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CHEMISTRY AND HUMAN HEALTH DIVISION*

EXPLANATORY DICTIONARY OF KEY TERMS IN TOXICOLOGY

(IUPAC Recommendations 2007)

Prepared for publication by
MONICA NORDBERG[1,‡], JOHN H. DUFFUS[2], AND DOUGLAS M. TEMPLETON[3]

[1]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden; [2]The Edinburgh Centre for Toxicology, Edinburgh, Scotland, UK; [3]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada

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‡Corresponding author: Address: Institute of Environmental Medicine, Karolinska Institutet, SE-171 77 Stockholm, Sweden: E-mail: monica.nordberg@ki.se

Explanatory dictionary of key terms in toxicology

(IUPAC Recommendations 2007)

Abstract: The objective of the "Explanatory Dictionary of Key Terms in Toxicology" is to give full explanations of the meaning of toxicological terms chosen for their importance and complexity from the point of merging chemistry and toxicology. This requires a full description of the underlying concepts, going beyond a normal dictionary definition. Often linguistic barriers lead to problems in obtaining a common understanding of terminology at the international level and between disciplines. The explanatory comments should help to break down such barriers. The dictionary consists of about 68 terms chosen from the IUPAC "Glossary of Terms Used in Toxicokinetics" organized under 22 main headings. The authors hope that among the groups which will find this explanatory dictionary helpful are chemists, pharmacologists, toxicologists, risk assessors, regulators, medical practitioners, regulatory authorities, and everyone with an interest in the relationship of chemistry to toxicology. It should also facilitate the use of chemistry in relation to risk assessment.

Keywords: toxicology; dictionary; toxicokinetics; risk assessment; explanatory dictionary; IUPAC Chemistry and Human Health Division.

CONTENTS

INTRODUCTION

Within the framework of the IUPAC Chemistry and Human Health Division, the project to develop an "Explanatory Dictionary of Concepts in Toxicokinetics" was initiated in 2003. Following the preparation of the "Glossary of Terms Used in Toxicokinetics", the Working Group came to the conclusion that further explanation of selected terms was needed for the reader to understand fully the concepts underlying the definitions. The approach has been broadened beyond toxicokinetics because it became clear that fundamental ideas in toxicology had to be addressed.

Toxicology, including toxicokinetics, has grown rapidly over recent years. The importance of the subject is highlighted by the EU REACH (European Union Registration, Evaluation and Authorisation of CHemicals) program. Like many IUPAC bodies, the Chemistry and Human Health Division is concerned with promoting world-wide "regulation, standardization, or codification" in relevant areas of chemistry. In this context, a lack of understanding of the terminology used in toxicology has constituted a problem in the relationship of chemistry to toxicology and its consequences for the regulation of the safe use of chemicals.

This explanatory dictionary is compiled for those from related disciplines who now find themselves working in toxicology or requiring a knowledge of the subject. Terms are not always defined in accessible dictionaries, and newcomers to the subject can have great difficulty in obtaining the background knowledge essential for their work. There are also regulators and managers who have to interpret toxicological information and therefore need to understand the internationally accepted definitions of relevant terms in common use.

In order to satisfy the requirements of the various groups now concerned with toxicology and toxicokinetics, the terms included in this explanatory dictionary have been chosen because of their frequent use in the literature reflecting current knowledge. The compilers have deliberately included explanations of terms known to cause confusion among users and of sufficient importance to cause significant problems for a newcomer to toxicology.

The explanations have been compiled to show the relation of terms to each other and also to clarify apparently contradictory definitions. All entries start with the IUPAC-approved definitions from the IUPAC "Gold Book" [1] and from the glossaries of terms in toxicology and toxicokinetics published in *Pure and Applied Chemistry* [2,3]. The explanatory definitions attempt to explain significant differences between related disciplines in the relevant concepts.

We are grateful to all those who have contributed to this explanatory dictionary with constructive criticism and who have suggested modifications for its improvement. Their valuable comments have been incorporated, and they are listed on the title page. There will still be flaws, but we hope that the final version will be sufficiently close to achieving the original objectives to justify the very widespread support that we have received.

ACKNOWLEDGMENTS

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1. ABSORPTION

IUPAC definitions

absorption
1. general
Process of one material (the absorbent) being retained by another (the absorbate); this may be the physical dissolution of a gas, liquid, or solid in a liquid, a gas or liquid in a solid, attachment of molecules of a gas, vapor, liquid, or dissolved substance to a solid surface by physical forces, etc. In spectrophotometry, absorption of light at characteristic wavelengths or bands of wavelengths is used to identify the chemical nature of molecules, atoms, or ions and to measure the concentrations of these species [1,3].

Note: The above definition from the IUPAC "Gold Book" requires that "light" be interpreted as referring to all forms of electromagnetic radiation.

2. of radiation
Phenomenon in which radiation transfers to matter which it traverses some or all of its energy [3].

3. in biology
Penetration of a substance into an organism by various processes, some specialized, some involving expenditure of energy (active transport), some involving a carrier system, and others involving passive movement down an electrochemical gradient.

Note: In mammals, absorption is usually through the respiratory tract, gastrointestinal tract, or skin [3].

4. systemic
Uptake to the blood and transport via the blood of a substance to an organ or compartment in the body distant from the site of absorption [3].

Explanatory comment

absorption (of radiation)
Absorption of radiation (2 above) is the prime consideration in radiation toxicology. Radiation can reach all tissues of the body directly from an external source, but the capacity to penetrate body tissues varies with the type of radiation. Radiation may be emitted as particles (α-, β-, or neutron particles) or as high-energy electromagnetic waves such as X-rays or γ-radiation. Radiation may be ionizing or non-ionizing. Ionizing radiation is particle radiation in which an individual particle (e.g., a photon, an α-particle, or a β-particle) carries enough energy to ionize an atom or molecule (that is, to completely remove an electron from its orbit).

α-Particles are a highly ionizing form of particulate radiation, which has low penetration. Each particle consists of two protons and two neutrons, which is identical to a helium nucleus; hence, the α-particle can be written as He^{2+}. α-Particles can be stopped by a thin sheet of paper and by the dead layer of the skin. Although not an external radiation hazard, α-particles released by radionuclides are dangerous if they are taken into the body by inhalation (breathing in) and/or ingestion (eating and drinking) as discussed further below. The adverse health effects caused by radon, an α-emitter, are explained by α-particles that are absorbed in the lung, thus becoming an internal radiation source. Indoor radon exposure can lead to lung cancer. Exposure from radon in drinking water is also of concern. Many countries have set exposure limit recommendations.

β-Particles are electrons that emanate from the nucleus of an atom. there are two forms of β-particles. They are the electron given the symbol β^-, and the positron, given the symbol β^+. The depth to which β-particles can penetrate the body depends upon their energy. High-energy β-particles of several MeV may penetrate approximately 1 cm of tissue, although most are absorbed in the first few millimetres. As a result, β-emitters outside the body are hazardous only to surface tissue such as the skin of the lens of an eye. When β-emitters are taken into the body, they irradiate internal tissues and become a more serious hazard.

The effect of radiation depends on the amount received and the exposure time. The amount of radiation received is expressed as a dose, and the measurement of dose is known as dosimetry. What is important is not so much the total dose to the whole system as the dose per mass of body tissue. This unit is the gray (Gy), equal to J kg^{-1}, and named in honor of the British physicist, Louis Gray. The gray is a large dose, and for most normal situations we use the milligray (mGy) and the microgray (μGy). Absorbed dose is given the symbol *D*.

The gray is a numerical unit that quantifies the physical effect of the incident radiation (the amount of energy in joules deposited per kilogram), but it tells us nothing about the biological consequences of such energy deposition in tissue. Studies have shown that α- and neutron radiation cause greater biological damage for a given energy deposition per mass of tissue than, for example, γ-radiation. One Gy of α- or neutron radiation is more harmful than 1 Gy of γ-radiation.

Weighting factors are used to compare the biological effects of different types of radiation. For example, fast neutron radiation is more damaging than X-rays or γ-radiation. This leads to the idea that weighting factors can be used to quantify the fact that fast neutrons are more biologically damaging by expressing the statement that a lower absorbed dose produces equivalent biological effects. This is expressed in terms of a radiation weighting factor, W_R, which is a function of energy deposition. The W_R of a certain type of radiation is related to the density of the ion tracks it leaves behind in the tissue; the closer the ion pairs, the higher the weighting factor. Another weighting factor used to further express biological effects is the tissue weighting factor, W_t.

The radiation weighting factor, W_R, for various types of radiation is listed in Table 1. They are valid for relatively long-term exposures; they do not apply to very large life-threatening doses received in a short period of time such as minutes or hours.

Table 1 The quality factors for the various types of radiation.

Radiation	Energy	W_R (ICRP60)
γ	All	1
β	All	1
neutrons	Slow	5
neutrons	Fast	10–20
α	All	20

The absorbed radiation dose, when multiplied by the W_R of the radiation delivering the dose, will give us a measure of the biological effect of the dose. This is known as the dose equivalent, or dose equivalent index. Dose equivalent is given the symbol *H*. The unit of *H* is the sievert (Sv), also equal to J kg^{-1}. It was named after the Swedish scientist Rolf Sievert. An equivalent dose of 1 Sv represents that dose of radiation that is equivalent, in terms of specified biological damage, to 1 Gy of X- or γ-rays. In practice, the millisievert (mSv) and microsievert (μSv) are the units in common use. Dose equivalent, quality factor and absorbed dose are related by the expression following immediately below:

$$H = D\,W_R$$

Most of the instruments used to measure radiation doses or dose rates display the values in mSv or μSv or in mSv h^{-1} or μSv h^{-1}, respectively. The collective dose to which a population is exposed is commonly quoted in "man-sieverts" (man-Sv). However, this term should be avoided since it confuses a physical quantity with its units. Thus, sieverts alone is sufficient. The natural background effective dose rate varies considerably from place to place, but typically is around 3.5 mSv/year. For comparison, more than 6 Sv will lead to death in less than two months in more than 80 % of cases, and much over 4 Sv is more likely than not to cause death.

For non-ionizing radiation, exposure standards are based on a measurement called the "specific (standard) absorption rate" (SAR). The specific absorption rate is defined by the Institute of Electrical and Electronics Engineers (IEEE) as: *the time derivative of the incremental energy (*d*W) absorbed by (dissipated in) an incremental mass (*d*m) contained in a volume element (*d*V) of a given density.* The specific absorption rate as defined by the American National Standards Institute (ANSI) standard reads: *SAR is the time rate at which radio frequency electromagnetic energy is imparted to an element or mass of a biological body. SAR is expressed as energy flow (power) per unit of mass in units of W/kg.* When referring to human tissue, this means that the SAR is a measurement of the heat absorbed by the tissue.

absorption (in toxicology)

In toxicology, we are mainly concerned with absorption as defined in 3 and 4 above. In other words, we are concerned with the processes by which a chemical crosses the various membrane barriers of a living organism and especially those processes by which a chemical is taken up from environmental media, including food and other ingested material, such as drinking water, liquid refreshments, and medication of all kinds. Absorption is the first step in the series of processes analyzed in the study of toxicokinetics. The others are distribution (including storage), metabolism, and excretion. Together, these are usually referred to by the acronym ADME.

The main barrier to uptake is the phospholipid bilayer that forms the core of biological membranes. This prevents passive diffusion of water and water-soluble molecules but permits passive diffusion of fat-soluble molecules. Passive diffusion is driven by the electrochemical gradient of the substances involved. Fat-soluble substances move down their gradient of chemical activity (proportional to concentration of the substance) and/or the gradient of electrical charge (positive or negative).

Chemicals (foods, medicines, drugs of abuse, industrial chemicals, and environmental chemicals) can enter the human body by various routes following ingestion, inhalation, injection (intravenous, subcutaneous, intramuscular), skin application, use of suppositories, and uptake through mucous membranes of the eye or oral or nasal cavities.

Except for injection directly into the bloodstream, chemicals must pass through a complex system of cell membranes before they can enter the bloodstream. For example, chemicals that enter the digestive tract in solution or after solubilization may be absorbed by the cells lining the small intestine and then transferred through the cell to the other side (the transcellular route; Fig. 1) where they cross the endothelial barrier into the bloodstream. Some chemicals may also pass between the epithelial cells of the intestine by what is called the paracellular route. Such transport is restricted by the junctions between cells, and this provides selectivity for the chemicals that can use this route. Chemicals that are inhaled must pass through the alveolar cells to the adjacent capillary cells and through them to the bloodstream.

