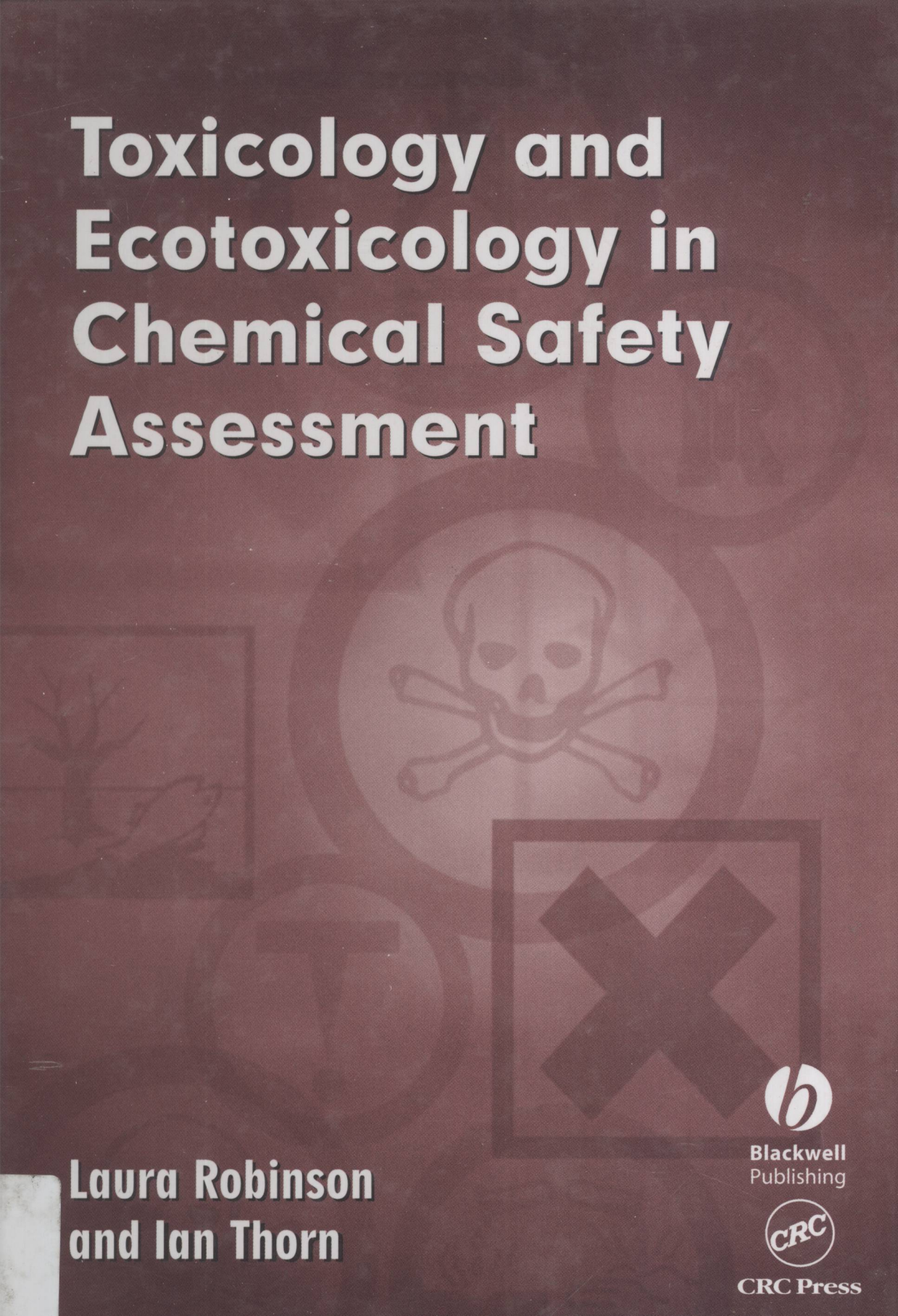
Toxicology and Ecotoxicology in Chemical Safety Assessment



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Toxicology and Ecotoxicology in Chemical Safety Assessment

by

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Preface

Toxicology and ecotoxicology in chemical safety assessment aims to provide the suppliers and recipients of chemicals with the means to interpret and apply toxicological and ecotoxicological information. This information, primarily provided by the supplier, should be employed to improve the understanding, and therefore the awareness, of the implications of handling a given chemical and the possible effect that handling could have on the environment or upon human health. Central to the awareness of the intrinsic hazards of these chemicals is the knowledge of test protocols and the legal requirements of various regulatory bodies.

