Surface tension $N\ m^{-1}$ (...°C)  |
| 3.6                         | Water solubility $mg\ l^{-1}$ (...°C)  |
| 3.7                         | Fat solubility<br>Solvent oil (to be specified) $mg\ 100\ g^{-1}$ solvent (...°C)  |
| 3.8                         | Partition coefficient <i>n</i> -Octanol/water  |
| 3.9                         | Flashpoint ... °C. Open cup and closed cup   |
| 3.10                        | Flammability   |
| 3.11                        | Explosive properties   |
| 3.12                        | Self ignition temperature ... °C   |
| 3.13                        | Oxidizing properties   |
| 3.14                        | Granulometry (particle size distribution)  |
| 4                           | Toxicological studies  |
| 4.1                         | Acute toxicity<br>Substances other than gases shall be administered <i>via</i> two routes at least one of which should be the oral route. The other route will depend on the intended use and on the physical properties of the substance. Gases and volatile liquids should be administered by inhalation. In all cases, observation of the animals should be carried out for at least 14 days. Unless there are contraindications, the rat is the preferred species for oral and inhalation experiments. The experiments in 4.1.1, 4.1.2, and 4.1.3 shall be carried out on both male and female subjects. |
| 4.1.1                       | Administered orally<br>$LD_{50}$ ( $mg\ kg^{-1}$ ) or acceptable alternative<br>Effects observed, including in the organs  |
| 4.1.2                       | Administered by inhalation<br>$LC_{50}$ (ppm) or acceptable alternative<br>Duration of exposure in hours<br>Effects observed, including in the organs  |
| 4.1.3                       | Administered cutaneously (percutaneous absorption)<br>$LD_{50}$ ( $mg\ l^{-1}$ ) or acceptable alternative<br>Effects observed, including in the organs  |
| 4.1.4                       | Skin irritation<br>The substance should be applied to the shaved skin of an animal, preferably an albino rabbit.<br>Duration of exposure in hours  |
| 4.1.5                       | Eye irritation. The rabbit is the preferred animal. Duration of exposure in hours  |
| 4.1.6                       | Skin sensitization. To be determined by a recognized method using a guinea pig.  |

**Table 1.3** (Continued)

| <i>Base set information</i> |   |
|-----------------------------|---|
| 4.2                         | <p>Repeated dose toxicity</p> <p>The route of administration should be the most appropriate considering the intended use, the acute toxicity and the physical and chemical properties of the substance. Unless there are contraindications, the rat is the preferred species for oral and inhalation experiments.</p> |
| 4.2.1                       | <p>Repeated dose toxicity (28 days)</p> <p>Effects observed on the animal and organs according to the concentrations used, including clinical and laboratory investigations.</p> <p>Dose for which no toxic effect is observed.</p>   |
| 4.3                         | <p>Other effects</p>  |
| 4.3.1                       | <p>Mutagenicity (including carcinogenic pre-screening test)</p> <p>The substance should be examined during a series of two tests, one of which should be bacteriological, with and without metabolic activation, and one non-bacteriological with and without metabolic activation</p>                                |
| 4.3.2                       | <p>Screening for toxicity related to reproduction</p>   |
| 4.3.3                       | <p>Assessment of toxicokinetic behaviour of the substance</p>   |
| 5                           | <p>Ecotoxicological studies</p>   |
| 5.1                         | <p>Effects on organisms</p>   |
| 5.1.1                       | <p>Acute toxicity for fish</p>  |
| 5.1.2                       | <p>Acute toxicity for Daphnia LC<sub>50</sub></p>   |
| 5.1.3                       | <p>Growth inhibition test on algae</p>  |
| 5.1.4                       | <p>Bacteriological inhibition</p>   |
| 5.2                         | <p>Degradation: biotic and abiotic</p>  |
| 5.3                         | <p>Absorption/desorption screening test</p>   |
| 6                           | <p>Possibility of rendering the substance harmless</p>  |
| 6.1                         | <p>For industry/skilled trades</p>  |
| 6.1.1                       | <p>Possibility of recycling</p>   |
| 6.1.2                       | <p>Possibility of neutralization of harmful effects</p>   |
| 6.1.3                       | <p>Possibility of destruction:</p> <ul style="list-style-type: none"> <li>– Controlled discharge</li> <li>– Incineration</li> <li>– Water purification station</li> <li>– Others</li> </ul>   |
| 6.2                         | <p>For the public at large</p>  |
| 6.2.1                       | <p>Possibility of recycling</p>   |
| 6.2.2                       | <p>Possibility of neutralization of harmful effects</p>   |
| 6.2.3                       | <p>Possibility of destruction:</p> <ul style="list-style-type: none"> <li>– Controlled discharge</li> <li>– Incineration</li> <li>– Water purification station</li> <li>– Others</li> </ul>   |

factors hypothesized to influence the probability of occurrence of a given disease or other outcome. Alternative terms for such a study – follow-up, longitudinal and prospective study – describe an essential feature of the method, observation of the population for a sufficient number of person-years to generate reliable incidence or mortality rates in the population subsets. This generally means studying a large population, studying for a prolonged period (years), or both.

Cohort studies involve cohort analysis. Cohort analysis is the tabulation and analysis of morbidity or mortality rates in relationship to the ages of the members of the cohort, identified by their birth period, and followed as they pass through different ages during part or all of their life span. Under certain circumstances, such as studies of migrant populations, cohort analysis may be performed according to duration of residence in a country rather than year of birth, in order to relate health or mortality experience to duration of exposure.

### **1.7.2 Retrospective Study**

A retrospective study is used to test hypotheses of cause (aetiological hypotheses) in which inferences about exposure to the putative causal factor(s) are derived from data relating to characteristics of the persons or organisms under study or to events or experiences in their past: the essential feature is that some of the persons under study have the disease or other outcome condition of interest, and their characteristics and past experiences are compared with those of other, unaffected persons. Persons who differ in the severity of the disease may also be compared.

### **1.7.3 Case Control Study**

A case control study starts with the identification of persons with the disease (or other outcome variable) of interest, and a suitable control (comparison and reference) group of persons without the disease. The relationship of an attribute to the disease is examined by comparing the diseased and non-diseased with regard to how frequently the attribute is present or, if quantitative, the levels of the attribute, in the two groups.

### **1.7.4 Cross-Sectional Study (of Disease Prevalence and Associations)**

A cross-sectional study examines the relationship between diseases (or other health-related characteristics) and other variables of interest as they exist in a defined population at one particular time. Disease prevalence rather than incidence is normally recorded in a cross-sectional study and the temporal sequence of cause and effect cannot necessarily be determined.

### **1.7.5 Confounding**

Confounding is one of the biggest difficulties in carrying out a successful epidemiological investigation.

Confounding can occur in a number of different ways. First, there is the situation in which the effects of two processes are not distinguishable from one another: this

leads to the situation where the distortion of the apparent effect of an exposure on risk is brought about by the association of other factors that can influence the outcome. Secondly, there is the possibility of a relationship between the effects of two or more causal factors as observed in a set of data, such that it is not logically possible to separate the contribution that any single causal factor has made to an effect. Finally, there is the situation in which a measure of the effect of an exposure on risk is distorted because of the association of exposure with other factor(s) that influence the outcome under study.

A confounding variable (confounder) is defined as a changing factor that can cause or prevent the outcome of interest, is not an intermediate variable, and is not associated with the factor under investigation, such a variable must be controlled in order to obtain an undistorted estimate of the effect of the study factor on risk.

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