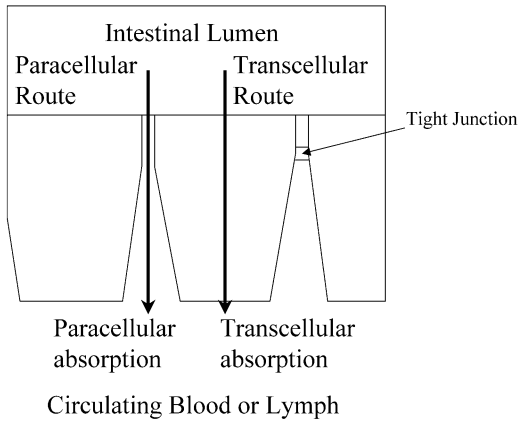

Fig. 1 Transcellular and paracellular routes of absorption from the intestine.

As chemicals pass into and out of cells, they must cross the cell membrane. The membrane defines the shape of the cell, controls the chemistry of the cell interior by regulating passage of substances into and out of the cell, and acts as a transducer for extracellular chemical regulators such as hormones. The cell membrane consists mainly of phospholipid and protein in the form of a lipid bilayer. Two phospholipid layers face each other inside the membrane with the more water-soluble parts of the phospholipid molecule (phosphate groups) facing the aqueous media inside the cell (cytoplasm) and outside the cell (extracellular fluid). The resultant structure is termed the fluid mosaic model (Fig. 2), and the fluidity is crucial to its function. The membrane proteins provide some rigidity to the structure, and some may act as transporters. Some "float" in the membrane, binding to external substances and diffusing with them from one side of the membrane to the other. Others may traverse the membrane structurally and, on binding to a substance, change shape so that the substance is transported across the membrane. This process may be associated with input of energy from nucleoside triphosphate breakdown, permitting transfer against an electrochemical gradient, referred to as active transport (see below).

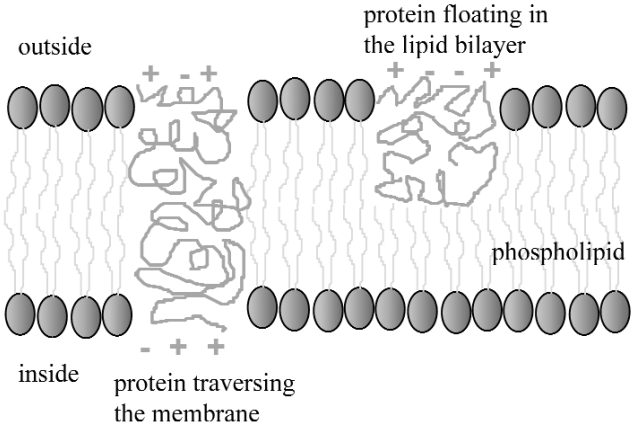

Fig. 2 The fluid-mosaic cell membrane.

The most fundamental mechanism for transport of either foreign chemicals or ordinary ions through the cell membrane is passive diffusion. The driving force for passive diffusion of a chemical is based on the difference between the concentration (or better chemical activity) of the chemical (or chemicals of the same sign of electrical charge if ionized and experiencing a charge gradient established by active transport, see below) on the outside of the cell and that on the inside of the cell. This is properly called the electrochemical gradient. The greater the difference in the relevant electrochemical activity between the outside and the inside of the cell, the greater the diffusion of the chemical down the resultant gradient, in or out. Since the membrane barrier to chemical movement is mainly lipid, the ability of a chemical to diffuse across the membrane is largely dependent on its lipophilicity, solubility in lipid. This is measured in practice in terms of its octanol–water partition coefficient, the ratio of the concentration in octanol to that in water after the substance is mixed thoroughly with both and they are allowed to come to equilibrium.

Because they are not lipid-soluble, charged molecules do not readily diffuse across the plasma membrane. The pH of the fluid surrounding the cell is important in this respect because it influences ionization and hence molecular charge. Weak acids in their un-ionized form may be lipid-soluble and will diffuse across membranes quite easily. The degree of ionization of a molecule at different pHs is dependent on its pK_a, i.e., the pH at which 50 % of the chemical is ionized and 50 % is un-ionized. This is important in the gut. In the human and, in general, carnivore stomach lumen, the pH may be as low as 1.5 to 2.0. In other species (e.g., in rats), the pH may be much higher (e.g., pH = 4). However, the pH inside the small intestine is about 7.0 to 8.0. Thus, the ratio of ionized to un-ionized chemical differs for any chemical in these two environments depending on its pK_a and the amount of ionizable chemicals absorbed from the stomach and the small intestine is different. This property may be used in designing drug molecules to ensure preferential absorption from the stomach by giving the molecule a structure with the appropriate pK_a.

Water-soluble biomolecules and other chemicals may be transported across the membrane with the aid of carrier proteins to which they become bound (see also carrier). One possibility is facilitated diffusion in which the molecules move down the electrochemical gradient exactly as happens with normal diffusion, although the process may be limited by the availability of carrier molecules and the kinetics of the binding/unbinding reaction between carrier and chemical. It is also possible for chemicals to compete for the binding sites on carrier proteins. A particularly important carrier is multidrug resistance carrier, which has been demonstrated to transport a wide range of xenobiotics conjugated to glutathione, glucuronate, or sulfate as well as unmodified anionic compounds such as the antifolate agent, methotrexate [4].

Chemicals may also cross the cell membrane by diffusion through water-filled membrane pores. This diffusion is dependent on the size of the pore and the molecular size and shape of the chemical.

Some inorganic ions, such as sodium and potassium, and many drugs, move through the cell membrane by a process called active transport. This process moves substances against the electrochemical gradient and requires input of energy, usually in the form of adenosine triphosphate (ATP). Thus, active transport absorption by any cell will reflect its metabolic activity and, in some circumstances, may stress this so much as to have adverse effects. A good example of active transport, the sodium pump, is illustrated in Fig. 3.

ATPase binds Na^+ and ATP in the E_1 conformational state (step 1) and is phosphorylated at an aspartate residue by the γ-phosphate of ATP. This leads to the occlusion of three Na^+ ions (step 2) and then to their release to the extracellular side (step 3). This new conformational state (E_2-P) binds K^+ with high affinity (step 4). Binding of K^+ leads to dephosphorylation of the enzyme and to the occlusion of two K^+ cations (step 5). K^+ is then released to the cytosol after ATP binds to the enzyme with low affinity (step 6). The dashed box highlights the electrogenic steps of the catalytic cycle.

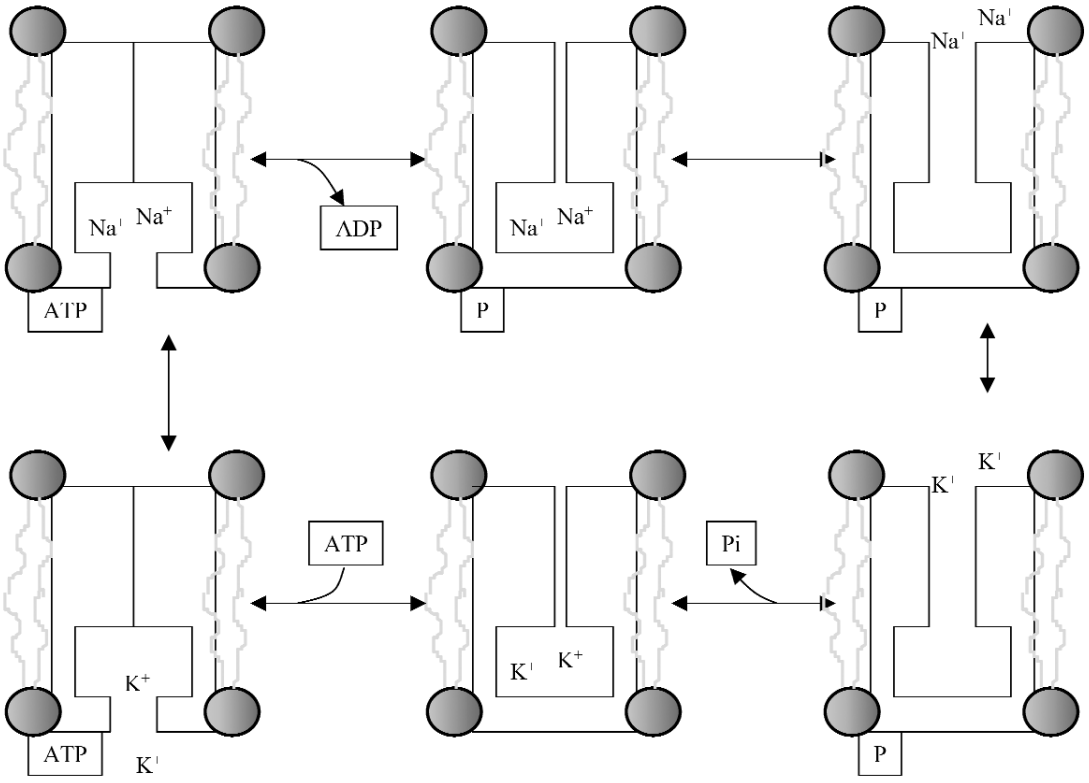

Fig. 3 Reaction cycle of the sodium pump (Na^+/K^+-ATPase).

Finally, the membrane can engulf the chemical, form a vesicle, and transport it across the membrane to the inside of the cell. This process is called endocytosis (Fig. 4) and is especially important for particulates. The same process can occur in reverse and then is known as exocytosis. It is energy-dependent and may result in the transport of a mixture of chemicals because, although it is usually induced by a specific process, it may take unspecifically enclosed substances into the cell. This process has been suggested to be more active in the new born.

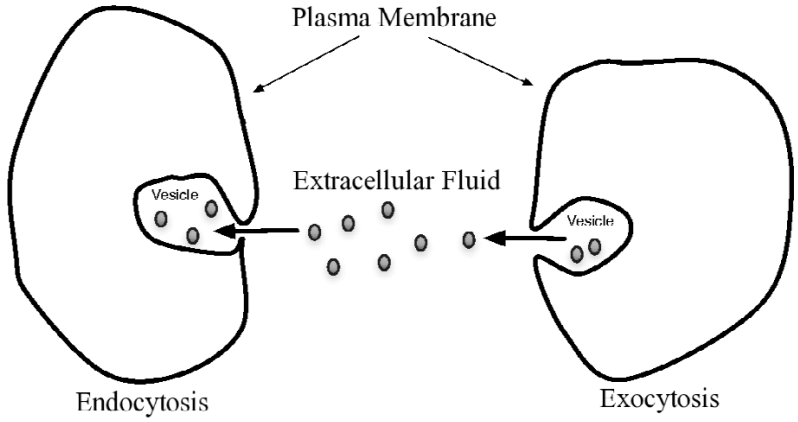

Fig. 4 Endocytosis and exocytosis.

Once the chemical has entered the bloodstream or the lymph, it is distributed to organs distant from the site of absorption. Initially, simply because of the electrochemical gradients between the blood or lymph and the organs that it perfuses, many chemicals will tend to leave the blood (or lymph) passively and enter the surrounding cells (uptake). The pH of the blood (or lymph), pH = 7.4, will determine the ionization state of polar organic chemicals, and this will influence passage through the cell membranes of the cells separating the blood (or lymph) from the organs.

Anatomical and physiological factors may affect the movement of a chemical around the body. For example, the cells surrounding the capillaries in the brain have tight junctions that impede the flow of materials between cells. One type of glial cell in the central nervous system, the astrocyte, forms a tight covering on the brain's capillaries and prevents or retards large molecules from entering the brain. This structure constitutes what is known as the "blood–brain barrier".

The placenta is an organ that permits nutrients to pass from the mother's bloodstream to that of her fetus but does not allow the passage of all chemicals. The maternal blood and the fetal blood do not have direct contact. Generally, molecules with a molecular mass greater than 1000 Da have difficulty entering the fetal blood supply. The placenta has the ability to metabolize chemicals, and the derivatives may be responsible for effects on the embryo and fetus.

Other factors to be considered in relation to the availability of chemicals to cells in the human body are chemical affinity and the resultant long-term incorporation into tissues such as fatty tissue and bone. Chemicals that are lipophilic have an affinity for, and a tendency to be absorbed by and accumulate in fat cells, from which they are released very slowly under normal circumstances. Such chemicals may cause no problems for years, but the fat cells can break down quite rapidly during pregnancy or illness or in old age. Any stored lipophilic xenobiotics may then flood the bloodstream and cause illness as a result of uptake of toxic doses into susceptible organs, particularly into the critical organ. The same effect occurs in birds and wild animal species during the winter when food supplies are short.