The implications of chemical usage on human health and the environment are constantly under review in the media, e.g. the effect of aerosols on the ozone layer and the ongoing suffering caused by asbestos use. The media has also highlighted the dangers of gene-modified foods. In this atmosphere, it is not surprising that there is a growing awareness and concern about the handling and use of chemicals.

Responsible chemical suppliers continually supply their customers with the information necessary for the safe handling and end-use of their products. Those purchasing chemicals are normally provided with a Safety Data Sheet, which should indicate any possible adverse effects on those handling chemicals, both directly and indirectly, and on the environment. However, as chemicals are often manufactured in different parts of the world, the information provided by the test results in the Safety Data Sheet may be based on different guidelines to those required by the regulations current in the country of use. This can result in some confusion and may hinder interpretation of the latent hazards.

This book provides health and safety managers, technical managers and anyone supervising the handling of chemicals with the means of interpreting the data provided by the suppliers for the safe handling of chemicals and the background and test methods used to prepare them.

In conclusion, we hope that this book makes a contribution to the safety of workers in the industry and the environment surrounding it.

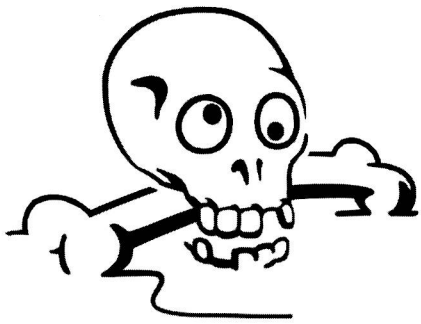
Contents

1 Toxicology

1.1 Introduction

Toxicology can be defined as the study of poisons, which to many probably conjures up images of witches hovering around a cauldron, cooking up a lethal potion for some unsuspecting victim!

Therefore, perhaps a better and more appropriate definition for the purpose of this book is:



Toxicology is the study of adverse effects of chemicals on living organisms.

Owing to the strict legislation that operates in most countries, chemical companies wishing to introduce their products onto the market have to assess firstly the hazards associated with them. (In this context, 'hazards' means adverse health effects.)

Toxicity can be defined as the 'inherent capacity of a chemical to cause adverse health effects'. Therefore, with relevance to humans, toxicologists are employed to investigate the potential adverse effects and associated mechanisms of toxicity arising from exposure to chemicals.

For toxicity to occur there has to be exposure to the chemical and there are three main ways in which this can occur: inhalation, skin contact and ingestion. Some chemicals cause toxicity directly whereas others require conversion to a more reactive form. Once *exposure* has taken place a chemical can cause *local effects* such as irritation at the site of contact, otherwise it may be *absorbed* into the bloodstream and distributed to other parts of the body which could result in *systemic effects* (see Table 1.1). Note that it is not uncommon for a chemical to cause both local and systemic effects.

Table 1.1 Two common types of adverse effects caused by chemicals.

Local effects	These occur at the point of exposure. Examples include irritation and corrosivity.
Systemic effects	Effects that occur in cells, tissues or organs. Effects occurring in specific organs are called 'Target Organ Effects'. For instance, carbon tetrachloride (tetrachloromethane) is a hepatotoxin (causes toxicity in the liver), although a chemical may affect more than one target organ.

Not all chemicals can be absorbed, and there can be large differences in the extent and rate at which absorption occurs. As an example, there is nearly no absorption whatsoever for most polymers, whereas for other chemical substances such as alcohol, full absorption occurs.

Once inside the body a chemical may be *metabolised*, stored or else *excreted*. Metabolism involves enzymes that convert the chemical into a form (called a metabolite) which is more readily excreted. However, the problem with metabolism is that it can sometimes convert the chemical into a more reactive toxic form. Some chemicals can also be stored within the body, which can in some cases cause cumulative toxicity. Excretion (in the breath, urine or faeces) leads to the removal of the chemical or its metabolites from the body.

1.1.1 Toxicity testing

In order to protect the general population, many countries have strict requirements for the hazardous nature of chemicals to be established prior to their use. The aim of toxicological testing is to predict the toxicity in man by using animals or cell cultures as surrogates. The tests themselves aim to mimic as closely as possible the likely exposure scenario that a human being would face, using the most appropriate animal model (or in some cases, cell cultures). There are standard test procedures, which can be used for toxicological evaluations and some of these procedures use animals, whilst others use cultured cells and other *in vitro* techniques. Throughout this book, the Organisation for Economic Co-operation and Development (OECD) test guidelines will be referred to as these are accepted in most countries.