Affinity for proteins in the blood may also be important in determining availability of chemicals to susceptible tissues. In particular, some chemicals may become strongly bound to plasma proteins (such as albumin), and the rate of release from such binding will determine how long the chemical is available to exert its biochemical and physiological effects. Serum albumin is important in the transport of bile pigments such as bilirubin. Some drugs, such as sulfasoxazole and ceftriaxone, can compete for bilirubin-binding sites on the albumin molecule, causing it to be released and deposited in tissues where it causes damage. This is just one example of many hundreds that are of clinical importance.

For some inorganic species, such as fluoride ion, lead ion, and strontium ion, incorporation into bone may occur. The elements may stay there for long periods of time. As bone slowly renews itself or is partly broken down during pregnancy, illness, or old age, these chemicals may be released. If this occurs during pregnancy, the resultant toxicity can affect both the mother and her child. Similar toxic effects may be seen in the sick and the elderly. Since these effects may occur some time after exposure to the chemicals, diagnosis of the cause depends upon their detection in blood or urine samples.

For some toxic substances, it is important to evaluate if the adverse effect (i.e., the toxicity caused by the substance) results from its radioactivity or from its direct toxicity as a result of its other properties, or from both. For example, ^{203}Hg and ^{109}Cd may cause damage as a result of both radiation effects and toxic effects due to direct chemical reactions.

2. ACUTE AND CHRONIC

A. Acute

IUPAC definitions

acute
1. Of short duration, in relation to exposure or effect.

In experimental toxicology, acute refers to studies where dosing is either single or limited to one day, although the total study duration may extend to two weeks [3].
2. In clinical medicine, sudden and severe, having a rapid onset [3].

Antonym: chronic

acute effect
Effect of finite duration occurring rapidly (usually in the first 24 h or up to 14 d) following a single dose or short exposure to a substance or radiation [3].

acute exposure
Exposure of short duration [3].
See also acute, exposure.
Antonym: chronic exposure

acute toxicity
1. Adverse effects of finite duration occurring within a short time (up to 14 d) after administration of a single dose (or exposure to a given concentration) of a test substance or after multiple doses (exposures), usually within 24 h of a starting point (which may be exposure to the toxicant, or loss of reserve capacity, or developmental change, etc.) [3].
2. Ability of a substance to cause adverse effects within a short time of dosing or exposure [3].

Antonym: chronic toxicity

Explanatory comment

acute (in toxicology)
In toxicology, "acute" is a word that is used in combination with exposure, toxicity, and effect. Acute exposure is a single or very short-lasting dosing by any route. Talking about acute toxicity addresses adverse effects (see explanatory comment below), i.e., harmful effects, unwanted negative effects that occur immediately after or within a short time after administration of a single dose of a substance, or following short exposure or concurrently with continuous exposure, or recurrently following shortly after multiple doses. "Short" implies a time of 24 h or less. Some effects considered to be acute can occur up to as long as after 96 h after exposure. Uremia can be an acute effect, but it takes almost 96 h to see such an outcome. In toxicity testing, it is most important to be aware of this in order not to draw any false conclusions from animal studies with agents that give rise to such an acute effect. Acute effects occur or develop rapidly after a single exposure. However, acute effects can also appear immediately after, or during, repeated or prolonged exposure.

Historically, an important aspect of acute toxicity has been the identification of the lethal dose or exposure that kills an organism after a short exposure or a single dose. This has been established by a test in which selected organisms are exposed to a series of increasing dose levels until a dose is reached at which all the organisms die. For regulatory purposes, in order to permit extrapolation to humans, it is usually performed with at least two mammalian species. From such tests, the LD_{50} for the test species has been derived and used for the classification of the toxicity of chemicals to humans. Such tests involved killing large numbers of animals to obtain a toxicity classification based on lethality. However, this classification tells us nothing about sublethal effects such as immunotoxicity or teratogenicity. This

situation was clearly unsatisfactory and so acute toxicity testing is now designed in such a way as to obtain maximum information about all aspects of acute toxicity using the minimum number of animals.

In Europe, classification of new chemicals for toxicity is no longer based on the LD_{50}. The tests used for this purpose are based on survival rather than on lethality. For example, the method of fixed-dose testing is usually limited to a maximum dose of 500 mg per kg body weight. If 5 males and 5 females exposed to a dose of this magnitude survive with no evidence of toxicity, the chemical tested need not be classified as toxic. Toxicity classifications based on this approach can provide a similar classification of toxicity to the old LD_{50} system but with a huge reduction in the number of animals used and in animal suffering compared to the traditional LD_{50} tests. Another approach to achieve the reduction in numbers of animals used is the up-and-down procedure which produces a value approximating to the LD_{50}. This procedure uses sequential dosing together with sophisticated computational methods. It provides a point estimate of the LD_{50} while achieving significant reductions in animal use.

Since chronic toxicity testing (see below) is expensive and labor-intensive, there is a great need to replace it where possible with shorter-term predictive acute tests and early identification of biomarkers of toxicity. This has been possible to some extent in relation to carcinogenicity. In the past, a cancer study was designed to expose animals to the toxicant and to follow the animals during their lifetime. Each animal upon death was examined for occurrence and localization of tumors in the body. Since it is expensive to maintain animals over long periods, the need for new tools to identify carcinogens is clear. Many cancers start with mutations or chromosome damage, and this can be assessed with short-term tests such as the Ames test or the host-mediated (Legator) test. The Ames test is based on reversal of a point mutation in a *Salmonella* strain, which makes it unable to synthesize the amino acid histidine. Back-mutation can be detected by growth of the bacteria in a histamine-depleted medium. Rat liver microsomes are included in the test medium in order to simulate the metabolic activation of organic compounds that may take place in the intact animal. The host-mediated test looks for chromosome changes in vitro and (or) in vivo, including chromosome breaks and sister chromatid exchanges, in microbial cells introduced (e.g., by intravenous injection) into a host animal. The host animal receives the test compound orally and therefore acts as a source of chemical metabolism, distribution, and excretion. Another whole animal test involves looking for the production of micronuclei in animals exposed to possible carcinogens. The micronucleus test is less sensitive than bacterial tests but is a more realistic measure of likely chromosomal damage in mammals at risk.

It is also possible to test quickly for mutagenicity and the possibility of associated carcinogenicity by adding suspect substances to cell cultures and looking for chromosome damage and cell transformation. Another approach to carcinogenicity testing is to apply the substances to tissues in culture and/or to syngenic transplants and similarly assess the changes that occur.

While the acute tests for mutagenicity give a quick indication of the mutagenic potential of substances tested, it must be emphasized that the effects observed may not necessarily extrapolate to the intact organism. The bacterial strains used in the Ames test have been selected for the absence of DNA repair mechanisms so that they are much more sensitive to mutagenicity than any normal organism. Cultured cells and tissues lose differentiated properties and are abnormal in this way. Dedifferentiated cells tend to divide more rapidly than normal, and this may facilitate chromosomal damage. With regard to predicting carcinogenicity, not all mutations lead to cancer nor are all cancers the result of mutations. Thus, while these acute tests may indicate the possibility of carcinogenicity, they are not sufficient to prove it and can be regarded only as screening tests to select substances for further study in this regard.

B. Chronic

IUPAC definitions

chronic
Long-term (in relation to exposure or effect).
1. In experimental toxicology, chronic refers to mammalian studies lasting considerably more than 90 days or to studies occupying a large part of the lifetime of an organism [3].
2. In clinical medicine, long-established or long-lasting [3].

Antonym: acute

chronic effect
Consequence that develops slowly and/or has a long-lasting course: may be applied to an effect that develops rapidly and is long-lasting [3].
Antonym: acute effect
Synonym: long-term effect

chronic exposure
Continued exposures occurring over an extended period of time, or a significant fraction of the test species' or of the group of individuals', or of the population's lifetime [3].
Antonym: acute exposure
Synonym: long-term exposure

chronic toxicity
1. Adverse effects following chronic exposure [3].
2. Effects which persist over a long period of time whether or not they occur immediately upon exposure or are delayed [3].

Antonym: acute toxicity

Explanatory comment

chronic (in toxicology)
Chronic effects usually occur after repeated or prolonged exposures. However, chronic effects can also occur after single exposure if they develop slowly or are long-lasting. They are often irreversible. Chronic effects may follow accumulation of a toxic substance or of metabolites formed by biotransformation of the administered substance. They may also be the result of cumulative irreversible effects of toxicants. Chronic effects usually result in a progressive loss of organ function, for example, increasing liver damage following regular ingestion of ethanol. For humans, a particularly serious example of chronic toxicity may be the gradual loss of brain cells due, for example, to excessive exposure to ethanol or other neurotoxic agents. Brain cells do not divide and cannot be replaced once they are lost. Because we have a large reserve capacity of such cells, their gradual loss may not be apparent, but this added to the normal loss associated with aging may result in premature dementia and related adverse effects.

For the toxicologist, a particular problem arises when the dose or exposure is low or the effect develops a very long time after exposure as may happen with cancer. In these circumstances, it is very difficult to attribute a cause to the delayed effect. It is also difficult to test substances for such effects. Cancer in humans may take up to 40 years to develop after exposure to a carcinogen. Our normal test animals, rats and mice, have life spans of about 2 years and 18 months, respectively. In order to cause malignant tumours within such a short time, very large doses of suspect carcinogens must be applied. Thus, test doses are much higher than those to which humans may ever be exposed and may therefore overwhelm metabolic defense mechanisms that work well within likely human exposure ranges.

Subchronic (sometimes subacute) toxicity refers to the adverse effects observed when animals are administered a toxicant over a period of time, as a result of repeated daily dosing of a chemical, or ex-

posure to the chemical, for a significant part of an organism's lifespan (usually not exceeding 10 %). Observations of acute and subchronic toxicity indicate what the critical (target) organ and the critical effect are. With experimental animals, the subchronic period of exposure may range from a few days to 6 months. The terms "subchronic" and "subacute" suffer from many variations in their usage and are best avoided. It is better to replace them by giving precise definition of the times of administration and observation. Subchronic testing has usually been limited to 90 days. Chronic toxicity testing should be over the lifetime of the organism, which means 1.5 to 2 years in the case of the mouse or the rat.

Chronic toxicity testing in rodent and non-rodent species identifies not only general toxicity but also aspects of mutagenicity, carcinogenicity, and reproductive toxicity (in rats and rabbits) including specific effects on the reproductive organs, teratogenicity, and reproductive toxicity. Some strains of mice, for example, have different frequencies of naturally occurring effects. Most chronic studies are carried out with at least two animal species, usually rats and a non-rodent species such as dogs or primates. For cancer testing, it is important to choose a species known to have a low frequency of tumors. For example, the Syrian golden hamster has low background frequency of tumors in the trachea and lung. Currently there is great activity in developing alternatives to chronic animal testing, e.g., the use of stem cells, tissue culture, and in silico methods.

3. ADVERSE EFFECT AND TOXICITY

IUPAC definitions

adverse effect
Change in biochemistry, morphology, physiology, growth, development, or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increase in susceptibility to other environmental influences [3].

toxicity
1. Capacity to cause injury to a living organism defined with reference to the quantity of substance administered or absorbed, the way in which the substance is administered and distributed in time (single or repeated doses), the type and severity of injury, the time needed to produce the injury, the nature of the organism(s) affected, and other relevant conditions.
2. Adverse effects of a substance on a living organism defined as in 1.
3. Measure of incompatibility of a substance with life: this quantity may be expressed as the reciprocal of the absolute value of median lethal dose ($1/LD_{50}$) or concentration ($1/LC_{50}$) [3].

toxicity equivalency factor (TEF), f
Factor used in risk assessment to estimate the toxicity of a complex mixture, most commonly a mixture of chlorinated dibenzo-*p*-dioxins, furans, and biphenyls: in this case, TEF is based on relative toxicity to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TEF = 1) [2,3]. For mixtures of polycyclic aromatic hydrocarbons, the reference chemical is benzo(a)pyrene and other reference materials may be used for other mixtures as appropriate.

toxicity equivalent (TEQ)
Contribution of a specified component (or components) to the toxicity of a mixture of related substances. The amount-of-substance (or substance concentration) of total toxicity equivalent is the sum of that for the n components B, C … N. Toxicity equivalent is most commonly used in relation to the reference toxicant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin by means of the toxicity equivalency factor (TEF, f) which is 1 for the reference substance. Hence:

$$n(\mathrm{TEQ}) = \sum_{i=\mathrm{B}}^{N} f_i n_i \quad [3]$$

Explanatory comment

adverse effect (in toxicology)

Living organisms have evolved to adapt to a range of environmental conditions and to change in response to environmental changes, including chemical changes. Such changes in organisms in response to the environment constitute effects. These effects may be beneficial (as perhaps with essential nutrients), neutral, or harmful. Harmful effects of a substance in relation to dose define its toxicity. Extreme damage is easy to identify, but the toxicologist wants to define the earliest signs of harm and this is not so easy. If the effect resulting from exposure to a potentially toxic substance is small, it may be within the normal range of physiological variation required for life to adapt and cause no harm. In that case, it is not an adverse effect. On the other hand, it may have a small effect which causes no immediate harm but may contribute to harm in future if the organism lives long enough. For example, lead may replace calcium in bone with no immediate effect but may accumulate there with time to cause harm during illness, pregnancy, or old age. Thus, the apparently clear definition of an adverse effect may become difficult to apply in practice.