Different species of animals are used in toxicity testing because there is no one animal that is satisfactory for use in the evaluation of all toxicological effects. The choice will depend on the type of toxicity to be investigated, although other factors such as availability, cost and reliability in their response will also influence the choice. Both sexes are usually employed although in some cases tests may be gender-specific. In tests where more than one dose level is used, they are selected so as to establish a *dose–response relationship* and *threshold level* of effect. Note that these terms will be described more fully in subsequent sections.

The aim of the following sections is to describe some of the different toxicological effects that can arise as a result of chemical exposure. This will, hopefully, enable the reader to better understand and use the toxicological information provided by chemical suppliers.

1.2 Toxicity

Many people perceive all chemicals as being bad in some way or another. The expression 'toxic chemicals' is often used in everyday life, and questions concerning whether or not one particular chemical can cause more harm than another are frequently raised. The kinds of questions raised were studied as far back as the sixteenth century by a Swiss scientist named Paracelsus and the results of his work are now considered to be an underlying theme in the field of toxicology. He concluded that in sufficient quantities everything has the potential to cause adverse health effects and that the only thing that differentiates something from being harmful or not is the dose. In other words, 'it is the dose which makes the poison'.

This may seem like a strange concept as many of us have the idea that when dealing with a 'toxic chemical', its mere presence in our vicinity can cause harm to us. But this is not the case and, as was written by Paracelsus, it depends on the exposure dose as there will be a dose level below which this 'toxic chemical' will not cause us harm. (There are exceptions, however – genotoxic carcinogens are believed to be active at all dose levels.) For some chemicals such as arsenic, which features in so many murder/suspense movies, this dose level is very low, whereas for other chemicals such as sugar, the dose level is much higher because it is less toxic. Conversely, so-called 'harmless' chemicals, which are around us all the time, such as water, oxygen and even those which we may daily use in the kitchen such as table salt, have the potential to cause us harm should the amount to which we are exposed be sufficiently high.

1.2.1 Acute and chronic effects

Toxicity can be defined as the propensity to cause harm (or adverse health effects). There are two main types of toxicity that need to be taken into consideration when studying the adverse effects of chemicals. These are acute toxicity and chronic toxicity.

- *Acute toxicity* describes the adverse health effects following a single or limited number of exposures.
- *Chronic toxicity* describes the adverse health effects resulting from continuous or intermittent exposure over a lifetime. An example of chronic toxicity is organ damage, such as liver cirrhosis arising from long-term alcohol abuse.

Both acute and chronic toxicities are very different with respect to how they occur, i.e. their manifestation, the target organs involved and also the resulting adverse effects.

For example, acute effects tend to appear quickly and can be reversible, whereas chronic effects usually take a longer time to appear and are often irreversible. Therefore, it is not possible to predict the chronic toxicity of a particular compound based on its acute toxicity, or vice versa – which is a question that is often asked! In fact, both these forms of toxicity can be considered to be extremes of each other, the differences being based on the dose levels experienced and the exposure period. Between these two types of toxicity lie two other types of toxicity, and two other

terms, which are often seen in relevant literature. These are *subacute toxicity* and *subchronic toxicity*.

Subacute and *subchronic* toxicity describes the adverse health effects arising from daily or frequent exposures (to smaller levels of chemical relative to acute toxicity) over part of a lifetime. The difference between these two toxicities lies in the duration of exposure.

1.2.2 What factors influence toxicity?

Every day we are exposed to a wide variety of chemicals such as detergents, hand cream, shampoo, chemicals in the food we eat and the air we breathe, etc. However, what should be noted is that chemical exposure does not always give rise to an adverse health effect. There are a number of factors that will influence the outcome of chemical exposure and whether or not a toxic effect will occur. These are considered below.

1.2.2.1 *The dose*

The dose or amount of chemical to which an individual is exposed is the one factor that has the greatest influence on toxicity, and this had already been discovered by Paracelsus way back in the sixteenth century. What is usually seen experimentally is that at lower chemical dose (or exposure) levels there are no toxic effects. However, as soon as the dose or exposure increases, so does the possibility of the occurrence and severity of a toxic response. This can be expressed graphically in the form of a dose–response curve and this is a major concept in toxicology and forms the basis for all toxicity tests.

The OECD guidelines define dose–response as 'the relationship between dose and the proportion of a population sample showing a defined effect'. Experimentally, the magnitude of the effect and/or the number of test animals affected will increase with increasing dose as can be seen in Fig. 1.1 which shows the effect of increasing dose against the percentage response in terms of mortality. Theoretically, a classic sigmoid-shaped curve is obtained, although in practice this is not often seen.

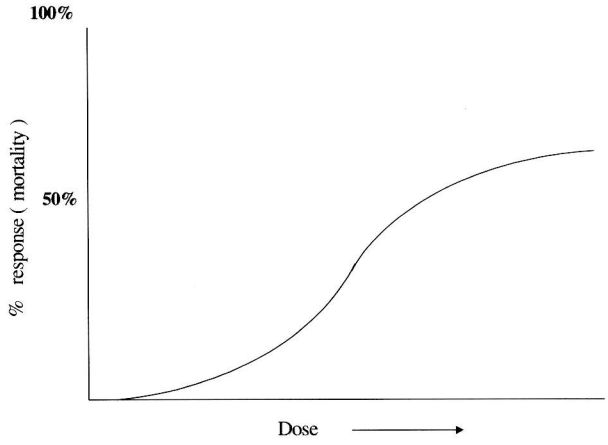


Fig. 1.1 A typical dose–response curve where, in this case, the response is mortality.

1.2.2.2 *Chemical structure*
This will also be a significant factor because the chemical structure will dictate how the chemical reacts with the body (i.e. toxic effects) and how it is both metabolised and excreted (if at all). In the paper-making process polyacrylamides are often used as a retention aid. The polymer itself is a relatively toxicologically inert structure, made up of acrylamide monomers. However, the monomers are highly reactive (all monomers are by their very nature). In Europe the acrylamide monomer is classified as 'Toxic', whereas the polymer is not considered to be hazardous to health.

1.2.2.3 *Route of exposure*
The most common routes of exposure are inhalation, ingestion (to a lesser extent in the industrial setting) and skin contact. For each exposure route there are usually different target organs involved and fortunately only very few chemicals are toxic by all routes of exposure.

1.2.2.4 *Host factors*
Very young or old people are more susceptible to toxic chemicals, one principal reason being that their metabolism and excretion function is not very efficient, which means that there is a risk of toxic chemicals building up in the body leading to cumulative toxic effects. Any predisposing illnesses, which may affect metabolism and/or excretion, will also influence the outcome. Likewise, those who are poorly nourished are more susceptible to the toxic effects of chemicals, again because they are less effective in metabolising and excreting undesirable compounds.

1.2.3 Testing for these different types of toxicity and the information obtained

When it comes to conventional toxicity testing, the types of toxicity are often categorised into three groups.

(1) Acute studies – for acute toxicity.
(2) Short-term (repeated dose) studies – for subacute or subchronic toxicity.
(3) Long-term studies – for chronic toxicity.

1.2.4 Which exposure route will be chosen?

As with any toxicological test, we ideally want to mimic the likely route of exposure when handling or using chemicals in the occupational setting (excluding other areas where chemicals are used). When it comes to handling chemicals in the workplace, it probably comes as no surprise to learn that the most likely routes of exposure are either by skin contact or by inhalation. Ingestion is itself not usually considered to be a relevant route of exposure in the workplace, although of course, it can never be totally ruled out, as there will always be incidences of accidental ingestion.

Any literature survey will show that it is the oral route that is the most common route of exposure used in these studies. This is partly because dosing by the oral route is inherently easier to carry out, and is also cheaper when compared to the other test routes. Also, in the past, most interest was focused on the use of chemicals in the food industry and of course the logical dosing regime involved the oral route. It has only

really been in recent years that the importance of exposure by both inhalation and by skin contact, especially in the workplace, has come to light and that consequently, these routes have also been taken into consideration when carrying out toxicity tests. However, the oral route is still used as a 'standard' when attempting to compare relative toxicities of different chemicals and this is what regulators will consider as one of their requirements.

Acute inhalation studies are not at all common because they can only be performed on certain types of chemicals and they are also very expensive to carry out because specialised staff are required both to do the tests and to analyse the results.

1.2.5 Acute studies

1.2.5.1 *Acute toxicity testing*

The purpose of acute toxicity tests is the same, regardless of the chosen route of exposure. That is, they are undertaken to investigate the potential adverse effects arising from exposure to a given chemical over a short period of time. There are many different types of acute effects that could be studied, but the one acute effect, or end-point, which all chemicals will demonstrate is lethality and it is this which is therefore used as the end-point in this type of study. The results from acute toxicity tests are graphically represented as a dose–response curve which is often converted to a straight-line plot as it makes the data easier to handle and interpret.