Once an adverse effect has been identified, it is important to know whether it is reversible or irreversible. It may be possible for an organism to recover completely from a reversible effect, but irreversible effects can accumulate with time and repeated exposures.

In assessing the consequences of adverse effects, the organ most affected, the critical organ, is a key factor, together with the dose–effect relationship. If one knows the initiating reaction for the adverse effect, this may help not only to assess the likely outcome but also to suggest treatment for alleviating the effect, for example, by blocking the active site for the toxicant on a receptor molecule.

Distinguishing between adverse and nonadverse effects

The simplest definition of an adverse, or "abnormal" effect experimentally is a measured effect that is outside the "normal" range. The normal range is usually defined on the basis of values observed in a group of presumably healthy individuals, and expressed statistically as a range representing the 95 % confidence limits (CLs) of the mean or, if the mean has been determined on the basis of a very large sample, the 95 % limits will be equal to $\mu \pm 1.96\,\sigma$ where μ (mu) and σ (sigma) are the population values of the mean and the standard deviation (SD), respectively. An individual with a measured value outside this range may be either genuinely "abnormal" or one of a small group of "normal" individuals who have extreme values. This distinction between "normal" and "abnormal" values based on statistical considerations may be used as a criterion for adverse effects, if the exposed population consists of adult, generally healthy individuals, subject to periodical medical examination, such as workers. In these circumstances, departures from normal values associated with a given exposure can be considered as adverse effects, if the observed changes are:

(a) statistically significant ($P < 0.05$) in comparison with a control group, and outside the limits ($\mu \pm 2\sigma$) of generally accepted "normal" values;
(b) statistically significant ($P < 0.05$) in comparison with a control group, but within the range of generally accepted normal values, provided such changes persist for a considerable time after the cessation of exposure; and
(c) statistically significant ($P < 0.05$) in comparison with a control group, but within the normal range, provided statistically significant departures from the generally accepted normal values become manifest under functional or biochemical stress.

The Student's *t*-distribution is usually important and can sometimes be applicable for datasets with a small number of components rather than the Gaussian distribution.

The preceding statistical considerations will not be appropriate if the available data are nonparametric, i.e., not consistent with a Gaussian (normal) distribution. For nonparametric data, the median replaces the mean as a measure of the central value. Two of the simplest nonparametric procedures are the sign test and median test. The sign test can be used with paired data to test the hypothesis that dif-

ferences are equally likely to be positive or negative (or, equivalently, that the median difference is 0). The median test is used to test whether two samples are drawn from populations with the same median. The median of the combined dataset is calculated, and each original observation is classified according to its original sample (A or B) and whether it is less than or greater than the overall median. The chi-square test for homogeneity of proportions in the resulting 2-by-2 table tests whether the population medians are equal. The major disadvantage of nonparametric techniques is clear from its name. Because the procedures are nonparametric, there are no parameters to evaluate and it becomes more difficult to make quantitative statements about the actual difference between populations. For example, when the sign test says two treatments are different, there is no confidence interval and the test does not say by how much the exposures differ. However, it is sometimes possible with the right software to compute estimates (and even confidence intervals!) for medians and differences between medians. The second disadvantage of nonparametric procedures is that they throw away information. The sign test uses only the signs of the observations. Ranks preserve information about the order of the data but discard the actual values. Because information is discarded, nonparametric procedures can never be as powerful (able to detect existing differences) as their parametric counterparts when parametric tests can be used.

The statistical definition of adverse effects is likely to be inappropriate for a population which includes groups that may be specially sensitive to environmental factors, particularly the very young, the very old, those affected with disease, and those exposed to other toxic materials or stresses. For such a population, which is the norm for humans, it is practically impossible to define "normal" values, and any observable biological change may be considered as an adverse effect under some circumstances. Thus, it is important to establish criteria for adverse effects based on biological considerations as well as on statistically significant differences with respect to an unexposed population (control group).

There are no generally agreed-upon biological criteria, and so ultimately the decision on what is an adverse effect tends to depend on experience and expert judgment. It may nevertheless be useful to give examples of such criteria, illustrating at the same time the difficulties in applying these criteria. One approach is to try to define which effects are non-adverse and to eliminate them from further consideration. Non-adverse effects have been defined negatively as the absence of changes in morphology, growth, development, and life span. In addition, non-adverse effects are those which do not result in impairment of the capacity to compensate for additional stress. Non-adverse effects should be reversible following the end of exposure without any detectable reduction in the ability of the organism to maintain homeostasis, and should not enhance its susceptibility to the harmful effects of other environmental influences. Thus, in contrast, adverse effects may be defined as changes that

1. follow single, intermittent or continued exposure and that result in loss of functional capacity (as determined by anatomical, physiological, and biochemical or behavioral parameters) or in a decrease in the ability of the organism to compensate for additional stress;
2. are irreversible during exposure or following the end of exposure if such changes cause detectable loss in the ability of the organism to maintain homeostasis; and
3. enhance the susceptibility of the organism to damaging effects of other environmental influences.

Application of the above criteria may be based on overt pathology (e.g., inflammation, necrosis, hyperplasia), or on metabolic and biochemical changes. Such changes may be considered to be adverse if, for example, enzymes that have a key significance in metabolism are inhibited or if there are changes in subcellular membranes (e.g., lysosomal membranes) resulting from the action of toxic substances. However, such changes may be within the limits of homeostasis or have no resultant pathology. Thus, the degree of change becomes a crucial measurement. One may debate, for example, what percentage inhibition of an enzyme must occur before harm results to an organism. Differentiating between "non-adverse" and "adverse" effects requires considerable knowledge of the reversible changes, which may be part of normal homeostasis. It also requires understanding of the subtle changes from "normal" physiology and morphology, which may alter biological properties such as the ability to adapt to stress, and life expectancy. In considering possible harm to humans, the psychological and behavioral changes ac-

companying small effects on the nervous system may be particularly important. Such changes may follow exposure to certain metals and their derivatives. Examples of substances which may cause these changes are lead(II) ions and methylmercury. Special attention must be paid to their effects in children.

Of course, none of the above considerations or criteria can be applied if the data available for analysis are inadequate. One must be sure that the animal data have been obtained under test conditions that would show up all the effects that might occur. Key considerations here are the number of animals studied and the time and environmental conditions of exposure and observation. Too few organisms or too short a study may result in lack of statistical power to identify effects that occur in only a small susceptible group in the population. Some effects may occur under conditions of environmental stress which are rarely simulated in toxicity testing. It may be that more flexibility of test conditions should be introduced to simulate specific conditions associated with high risk of adverse effects on humans in order to provide the information we need to avoid toxic exposures to certain chemicals.

4. BENCHMARK CONCENTRATION AND BENCHMARK DOSE

IUPAC definitions

benchmark concentration (BMC) (Fig. 5)
Statistical lower CL on the concentration that produces a defined response (called the benchmark response or BMR, usually 5 or 10 %) for an adverse effect compared to background, defined as 0 % [3].

benchmark dose (BMD)
Statistical lower CL on the dose that produces a defined response (called the benchmark response or BMR, usually 5 or 10 %) of an adverse effect compared to background, defined as 0 % [3].

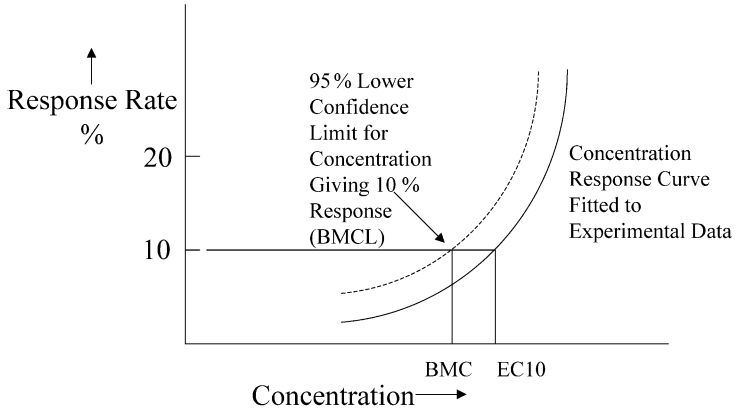

Fig. 5 Dose–response curve showing BMC and BMCL (BMC 10 % lower CL).

Explanatory comment

benchmark concentration and benchmark dose (BMC, BMD)

General considerations
The benchmark concept has been introduced in risk assessment in order to reduce numbers of animals used in testing and to move away from compulsory LD_{50} determination. The aim is to be able to define a concentration between no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL), which can substitute for them in risk assessment. In particular, the benchmark concentration (BMC) or dose (BMD) is proposed as an alternative to the NOAEL. Using the NOAEL in

determining acceptable human exposure values such as reference doses (RfDs) and reference concentrations (RfCs) has long been recognized as having limitations in that it: (1) is limited to one of the doses in the study and is dependent on study design; (2) does not take account of the variability in the estimate of the dose–response; and (3) does not take account of the slope of the dose–response curve. The NOAEL value is highly dependent on the quality of the data from which it is derived. The less precise these data are, the larger the NOAEL value tends to become. This means that any derived permissible exposure level (PEL) may be too high for safety. Exposure to levels equal to (or even below) the NOAEL may still permit the occurrence of adverse health effects. This is why health-based recommended exposure limits are derived by dividing the NOAEL by an uncertainty factor to ensure adequate health protection. Such uncertainty factors are largely chosen ad hoc by the regulatory toxicologists involved.

Determination of the BMC or BMD requires quantitative analysis of the data relating level of exposure to the effects of a chemical on animal or human health. The aim of the data analysis is to determine, as accurately as possible, the relationship between a given exposure and the likelihood of its producing a defined harmful effect as measured by the response (the percentage of a test population showing the defined effect). The statistical uncertainty to which any data is invariably subject is incorporated into the calculations. The dose–response relationship obtained is plotted graphically and used to calculate the BMC or BMD: this is the concentration or dose which corresponds to a chosen statistical percentage likelihood of health impairment in the exposed population, for instance, 5 or 10 %. The BMC or BMD is divided by an uncertainty factor to yield a health-based recommended permissible exposure limit, chosen with the aim of protecting the whole population at risk. Software for calculating the BMD is available on the Internet (see below).

The BMC or BMD method takes account of research data uncertainties which are largely ignored in the NOAEL method, and, while the NOAEL is by definition the highest experimental dose applied that does not give rise to an adverse effect, the BMD is a quantity derived from all the available experimental values. The BMD method can also give information about the risks associated with exposure exceeding the health-based recommended exposure limits because it is related to a statistically modeled dose–response curve.

The BMC or BMD method requires the following three choices to be made:

1. the statistical likelihood of an effect occurring in the test population that would be used in the determination of the BMC or BMD;
2. the dividing line between an effect considered to be tolerable and one considered to be harmful; and
3. the choice of a model function with which to describe the relationship between dose and response.

With current methodology, these choices have to be made and justified on a substance-by-substance basis. Derivation of uncertainty factors used to derive health-based permissible exposure limits is still ad hoc.

BMC and BMD refer to the central estimates, for example, the effect concentration (EC_x) or the effect dose (ED_x) for dichotomous end-points (with x referring to some level of response above background, e.g., 5 or 10 %). BMCL or BMDL refers to the corresponding lower limit of a one-sided 95 % confidence interval on the BMC or BMD, respectively. This is consistent with the terminology used in the EPA's BMD software (BMDS) which is freely available on the Internet at <http://www.epa.gov/ncea/bmds.htm>.

Determination of appropriate studies and end-points on which to base BMD calculations

Following hazard characterization and selection of appropriate effect end-points for use in dose–response assessment, studies appropriate for modeling and BMC or BMD analysis should be evaluated. All studies that show a graded monotonic response with concentration or dose are likely to be useful for BMC or BMD analysis. The minimum dataset for calculating a BMD should show a significant

dose-related trend in the selected effect end-point(s). It is preferable to have studies with one or more doses near the level of the benchmark response (BMR), usually 5 or 10 %, in order to give a better estimate of the BMC or BMD, and thus, a smaller confidence interval. Studies in which all of the concentration or dose levels show changes compared to the control values (unsuitable for NOAEL determination) can be used for BMC or BMD estimation, provided the lowest response level is reasonably close to the BMR.

There are at least three types of end-point data which may be available from toxicity testing: quantal (dichotomous), continuous, or categorical. A quantal (dichotomous) response may be reported as either the presence or absence of an effect, a continuous response may be reported as an actual measurement, or as a comparison (absolute change from control or relative change from control). In the case of continuous data, the number of subjects, mean of the response variable, and a measure of response variability (e.g., standard deviation (SD), standard error (SE), or variance) are needed for each group. For categorical data, the responses in the treatment groups are often characterized in terms of the severity of effect (e.g., mild, moderate, or severe histological change).

Selection of end-points should not be limited to only the one with the lowest concentration for giving rise to the lowest-observed adverse effect a kind of minimum value for the LOAEL. In general, end-points that have been judged to be appropriate and relevant to the exposure should be modeled if their LOAEL is up to 10-fold above the lowest LOAEL. This will help ensure that no end-points with the potential to have the lowest BMDL are excluded from the analysis on the basis of the value of the LOAEL or NOAEL. Selected end-points from different studies that are likely to be used in the dose–response assessment should all be modeled, especially if different uncertainty factors may be used for different studies and end-points. As indicated above, the selection of the most appropriate BMCs (BMDs) and/or NOAELs (if some end-points cannot be modeled) to be used for determination of health-based PELs will be a matter of scientific judgement.

Selection of the BMR value

Calculation of a BMD is directly determined by the selection of the BMR, the increase in the incidence of a given adverse effect in a population subjected to exposure to a toxicant. For quantal effects such as cancer or mortality, an excess incidence of 10 % is usually the default BMR, since the 10 % increase in detectable response in a given population is at or near the limit of sensitivity in most cancer bioassays and in some non-cancer bioassays as well. If a study has greater than usual sensitivity, then a lower BMR may be used, although the ED_{10} and LED_{10} should always be presented for comparison purposes.

For continuous data, if there is an accepted level of change in the end-point that is considered to be biologically significant, that amount of change should be selected as the BMR. Otherwise, if individual data are available and a decision can be made about what individual levels should be considered adverse, the data can be "dichotomized" based on that cut-off value, and the BMR can be set as above for quantal data. Alternatively, in the absence of any other idea of what level of response to consider adverse, a change in the mean equal to one control SD from the control mean can be used. The control SD can be computed including historical control data, but the control mean must be from data concurrent with the treatments being considered. Regardless of which method of defining the BMR is used for a continuous dataset, the effective dose corresponding to one control SD from the control mean response, as would be calculated for the latter definition, should always be presented for comparison purposes.

Choice of the model to use in computing the BMD

The goal of the mathematical modeling in BMD computation is to fit a model to dose–response data that describes the dataset, especially at the lower end of the observable dose–response range. In practice, this involves first selecting a family or families of models for further consideration, based on characteristics of the data and experimental design, and fitting the models using one of a few established methods. Subsequently, a lower bound on dose is calculated at the BMR. USEPA guidance on BMC and BMD calculation recommends that 0.1 be used to compute the critical value for goodness of fit, in-

stead of the more conventional values of 0.05 or 0.01, and that a graphical display of model fit be examined as well. For comparison of models and selection of the model to use for BMDL computation, the USEPA recommends the use of Akaike's Information Criterion (AIC); see the Web site referred to below.

Computation of the confidence limit for the BMD (BMDL)

The USEPA benchmark dose guidance document (available at the USEPA Web site <http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=20871>) discusses the computation of the CL for the BMD, the fact that the method by which the CL is obtained is typically related to the data type, and the manner in which the BMD is estimated from the chosen model. Details for approaches to CL computationally specific to particular data types (quantal, clustered, continuous, multiple outcomes) are provided in the USEPA document.

Advantages of the benchmark dose (concentration) method

The advantages of using the BMD approach are many. First, all the experimental data are utilized to construct the dose–response curve. Secondly, the variability and uncertainty are taken into account by incorporating SDs of means, and thirdly, the method represents a single methodology which can be applied to cancer and non-cancer end-points. It may also be possible to use fewer animals in testing.

5. BIOMARKER

IUPAC definitions

biomarker

Indicator signalling an event or condition in a biological system or sample and giving a measure of exposure, effect, or susceptibility [3].

Note: Such an indicator may be a measurable chemical, biochemical, physiological, behavioral, or other alteration within an organism.

biomarker of effect

Biomarker that, depending on its magnitude, can be recognized as associated with an established or possible health impairment or disease [3].

biomarker of exposure

Biomarker that relates exposure to a xenobiotic to the levels of the substance or its metabolite, or of the product of an interaction between the substance and some target molecule or cell that can be measured in a compartment within an organism [3].

biomarker of susceptibility

Biomarker of an inherent or acquired ability of an organism to respond to exposure to a specific substance [3].

Explanatory comment

The initial changes in enzymes and other biological substances or physiological responses affected by a substance are called early effects, and some may be used as biomarkers of exposure and to give a measure of internal dose. The term "biomarker" may cover any one of a range of biological effects reflecting the interaction between a toxicant and the organism affected. The term may be applied to a functional, biochemical, or physiological change or it may be applied to a specific molecular interaction. The best biomarkers provide direct evidence for the exposure of individuals in a population to a particular substance, e.g., lead in bone, cadmium in the kidney (both in vivo determinations), mercury in urine, or trichloroethylene in exhaled air. Quantitative measurements may permit the determination

of a dose–effect relationship, particularly if the toxicokinetics of the substance are well established. Mostly, samples of blood, urine, and exhaled air are used but hair, teeth, and nail clippings may sometimes be analyzed. Noninvasive methods should be used where possible. Such measurements may be used for screening or for monitoring either an individual or a group for absorbed dose.

Biomarkers of exposure, where applicable, are to be preferred to monitoring ambient media for exposure assessment because they reflect internal dose directly. An advantage of biomarkers of exposure is that they are an integrative measure, i.e., they provide information about exposure through all routes including those of nonoccupational exposure. An example where this is important is the combination of occupational exposure to lead with exposure to lead through hobbies (such as in soldering, shooting, or glazing with lead) and with environmental exposure to leaded gasoline. Another example is occupational exposure to solvents combined with exposure to solvents at home during painting or while engaged in hobbies involving paint and glue.

Biomarkers related directly to exposure can be classified into two groups: (a) biomarkers of exposure (biological monitoring), (b) biomarkers of effect (biological effect monitoring).

Use of biomarkers of exposure in monitoring individual dose of toxicants (biological monitoring)

To assess internal dose and body burden, the amounts of toxicants of concern or their metabolites and/or derivatives in cells, tissues, body fluids, or excreta are measured. In addition or alternatively, an indirect biomarker of exposure may be determined such as cytogenetic change or reversible physiological change in exposed individuals. For example, if lead effects on heme synthesis are detected, they may be used in addition to measurement of lead in blood as a measure of internal dose (integrated in time) for this particular effect. In the case of cadmium in blood, there is no effect on the blood and thus cadmium in blood has to be used exclusively as a measure of internal dose. An early effect of cadmium is on the kidney, causing the leakage of proteins into urine. A good dose measure to relate to this effect is the concentration of cadmium in urine, adjusted for urinary dilution. This measure also gives an indication of the body burden.

For assessment of internal exposure and dose, sampling technique must be precisely controlled since it can profoundly affect results. Data for substances which disappear rapidly from the blood cannot be interpreted unless there is a documented standard time at which samples are taken. A standard time for sampling is especially essential if the half life of the toxicant of concern in the body is short. Contamination is the major source of errors when analyzing many substances, especially metals such as nickel, chromium, and cadmium. Contamination can come from the air, from the skin and sweat, sample containers, and anticoagulants (for blood samples). The risk of contamination from skin, clothes, and hair, as well as from the air at the workplace is particularly great when collecting urine samples. Precipitation and adsorption are problems when collecting and storing urine samples. Certain chemicals, for example, aluminum and volatile organic compounds, are adsorbed on glass and plastic.

The most common sample materials are anticoagulated whole blood, serum or plasma, urine, and exhaled air. Saliva, sweat, hair, and nails may also be used in biological monitoring for certain substances. Urine samples are frequently used as urine is easy to collect in large amounts. Variations in liquid intake and fluid loss (e.g., in a warm working environment where a lot of fluid is lost through sweat) result in large variations in concentrations of substances in urine. This variation is often corrected using the creatinine concentration of the urine or by measuring the urinary 24-h volume output. Relative density of urine can also be used for such corrections. Because of the differences between men and women, results for each gender should be reported separately.

Biomarkers of effect (biological effect monitoring)

Biomarkers of effect are measurable biochemical, physiological, or other alterations within an organism that can be recognized as associated with an established or potential health impairment or disease. Biomarkers of effect are often not specific for a certain substance but sometimes may be sufficiently specific to be used as surrogate measures of exposure and of dose. This will certainly be true if it is

known that only one of the possible causes of a given effect can be involved in a given exposure situation. However, in some circumstances, single biomarkers of effect may be useful in blanket monitoring of multiple exposure, where those exposures have a common effect. If multiple biomarkers are used, and one or more markers are positive, additional markers or environmental monitoring can be used to determine the substance(s) causing the effect.

Examples of biomarkers of effect are:

1. The inhibition of certain enzymes of the heme synthesis pathway, which is caused by lead ions (or by dioxins), resulting in elevated concentrations of the precursors protoporphyrin and δ-aminolaevulinic acid dehydratase in blood and δ-aminolaevulinic acid and coproporphyrin in urine.
2. The leakage into urine of certain proteins such as β_2-microglobulin, α_1-microglobulin, retinol-binding protein, and albumin, which is caused by a number of metal ions and solvents; in addition, there is inhibition of the activity of certain enzymes in the urine, e.g., *N*-acetyl-D-glycosaminidase (NAG), with specific isoforms of NAG-A and NAG-B.
3. The inhibition of the enzyme acetylcholinesterase, which occurs following exposure to a number of organophosphate and carbamate insecticides (e.g., parathion).
4. An increase in hemoglobin adducts, which follows exposure to aromatic amines, ethylene oxide, propylene oxide, butadiene, and alkylating or arylating agents of all kinds. Such adducts may also be used as biomarkers of exposure.

6. BIOTRANSFORMATION

IUPAC definition

biotransformation
Chemical conversion of a substance that is mediated by living organisms or enzyme preparations derived therefrom [3].

Explanatory comment

Chemical conversion refers to the transformation of one chemical species into another. The main point in using the term biotransformation is that the conversion must be carried out by (i) a living system or (ii) enzymes derived therefrom. In general, biotransformation refers to conversions that are carried out by enzymes. Conversion carried out by a living system includes all chemical conversions that go on within the cell or the body, either as part of the metabolism of endogenous substances or of exogenous xenobiotics, drugs, toxic substances, etc. However, interconversion of intermediates in a defined metabolic pathway would more usually be referred to as metabolism than as biotransformation. Thus, the term "biotransformation" normally refers to a xenobiotic, unless the xenobiotic is a drug when the term "drug metabolism" would be preferred.

The biotransformation need not occur within the organism. For instance, microorganisms are often employed in waste management and environmental clean-up, i.e., in remediation. Including derivative enzyme preparations in the definition reinforces the intent that the term refers to enzyme-catalyzed conversions. In principle, one could manufacture an enzyme by total chemical synthesis and then use it for chemical conversion of a substance; this would qualify as biotransformation. However, this proviso in the definition is really intended to include the more usual scenarios where a bacterial culture, a cell or tissue homogenate, or an enzyme purified therefrom, is used for a specific intended conversion. Biotransformations using purified enzymes or enzyme-enriched preparations are also useful in synthetic organic chemistry.

Biotransformation is one method of clearance of a substance, and it may result in a product of greater or lesser biological effect (toxic or therapeutic) than the starting material. In the therapeutic con-

text, biotransformation can be exploited to facilitate delivery to a site of effect, and activation at that site. Dopamine, used in the treatment of Parkinson's disease, does not readily cross the blood–brain barrier. However, L-dopa (3,4-dihydroxyphenylalanine) does, and is decarboxylated to dopamine in the central nervous system. The cell membrane is another important barrier. An acetoxymethyl ester (AM) derivative is often employed to mask a carboxylate functionality. Once inside the cell, endogenous esterase activity hydrolyzes the ester, and the charged parent acid is trapped within the cell by virtue of its negative charge. Chemotherapeutic agents are also designed with regard to biotransformations that might take place in the unique environment of the neoplastic tissue, based, for example, on the hypoxic nature of its core.