1.2.5.2 *The LD_{50} test*

This is probably one of the most well-known toxicological tests that you will come across. The term LD_{50} (lethal dose, 50) is used to describe the acute oral or dermal median lethal dose, i.e. the single lethal dose which will kill 50% of the test population. Its value is usually expressed in milligram or gram of test compound per kilogram of animal weight (mg kg^{-1}). However, for acute inhalation studies the value used is LC_{50}, which refers to the median lethal concentration in air. It is usually expressed either as parts per million (ppm) or mg m^{-3} (milligrams of chemical per cubic metre of air). Both the LD_{50} and LC_{50} values are determined from the dose–response graph as mentioned earlier.

1.2.5.3 *How is this test carried out?*

The tests involve the use of three groups of test animals (10 animals per group, usually rodents) which are administered increasing graduated doses of the test chemical, one per dose group. After an observation period of 14 days, where all mortalities are noted along with any behavioural effects, etc., all the animals are autopsied and a percentage response (lethality) against dosage administered is plotted. From this the LD_{50} value is derived as is shown in Fig. 1.2.

The OECD test guidelines provide the full details for these tests and they are quite 'reader-friendly'. It should be noted that once the dose–response curve has been established, other values can be derived, such as the LD_0, the dose at which no deaths occur, etc.

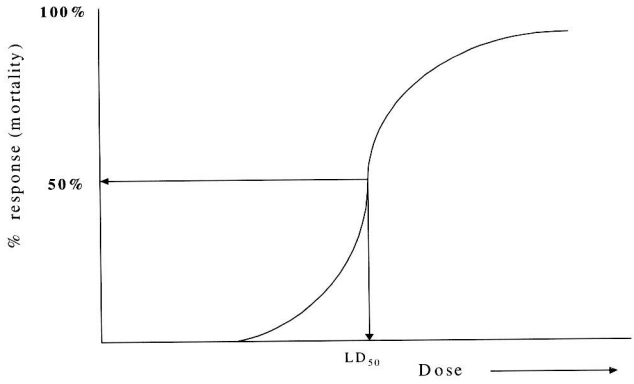


Fig. 1.2 Derivation of an LD_{50} value from a dose–response curve.

1.2.5.4 *What information do we get from an acute toxicity test?*
An acute toxicity (LD_{50}) test provides information on the magnitude of the acute toxic dose, i.e. the LD_{50}, which can then be used for classification and labelling purposes and can also be used as a rough measure of relative toxicity. The results from these types of studies can also provide some insight into any systemic toxicity arising from that particular exposure route, and can be used in order to establish the dosing strategy for other repeat-dose toxicological studies.

In the real world, i.e. in terms of human exposure, these results can be used in a risk assessment in order to help predict what might happen as a result of an accident involving contact with or inhalation of large amounts of chemical or accidental (or otherwise) ingestion. The LD_{50} value obtained in such tests will depend on the choice of test species (because some are more sensitive than others) and also on the chosen route of exposure. For example, some chemicals will exhibit toxicity by one exposure route but not by another. An example of such a chemical is vitamin D. This vitamin is not acutely toxic by the dermal route but it is acutely toxic by ingestion.

1.2.5.5 *How to interpret the results*
Interpreting the LD_{50} (or LC_{50} for acute inhalation) value is quite easy to do. Simply put, the lower the LD_{50} value the more toxic the test compound and the higher the LD_{50} value, the less toxic the test compound.

Therefore, a chemical with an LD_{50} value of 5 mg kg^{-1} is more toxic than one with an LD_{50} value of 300 mg kg^{-1} because fewer milligrams of chemical per kilogram of body-weight are needed to cause death (for that particular exposure route).

1.2.5.6 *Acute oral toxicity testing*
Over the years there has been a lot of criticism of the acute LD_{50} test. This has mainly been from an ethical standpoint because a large number of animals are used and the endpoint is lethality in order to obtain a numerical value (LD_{50}), which is only

relevant for those particular test conditions. The original OECD 401 test has now been deleted from OECD chemical test guidelines and replaced by three other alternative methods.