Biotransformation is an extremely important concept in toxicology, as it often involves systems that have evolved to detoxify xenobiotics. However, initial steps in detoxification may increase toxicity. An example would be epoxidation of an aromatic hydrocarbon, with the intent of increasing its solubility for excretion or creating functionality for conjugation, but in the process creating reactive chemical species that can damage biomolecules like lipids, proteins, and DNA by creating oxygen-centered radicals and forming adducts.

The above processes are the basis for a classification of reactions of drug metabolism into phase I and phase II. Phase I refers to the chemical modification of a substance by processes such as hydroxylation, oxidation, reduction, or chlorination. This introduces functionality for subsequent further metabolism. Phase II refers to conjugation of the phase I products, usually to enhance their hydrophobicity and facilitate urinary or biliary excretion. Whereas phase I processes increase hydrophilicity and add functionality at the risk of increased toxic potential, those of phase II almost invariably decrease toxicity.

Phase I metabolism

Phase I metabolism includes a variety of reactions that modify the core structure of the xenobiotic. These include oxidations by mixed-function oxidases (MFOs), reductions, hydrolyses, hydrations, and isomerisms. The MFO cytochrome P_{450} (P450) is so important for phase I oxidations that it is usual to consider oxidations by P450 separately from others. Non-CytP450 oxidations are catalyzed by enzymes such as alcohol dehydrogenase, xanthine oxidase, amine oxidases, and aromatases; they require oxygen. Reductive biotransformation includes reactions that are generally inhibited by oxygen and require NADPH. Substrates include azo- and nitro-compounds, halogenated compounds, nitrogen heterocycles, and epoxides. A number of enzymes effect hydrolysis, most significantly the esterases, while hydration without hydrolytic cleavage is the domain of less abundant hydratases. Simple reactions such as cyclization, isomerization, dimerization, transamidation, and decarboxylation are also considered phase I.

The P450 family of enzymes uses NADPH and molecular oxygen to hydroxylate many different substrates. These are heme-containing enzymes with molecular weights (relative molar masses) of about 45 to 55 kDa embedded in the membrane of the endoplasmic reticulum. The P450 genes are classified into families and subfamilies, and well over 30 human P450 proteins are uniquely identified. Some of these are primarily important for drug metabolism (e.g., P450's 2E1, 2D6, 2C9, 3A4); others function in sterol synthesis (e.g., P450's 11A1, 21A2). Genetic variation in P450 isoforms is associated with differing rates of drug metabolism (e.g., P450 2D6 variants give rise to different rates of debrisoquine metabolism), and potential susceptibility to some carcinogens.

Phase II metabolism

Phase II metabolism acts upon the products of phase I to render them even less toxic and more water-soluble by conjugation reactions, enhancing urinary and biliary excretion. These reactions include addition of glucuronic acid (glucuronidation), glycosylation, sulfation, methylation, and acetylation. Conjugation of many substances (generally strong electrophiles produced by phase I metabolism) to glutathione represents a major route of detoxification. This may occur spontaneously, or be catalyzed by glutathione-*S*-transferases. The glutathione (Gly-Cys-Glu) thioconjugate may be excreted directly through the bile or urine, or may become a substrate for sequential attack by glutamyl transpeptidase

and a second peptidase that results in release of the cysteine conjugate of the xenobiotic. This occurs particularly in the liver and kidney. Conjugations with fatty acids or cholesterol esters also occur, as with the conjugation of cannabinoids to stearic and palmitic acids. Glucuronidation of bilirubin is important in preventing build-up of this potentially toxic metabolite. In general, conjugations require an "activated" intermediate, either of the xenobiotic itself, or involving UDP-glucuronic acid (for glucuronidation), *S*-adenosyl methionine (for methylation), acetyl coenzyme-A (for acetylation), or phospho-adenosine phosphosulfate (for sulfation). Though not generally commonly used, the term "phase III metabolism" refers to metabolism of conjugates arising from products of phase II. An example would be the further metabolism of glutathione conjugates in the gut.

Among the most important sites of biotransformation from the perspective of toxicokinetics are the liver and the lung, although the gastrointestinal tract, kidney, and erythrocytes are also important in xenobiotic metabolism. The liver plays a central role in biotransformation and detoxification of xenobiotics; indeed, that is one of its major roles. The "first pass effect" refers to the fact that oral exposure to a substance absorbed in the gut will first meet the liver through the portal circulation, and undergo phase I processing. Most of the enzymes of phase I and phase II metabolism were initially identified in liver tissue. Phase I oxidative enzymes in liver and other cells are almost exclusively in the endoplasmic reticulum, whereas phase II enzymes, including the glutathione-*S*-transferases, are cytosolic: an exception is glucuronosyl transferase, which is in the endoplasmic reticulum. There are effective transfer systems for sulfate, glucuronic acid, glutathione, and glycine conjugates in liver cell membranes. These permit uptake of conjugates into liver cells and their secretion into bile. Similar transfer systems are found in kidney cell membranes.

The lung is the portal of entry for inhaled substances and has its own defensive barrier. Pulmonary alveolar macrophages are adapted to antioxidant defence. Paradoxically, the very defenses of lung tissue result in metabolic activation of many compounds. This is the consequence of a metabolically active, aerobic tissue. Many toxicants first enter the body into the circulation. The erythrocyte, as the major circulating cell, protects itself by detoxification through biotransformation. Erythrocytes lack nuclei and reproductive capacity, nevertheless serve a unique role in detoxification. They exist in a high O_2 and high iron environment, and must withstand this environment of high oxidative stress. Iron, hemoglobin, and oxygen (oxidative stress) participate in oxidative biotransformation reactions in the erythrocyte. Glutathione conjugates are actively transported outward across the red-cell membrane. A major protective enzyme against oxidative stress in erythrocytes is glutathione peroxidase. Characteristic biotransformation reactions in erythrocytes are glutathione transferase and N-oxidations of some substrates.

7. CARRIER

IUPAC definition

carrier

1. Substance in appreciable amount which, when associated with a trace of a specified substance, will carry the trace with it through a chemical or physical process [3].
2. Person who is heterozygous, that is, who carries only one allele, for a recessive disease, and hence does not display the disease phenotype but can pass it on to the next generation [2].

Explanatory comment

A carrier, in chemistry, refers to a substance that chaperones or carries another. In toxicology and toxicokinetics, it is used conventionally in several ways—subtly different, perhaps, but all related to the general concept. The parent definition mentions the carrier in "appreciable" amount, and a referent substance in "trace" amount. Without attempting a definition of these terms, we can note that a large dif-

ference in substance amount, perhaps a concentration gradient, is implied. The term is often used in describing preparations of radioisotopes, and this illustrates the concept nicely. If all the atoms of an element in a sample are radioactive, then the element is "carrier-free"; otherwise, we would say that the cold isotope was a carrier. "Carrying" in this sense refers only to dilution; a carrier here refers to a chemical species that changes the amount of another species from which it is distinguishable only by a particular analytical method. Combining the above two concepts, the occurrence of a large difference in amount of two substances that cannot be readily distinguished by available analytical techniques, we come to the concept of a carrier as a diluent.

In a different sense of the term, it is not dilution of one species by another, but a physical interaction that is specified. The English verb "carrying" is often used to describe a physical process of transport. Thus, if one molecule can interact with another, e.g., by adsorption or electrostatic attraction, one can be said to carry the other. It is in this sense that we refer to a carrier protein as interacting with a ligand in such a way that the ligand's distribution is determined by the protein. Again, concentration gradients would be important, but now from the point of mass action; a high concentration of the carrier will determine the relative distribution of the partner. This is what we mean, for instance, when we note that a carrier may compete with a substance's binding to a surface. A carrier substance (often a protein) is one which is used to deliver specifically another substance to an intended location, for instance, often referring to a substance that delivers a drug to its site of action.

In practice, we might add one substance to a sample to prevent another substance from being lost due to its presence in low quantities, for example, by adsorption to a surface. The former substance would then be described as a carrier, and could contribute to the recovery of the latter by all the above processes—direct binding, competition for binding, and dilution or displacement into a phase of interest. Albumin is often used as a "carrier protein" because of its ready abundance and mixed hydrophobic and hydrophilic nature.

A final meaning of the term "carrier" in biology is its use to describe a molecule or molecular complex that "carries" a substance across a biological barrier. Here, the term "transporter" is often substituted, though this may be a more specific term if it is intended to imply active transport requiring energy, as in the case of ATP hydrolysis, coupled transport, or conformationally coupled processes. Such transport frequently refers to delivery across a cell membrane. See also "1. Absorption" above.

8. COMPARTMENT

IUPAC definitions

compartment
Conceptualized part of the body (organs, tissues, cells, or fluids) considered as an independent system for purposes of modeling and assessment of distribution and clearance of a substance [3].

compartmental analysis
Mathematical process leading to a model of transport of a substance in terms of compartments and rate constants, usually taking the form $C = Ae^{-\alpha t} + Be^{-\beta t}\ldots$ where each exponential term represents one compartment. C is the substance concentration; A, B, … are proportionality constants; α, β, … are rate constants; and t is time [3].

physiologically based pharmacokinetic modeling (PBPK)
Mathematical modeling of kinetic behavior of a substance, based on measured physiological parameters [3].
Synonym: toxicologically based pharmacokinetic modeling

Explanatory comment

compartment

A compartment as used in toxico- or pharmacokinetics is an abstract concept that should be thought of in purely arithmetical terms; although the mapping of this concept onto a biological structure may be straightforward and in some cases may seem obvious, this is by no means the way to understand compartmental analyses, as the compartments need not have any anatomical reality. The need for compartments, then, is guided by the need to analyze the movement of substances, their accumulation and uptake, their clearance and elimination, and their redistribution. Because we often wish to describe a concentration of a substance and its change (e.g., the derivative of substance amount with respect to time) we need a representation of volume in the denominator. Thus, a compartment is, in its broadest meaning, any volume that can be used to define the concentration of a substance of interest.

Typically, one of the most useful compartments is the circulating blood volume, which may be defined specifically as blood (the fluid including all cellular components), or as the plasma (free of cells) or serum (plasma free of coagulating proteins). In this context, the blood behaves as a compartment, and we can consider the entry of a substance into this compartment or space, or its elimination therefrom. From the principle that first-order chemical reactions will occur at rates proportional to the amount of the reactant, a simple exponential equation usually describes the rate of appearance or disappearance in the compartment. A simple example of compartmental behavior, then, is the exponential rate of decay of a substance in the blood, i.e., its disappearance from the blood.

If compartments can be considered as hypothetical spaces between which substances move according to defined kinetic principles, we can introduce the idea of compartmental analysis as the mathematics that describes this movement. Complex multicompartment models can be employed to model complicated behavior, but the general principles are well illustrated by considering the cases of one- and two-compartment systems. Surprisingly, the intricate biology that determines the full toxicokinetics of a substance is often modeled very well by these simple compartmental descriptions based on first-order kinetics.

The simplest one-compartment model depicts the whole body as a single homogeneous unit. If a substance distributes rapidly throughout the body, this might be a rather accurate model, and the plasma might be the compartment of choice to describe the substance's behavior, because of its accessibility for sampling. Note that the criterion of homogeneity does not mean that the substance is assumed to be uniformly distributed throughout the body; rather, kinetic homogeneity means that the rate of the substance's appearance in, or disappearance from, the plasma well represents its rate of appearance or disappearance in any other part of the body. Important concepts are the maximum concentration reached in the compartment (c_{max}) and the time at which it occurs (t_{max}). The integration of the curve of concentration of the substance vs. time, from zero to infinity (area under the curve), depends upon the total amount of the substance that has entered the compartment, and is useful for describing dose or exposure. The rate, K, of addition to, or elimination from, a compartment is the sum of individual rate constants (k_i) of the various processes. In the example of an infused drug, X, of concentration [X], it is common to write the elimination rate as $d[X]/dt = k_o - K[X]$, where k_o is the rate of infusion and [X] = 0 at $t = 0$. Often in pharmacokinetics, one uses the Laplace transform to solve this and similar differential equations to eliminate the time variable, yielding equations that can be solved more easily.

Two-compartment (and higher-order) models are useful for describing exchange between pools. In the simplest sense, a pair of exchange rate constants, k_{12}/k_{21}, describe the flux between compartments, and an elimination rate constant, k_{10}, accounts for removal of the substance from a single compartment. As additional compartments and multiple sites of uptake and/or elimination come into play, the arithmetic becomes more complicated, but the underlying principles, and their utility, remain the same.

The description of transfer of a substance among compartments facilitates modeling of kinetic behavior, so-called pharmacokinetic or PK modeling. The resulting models are descriptive, giving a con-

cise mathematical description of empirical data, for instance. Predictive models, on the other hand, accommodate nonlinear aspects of ADME (see 1. Absorption) that are not well handled by classical compartment models. PBPK is one of the most successful among predictive models. Proposed mechanistic aspects of physiological phenomena are incorporated, and rigid assumptions, e.g., of steady-state or first-order kinetics, are avoided. For instance, successful PBPK (PBTK) models of lead, chromium, and methyl mercury in humans have been developed that take into account perfusion rates of various tissues where redox reactions, lipophilic partitioning, or selective ligation can occur.

9. DETERMINISTIC (NON-STOCHASTIC) AND STOCHASTIC

IUPAC definition

deterministic effect, deterministic process
Phenomenon committed to a particular outcome determined by fundamental physical principles [3].

stochastic
Pertaining to or arising from chance and hence obeying the laws of probability [3].

stochastic effect, stochastic process
Phenomenon pertaining to or arising from chance, and hence obeying the laws of probability [3].

Explanatory comment

Deterministic (non-stochastic) effects, also called threshold effects, are effects that have a threshold of chemical exposure below which they do not occur and above which the severity of the effect is related directly to the dose. Most toxic effects come into this category and, for these, determination of the threshold or some acceptable approximation to it (such as the benchmark dose described above) is the essential basis for setting the PELs which underlie all regulatory activities to protect people and their environment from harm that chemicals may cause.

Deterministic effects give the classical (or skewed) S-shaped dose–effect and dose–response curves, approximating to a linear relationship in the low dose area. The S-shaped curve is explained by the fact that both for receptor molecules and for individuals there is a range of sensitivity to any given toxicant. The variation in sensitivity is random (except in genetic extremes where a receptor or essential enzyme may be missing completely) and shows a normal (Gaussian) distribution.

If the population has genetic subgroups of markedly different susceptibility to the toxicant because of a missing or much altered receptor or activating enzyme, more than one such curve may be apparent in the test data, but this situation can be identified only if very large populations are studied.

Stochastic effects, sometimes referred to as quantal effects, are usually produced by a reaction between an agent and DNA causing a discrete genetic change, Thus, they are described as "all or none", either occurring or not occurring. For such reactions, no safe threshold of exposure can be determined, but the probability of their occurrence is related directly to increasing dose. Severity of the resultant toxic effect is not related to dose but to the consequences of the genetic change. The consequences of stochastic (or pseudo-stochastic) effects range from relatively minor metabolic deficiencies through immunologically mediated effects to mutagenicity, aspects of teratogenicity, genotoxic carcinogenicity, and ultimately death, The inability to determine a threshold of exposure for stochastic effects does not mean that one does not exist, simply that it cannot be demonstrated by current methods of testing. In this circumstance, the precautionary principle determines that the no threshold assumption must be applied to ensure safety. As a result, PELs are set with reference to a calculated low probability of the adverse effect occurring. This probability for humans is usually set at 1 in 100 000 or at 1 in 1 000 000. To some extent, this is based on the practicality that an increase in disease rate (see below) of this order is too low to be detected by current techniques of epidemiological monitoring.

A problem with stochastic effects is that the shape of the dose–response curve is uncertain in at least three ways. First, the curves derived from animal trials are usually based on no more than two central data points (out of perhaps four doses used). Secondly, there is an inherent statistical uncertainty if an extrapolation is made to those low dose levels which are likely for normal human exposure and therefore of regulatory importance. Thirdly, the curves are extrapolated to low doses using debatable mathematical models, usually chosen for precautionary purposes to give the highest estimate of probability of effect.

10. DOSE

IUPAC definitions

dose (of a substance)
Total amount of a substance administered to, taken up, or absorbed by an organism, organ, or tissue [3].

Explanatory comment

dose (of a substance in toxicology)

General considerations

In common pharmaceutical usage, the term "dose" is applied to the amount of medication taken by a patient at any one time. In the IUPAC definition, this is covered by the term "administered" but, ideally, the toxicologist wants to know the amount of a substance "taken up, or absorbed" in the same definition. In other words, the toxicologist would like to know the amount available for interaction with metabolic processes or biologically significant receptors after crossing the relevant biological boundary (epidermis, gut, respiratory tract, cell membrane). This "absorbed dose" is the amount crossing a specific absorption barrier and would be best defined in practice if it could be referred specifically to the target organ but this is rarely possible.

Scientifically, it would be best if the dose were always expressed in molar terms so that comparison could be made between the numbers of molecules involved and even related to numbers of receptor molecules. In practice, units of mass are more common in relation to prescription drugs.

A major problem for toxicologists is the relationship between exposure and internal dose. For a given exposure in a given medium, uptake of a substance into the body, and hence internal dose, varies from individual to individual depending upon physiology, behavior, and the presence of other substances which may prevent or enhance uptake. Because of our physiology, when air is contaminated with pollutants, people are advised to minimize exercise as this is associated with rapid deep breathing, resulting in greater uptake of the pollutants from the air than would occur in someone at rest exposed to the same air concentration. Because of behavior, soil contaminants at a given concentration may be absorbed more by children who put soil in their mouths than by adults who do not behave in this way. Finally, the presence of sufficient calcium ions in water prevents uptake of lead ions, minimizing the internal dose associated with a given lead ion concentration.

To determine the internal dose of a given chemical, analysis of tissues and body fluids can be carried out. In determining the internal dose, analysis is aimed at measuring amounts of the substance itself, and (or) of its metabolites. Since the internal dose is defined as the total amount of a substance absorbed, measurement of the substance of concern should be repeated over the full period of exposure and the measurement results should be integrated over this time.

11. EFFECT AND RESPONSE

IUPAC definitions

effect
Change in biochemistry, morphology, physiology, growth, development, or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increase in susceptibility to other environmental influences [3].

response
Proportion of an exposed population with a defined effect or the proportion of a group of individuals that demonstrates a defined effect in a given time at a given dose rate [3].

Explanatory comment

In the general toxicological literature, the terms "effect" and "response" are often used interchangeably to describe a biological change in individuals or in a population which can be caused by a given exposure or dose. However, there is a clear distinction to be made between the consequences of exposure to an individual and those for a population. This is most clearly seen when one considers the differences in approach to human toxicology and ecotoxicology. In human toxicology, the main concern is protection or treatment of the individual, and the aim of regulatory activity is to protect every individual in the population at risk. In ecotoxicology, the aim is to protect populations, communities, and ecosystems; the loss of a small number of individuals here is of little concern if the population can easily restore its numbers. Thus, it useful, as done in the Glossary, to be able to distinguish clearly between consequences for the individual and those for the population. This important distinction is facilitated if we differentiate between an effect and a response by applying the term "effect" to a biological change in an individual and the term "response" to the proportion of a population that demonstrates a defined effect. Following this convention, response means the incidence rate of an effect (see *rate* below). In this way, the LD_{50} value may be described as the dose expected to cause a 50 % response in a population tested for the lethal effect of a chemical.

It will be seen that the use of two different words distinguishing between effects on individuals and responses of populations makes for greater clarity of thought and communication. In human terms, effects on individuals must be understood for the application of toxicological knowledge to treat the problems of individual patients or to prescribe drugs properly. On the other hand, response of populations must be understood in order to regulate exposure to safe levels for both human populations and populations of other organisms.

12. ELIMINATION AND CLEARANCE

IUPAC definitions

clearance (in toxicology)

1. Volume of blood or plasma or mass of an organ effectively cleared of a substance by elimination (metabolism and excretion) divided by time of elimination.

   Note: Total clearance is the sum of the clearances of each eliminating organ or tissue for a given substance.

2. (in pulmonary toxicology) Volume or mass of lung cleared divided by time of elimination; used qualitatively to describe removal of any inhaled substance which deposits on the lining surface of the lung.

3. (in renal toxicology) Quantification of the removal of a substance by the kidneys by the processes of filtration and secretion; clearance is calculated by relating the rate of renal excretion to the plasma concentration [3].

elimination (in toxicology)
Disappearance of a substance from an organism or a part thereof, by processes of metabolism, secretion, or excretion [3].
See also clearance.

elimination rate
Derivative with respect to time of the concentration or amount of a substance in the body, or a part thereof, resulting from elimination [3].

Explanatory comment

"Clearance", and the related term "elimination", are used in toxicology in an attempt to describe, usually quantitatively, the rate of disappearance of a substance (drug, toxin, analyte, etc.) from an organism, organ, tissue, or compartment. "Elimination" refers simply to the disappearance of the substance. "Elimination rate" indicates the time over which this happens, and so is a derivative with respect to time (dc/dt) of the concentration (c, expressed in suitable units) of the substance in the compartment of interest. The use of these terms does not imply, or make any reference to, mechanism. The substance may be removed, or the compartment cleared, by mechanical means such as transport or filtration, or by conversion to another substance through metabolism or biotransformation.

Whereas elimination focuses on the substance and its rate of change of concentration with time, clearance generally refers to the compartment, such as blood or lung, in the numerator. (Renal clearance is somewhat different and is discussed below.) It is common to report clearance from blood (or from plasma if the substance does not enter the blood cells) as this is a measure of how long cells and tissues perfused by the circulation will be exposed to the substance. The numerator of the term, then, would be blood volume, and we would say for instance that so many millilitres of blood were cleared of the substance in a minute. This is quite different from an elimination rate where the expression is dc/dt. We do not mean that the substance is completely eliminated from the reference volume of blood in a given time. That is, we do not focus on 1 mL of blood and say that c drops to zero. Rather, if the concentration drops by one-half we would say that this has removed enough substance to clear one-half of the blood volume.

One reason it is useful to express clearance in this way is that it relates to the total volume of the compartment, e.g., blood. So if elimination is due to the functioning of a particular organ, for example, this gives us an indication of the organ's functional capacity. It also allows for consideration of changes in blood volume, for instance, due to transfusion or hemorrhage. This means of expression is also mathematically useful in compartment models.

A note is added to the definition of "clearance" that total clearance of a substance is the sum of individual clearances from each eliminating organ or tissue. This might seem intuitively obvious from conservation of mass. But it reminds us that (i) we may not always be able to identify all sources of elimination; (ii) in multicompartment models, subtraction from one compartment may result in addition to another; and (iii) this relation holds whether concentration of substance or volume of compartment is being divided by time.

Clearance as commonly used in pulmonary toxicology has a somewhat different nuance. When a substance is inhaled and deposits on the epithelial surface of the bronchi, bronchioles, or alveoli, clearance is expressed as disappearance from this surface. The value is only qualitative, for two reasons. (i) We cannot easily measure the surface area, so use lung mass or volume instead, but of course the surface-to-volume ratio is not known. (ii) We cannot really measure surface concentrations. The clearance will be derived by considering the amount of surface deposition that could arise from a certain expo-

sure, and the time it takes to drop to a presumed value of zero, determined by analytical detection, with inherent detection limits, following a procedure such as lavage. Because we define clearance in pulmonary toxicology on the basis of tissue surface, elimination may be either mechanical, through mucociliary transport and (or) the cough reflex, or by cellular uptake. When the substance is taken up by the cells, it may remain in the lung tissue or be eliminated by metabolism or absorption into the body, but it has been cleared from the lining surface of the respiratory tissues.

Clearance in renal toxicology has a somewhat specialized meaning, reflecting the unique physiology of the kidney. Renal clearance is quantitative. While it is often more useful to know the effect of removal a substance from the body fluids than to know the composition of the urine, plasma clearance frequently depends primarily on renal clearance, and the latter is an important measure of renal function. A substance with a molecular weight (relative molar mass) of less than about 65 kDa will be filtered from human plasma at the renal glomerulus, and then reabsorbed (or not) to varying degrees during subsequent passage through the remainder of the nephron. For a substance that is removed from plasma exclusively by urinary excretion, its concentration in urine divided by its concentration in plasma will equal its plasma clearance divided by the urinary rate of flow. With units as examples,

$$[\text{Plasma clearance/mL min}^{-1}]/[\text{Urine flow/mL min}^{-1}] = [\text{Concentration in urine/mol L}^{-1}]/[\text{Concentration in plasma/mol L}^{-1}]$$

Glomerular filtration rate (GFR) is a measure of glomerular function. If a substance is cleared from the plasma solely by the kidney and is not reabsorbed or secreted, then the GFR is equal to the plasma clearance. Inulin is a substance that behaves this way to a very good approximation, and its plasma clearance can be used to measure GFR. Creatinine is an endogenous substance produced by muscle metabolism at a fairly constant rate and shows a reasonable approximation to inulin with respect to its handling by the body. Thus, creatinine clearance is a good clinical measure of GFR. Creatinine is easier to measure than inulin and, as an endogenous substance, its use is less invasive. Glucose is completely reabsorbed in normoglycemic states. Thus, in renal physiology glucose clearance is often said to be zero, although it is of course non-zero in more general terms because it is taken up by cells and metabolized. Because the kidney can also secrete substances through the peritubular capillaries independently of glomerular filtration, plasma clearance can be greater than GFR. An important example of this latter principle is H^+. The opposite is true of a substance which is reabsorbed by the kidney tubules.