(1) *The fixed dose method (OECD 420)* This is an example of a reduction and refinement alternative to OECD 401. It was introduced in 1985 by the British Toxicological Society in a bid to address the ethical issues and validity of the LD_{50} test. The objective of this method is to identify a dose that produces clear signs of 'evident toxicity' but not lethality. The numbers of animals that are used are smaller than the traditional OECD 401 test, and only four fixed dose levels are used (5, 50, 300 and 2000 mg kg^{-1} body weight). The results of the first test will dictate whether or not further testing is carried out at a higher or lower dose level. This method is not intended to generate data for the estimation of an oral LD_{50} for the test substance.

(2) *The up and down procedure (OECD 425)* This guideline significantly reduces the number of animals that are required in order to establish the acute oral toxicity of a test substance. A single animal is exposed to subsequent doses adjusted up or down by a constant factor depending on the outcome of the previous result. If this exposure does not result in toxicity, the dose is increased by an equivalent constant factor until five animals have been dosed or the limit dose has been reached.

(3) *The acute toxic class method (OECD 423)* The acute toxic class method is a stepwise dosage procedure that is based on the assessment of lethality. This test uses three animals that are dosed with one of the three levels that correspond to the acute oral LD_{50} classification limits. Although lethality is still the endpoint, this method offers a significant reduction in the numbers of test animals that are used.

1.2.6 Short-term (repeated dose) studies

Subacute or subchronic studies are designed to investigate the adverse effects resulting from repeated exposures to smaller levels of chemical (relative to acute toxicity studies) over part of the organism's lifetime (usually under 10% of the lifespan). These tests aim to mimic the exposure pattern of humans who may daily work with and are exposed to low levels of chemicals, which in reality is probably more representative of the actual daily workplace exposure scenarios that can occur than is the case with acute toxicity studies.

1.2.6.1 *NOELs and LOELs – what are these?*

One feature that both short-term studies and long-term studies have in common is that in contrast to acute toxicity studies, these tests do not use lethality as the endpoint. Therefore, no 'LD_{50} type of value' is generated. Instead, what these studies attempt to do is to find the smallest, or lowest dose which produces any kind of detectable adverse effect, whether behavioural, some kind of organ damage or a change in weight, etc. This value is known as the *lowest observable effect level* (LOEL). As well as this LOEL value, it is also important to know the highest dosage level at

which there will be no detectable adverse effects. This is known as the *no observable effect level* (NOEL).

1.2.6.2 *How are short-term tests carried out?*
Groups of test animals (usually rodents) are daily administered graduated doses of the test chemical over a period usually amounting to one-tenth of their total lifetime. The doses administered to each group are varied so that a dose–response curve can be constructed. From this it is possible to determine the dose at which the first detectable toxic response occurs, which is of course the LOEL, and the highest dose where there is no toxic response, the NOEL. The objective of both subacute and subchronic studies is the same, but the test duration and animal numbers used differ. According to OECD guidelines and assuming that the rat is used as the test animal and dosing is by the oral route, subacute studies usually last for a period of 28 or 14 days, using at least 10 rodents per group (OECD 407), while subchronic studies last 90 days and use at least 20 test animals per group (OECD 408).

1.2.6.3 *What information do we get from short-term studies?*
These short-term tests enable the determination of both the LOEL and the NOEL. They can also help pinpoint any potential target-organ effects, i.e. those organs where the chemical produces its adverse effects, and help determine whether there is any possible accumulation effects. This is important because it is possible for a chemical to be of very low acute toxicity (high LD_{50} value) but as a result of accumulative effects it can interfere with critical bodily functions thereby producing some form of 'delayed toxicity' which would not have been detected by a short-term study. Of course, the results from such tests can be used in selecting the appropriate dose levels for longer-term studies.

1.2.7 Long-term studies

Chronic studies investigate the adverse effects arising from prolonged or repeated exposure to low levels of chemical over the whole or the greater part of a lifetime. These studies allow time for any adverse effects, which have a long latent period, to show up along with any cumulative effects.

As can be imagined, results from chronic toxicity studies will be of special interest to us as consumers when talking about food additives, pesticide residues on fruit and vegetables, etc. to which we are all daily exposed throughout our lives. The studies themselves are similar to the subacute/subchronic tests in terms of the design protocol, the major differences being of course the test duration, dosage levels and the number of test animals used. The reader is referred once more to the OECD guidelines for more information. Any literature survey will show that it is the oral route that is the most common route of exposure used in these studies. As can probably be recalled, this was also the case in acute toxicity studies and the reasons are the same.

Since chronic studies are extremely expensive to perform and involve the use of many test animals, it is actually not uncommon that they are combined with carcinogenicity studies. In this way the design of the test is such that the objectives of the two separate studies are completely fulfilled.