13. HALF LIFE

IUPAC definitions

half life, $t_{1/2}$
Time required for the concentration of a reactant in a given reaction to reach a value that is the arithmetic mean of its initial and final (equilibrium) values. For a reactant that is entirely consumed, it is the time taken for the reactant concentration to fall to one-half its initial value.

Note: The half life of a reaction has meaning only in special cases:

1. For a first-order reaction, the half life of the reactant may be called the half life of the reaction.
2. For a reaction involving more than one reactant, with the concentrations of the reactants in stoichiometric ratios, the half life of each reactant is the same, and may be called the half life of the reaction.

If the concentrations of reactants are not in their stoichiometric ratios, there are different half lives for different reactants, and one cannot speak of the half life of the reaction [3].
Synonym: half time

half time, $t_{1/2}$
See synonym: half life.

Explanatory comment

Half life in toxicology can be either radioactive or biological. Radioactive half life is the time taken for half the number of atoms in a radioactive substance to decay. Different radionuclides have different half lives and emit different forms of radiation and thus have different toxicological effects. In radiation toxicology, both types of half life must be taken into account. If a radionuclide has a long radioactive half life but a short biological half life in the tissues or cells of a given organism, the chances of harm resulting from the radioactivity in that organism are small because the radionuclide will probably be excreted before significant radioactive decay occurs. But the possibility of radiation damage is still there, however small it may be. An even more complex situation arises if the decay process follows a cascade mechanism through intermediate radioactive daughter isotopes. In this situation, every case must be considered individually and no generalizations are possible.

At its simplest, the biological half life is the time required for the amount of a particular substance in an organism to be reduced to one-half of its value by biological processes when the rate of removal is approximately exponential. Substances with a long biological half life will tend to accumulate in the body and are, therefore, particularly to be avoided. Knowing that they accumulate in the body is useful but not in itself sufficient to assess the consequences and to take steps to minimize them. Different organs have different half lives for the presence of the same substance. For example, methylmercury has been reported to have a half life of about 50 days in the liver and 150 days in the brain. Thus, the tendency for accumulation in the brain is greater and so is the risk of brain damage. Since the overall half life for methylmercury in the human body is only 70 days, it is clear that there is a danger of underestimating risk if only total body half life is assessed.

An increasingly important aspect of half life determination is the half life for disappearance from the natural environment, especially of man-made toxicants. Persistence in the natural environment is seen as a substance property which must eventually lead to problems simply because all substances are toxic at a given concentration. The toxic concentration will eventually be reached for even the least toxic substance if it persists sufficiently long and is constantly being added to the environment. This concept underlies the definition of persistent organic pollutants (POPs), which are defined as persistent if:

1. There is the potential for long-range transboundary atmospheric transport. Necessary evidence includes a half life in air of > 2 days;
2. there is aquatic persistence: half life in water > 2 months;
3. there is persistence in soils: half life in soil > 6 months; or
4. there is persistence in sediments: half life in sediments > 6 months.

There is a problem in applying these criteria in that laboratory tests to establish half lives for organic pollutants cannot simulate all the environmental possibilities contributing either to breakdown or stabilization of compounds. In particular, since most organic breakdown in the natural environment is due to microorganisms, the presence or absence of appropriate microorganisms is often the determining factor in half life assessment and so there may be a large difference between laboratory estimates and environmental behavior simply because of variability in this factor.

Further problems arise in assessing half lives of inorganic compounds in the natural environment. While "mineralization", breakdown of organic compounds to carbon dioxide and water is considered the "harmless" end of their natural life, no similar criterion is available for inorganics. Historically, most inorganics containing metallic elements have been defined toxicologically in relation to their content of the metallic element. Thus, toxicologists have talked loosely of the toxicity of chromium or nickel when in fact most of the toxicological effects relate to specific forms of these metals such as chromate anions

or nickel tetracarbonyl. In consequence, regulatory authorities have regulated for levels of the element in environmental media, sometimes defining a relevant oxidation state as with chromium(VI). Thus, since elements are persistent by definition, all inorganic elements regulated in this way automatically become persistent inorganic pollutants (PIPs). However, where testing has been done, most simple elements in the pure elemental state show low toxicity, mainly because they are not bioavailable. Thus, there is a need for a better approach to regulation of inorganic pollutants incorporating an appropriate method for determining environmental half life of those compounds which are genuinely toxic.

Substances with a short biological half life may nevertheless accumulate if a small amount becomes tightly bound or the organism makes more receptor molecules, even if most is cleared from the body rapidly. There is also the possibility that substances with a short biological half life may have cumulative toxic effects. This is the most difficult situation for the toxicologist to interpret, but it may be quite common in long-lived organisms such as human beings. Thus, care must be taken to understand the toxicokinetic metabolism of any substance before regarding it as relatively harmless on the basis of a short half life.

Similar reservations must be applied to consideration of substances with short half lives in environmental media. Half life is measured in relation to the bulk of the chemical present, and it may be that a small fraction persists in association with a component of the environment which is small in quantity but ecologically important or essential for a species which has colonized a unique niche. Again, interpretation of half life for risk assessment must be cautious and based on as full as possible an understanding of environmental chemistry and ecology.

14. HAZARD

IUPAC definition

hazard
Set of inherent properties of a substance, mixture of substances, or a process involving substances that, under production, usage or disposal conditions, make it capable of causing adverse effects to organisms or the environment, depending on the degree of exposure; in other words, it is a source of danger [3].

Explanatory comment

General considerations
The IUPAC definition may not make it clear that the term "hazard" may also be applied directly to the substance, agent, source of energy, or situation having hazardous properties.

Just because a substance has hazardous properties does not mean that these properties will necessarily be expressed. For most toxic effects, substances must be present in relevant media at a concentration above a threshold level before any toxicity will be apparent as a result of the threshold dose being exceeded. For mutagenic, carcinogenic, and teratogenic effects, for which it is assumed that there is no threshold, the hazardous properties must be considered in terms of risk (see explanatory definition below). This requires determining the relationship of risk of these properties being expressed to exposure in terms of concentration and/or dose. This process will be discussed under the definition of "risk".

Hazard assessment
Hazard assessment is the process designed to determine factors contributing to the possible adverse effects of a substance to which a human population or an environmental compartment could be exposed. The process includes three steps: hazard identification, hazard characterization, and hazard evaluation (see Fig. 6). Factors affecting toxicity may include metabolism, dose–effect and dose–response relationships, and variations in target susceptibility, amongst others.

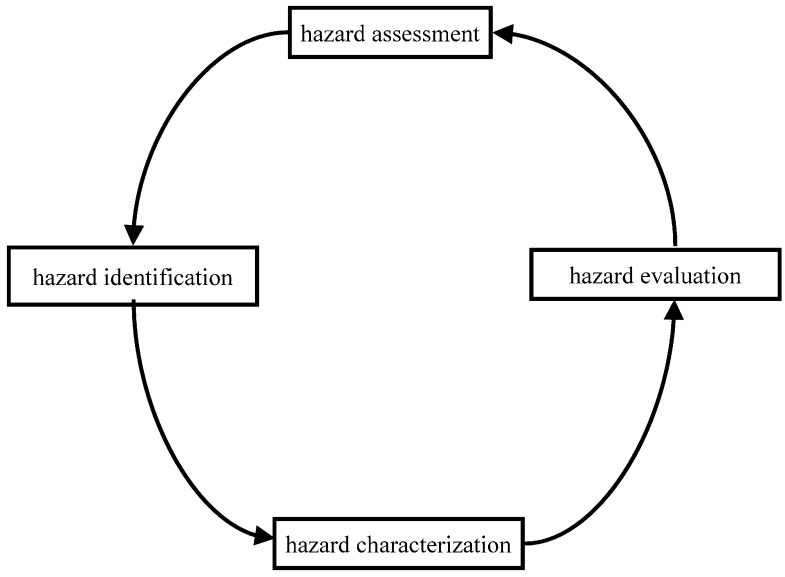

Fig. 6 Hazard assessment concept diagram.

Hazard identification

The first stage in hazard assessment is the determination of substances of concern and the adverse effects that they may have on target systems under defined conditions of exposure, taking into account all the data relating to toxicity, especially relevant physicochemical properties such as volatility and solubility and aerodynamic diameter, particle size. A list is made of these substances along all the available relevant information. Usually the relevant information will be available in the form of material safety data sheets (MSDS). In many countries there is a legal requirement that MSDS must be provided by chemical producers or suppliers. It is also a normal legal requirement that chemicals should be labeled in such a manner that hazardous properties are clearly identified. In the European Union, appropriate "risk" and "safety" phrases must be included on the label in the language of the country where the chemicals are to be used. These phrases are allocated by an international expert group involving representation from the International Programme on Chemical Safety (IPCS). The phrases are also to be found with other evaluated hazard information on the International Chemical Safety Cards produced by IPCS and available on the INCHEM Web site and the U.S. National Institute of Occupational Safety and Health (NIOSH) Web site.

Hazard characterization

This is the second step in the process of hazard assessment. It consists of the qualitative and, wherever possible, quantitative description of the nature of the hazard associated with the agent of concern. The description may cover various aspects of the hazard considered in a holistic way. Thus, attention is given to the mechanisms of action of the agent, the biological extrapolation of these mechanisms to physiological consequences, dose–response and dose-effect relationships, amongst other properties likely to be relevant to the specific circumstances under consideration. It is particularly important to define as precisely as possible any related uncertainties as these are essential for subsequent risk assessment.

Hazard evaluation

This is the third step in the process of hazard assessment aiming at the determination of the qualitative and quantitative relationship between exposure to a hazard and the resultant adverse effects under the defined conditions of exposure which may give concern. As in hazard characterization, it is important

to define and include any attendant uncertainties. In risk assessment, assessment of probability of exposure is related to hazard evaluation and a level of exposure likely to produce a significant level of harm (see Risk).

Hazard quotient (HQ)
Ratio of toxicant exposure (estimated or measured) to a reference value regarded as corresponding to a threshold of toxicity: if the total hazard quotient from all toxicants to a target exceeds unity, the combination of toxicants may produce (will produce under assumptions of additivity) an adverse effect.

15. INTERACTION

Terms related to interaction—additive effect, potentiation, synergism, antagonism

IUPAC definitions

additive effect
Consequence that follows exposure to two or more physicochemical agents which act jointly but do not interact: the total effect is the simple sum of the effects of separate exposures to the agents under the same conditions [3].

potentiation
Dependent action in which a substance or physical agent at a concentration or dose that does not itself have an adverse effect enhances the harm done by another substance or physical agent [3].

synergism (in toxicology)
Pharmacological or toxicological interaction in which the combined biological effect of two or more substances is greater than expected on the basis of the simple summation of the toxicity of each of the individual substances [3].

antagonism
Combined effect of two or more factors, which is smaller than the solitary effect of any one of those factors. In bioassays, the term may be used when a specified effect is produced by exposure to either of two factors but not by exposure to both together [3].

Explanatory comment

Terms related to interaction
When an organism is exposed to two or more substances that produce a particular physiological effect, these substances may or may not interact. If there is no interaction, the effects would be strictly additive; this is intuitively obvious, and the parent Glossary defines additive effect accordingly. The substances would in general each show a dose response–effect individually, and the effects would be strictly additive at any combination of concentrations. This shifts our focus from the substances to the effect: additivity or other descriptors of interaction do not describe the substances themselves, but rather the effects they elicit. A corollary is that to assert that two substances behave in an additive fashion requires that no statistically significant difference can be demonstrated between measurements made upon exposure to the substances together, compared to the sum of the individual exposures. With this reasoning, several points should be made:

1. Additivity (or lack thereof) refers only to what can be measured. Therefore, it refers only to specific effects. Two substances that may be additive with respect to a certain effect, may be non-additive with respect to other effects. For example, two drugs might be strictly additive with respect

to an effect on blood pressure, but have non-additive effects on liver function. So-called drug–drug interactions often refer to non-additivity with respect to side effects.

2. Additivity may occur at some concentration ratios and not others. Because any substance may be nontoxic at some levels and toxic at others (Paracelsus—it is only a question of the dose), non-additive effects might be observed only when one component reaches a critical, threshold concentration. For instance, two anticancer drugs might have additive effectiveness until one reached a threshold concentration for suppressing angiogenesis, at which point the effectiveness of the other might increase based on an ability to target hypoxic tissue.
3. Additivity, in the strictest sense, is in general probably not the norm. The complexity of biological systems is such that multiple effects will probably occur with any bioactive agent, and overlap with the effects of a second agent will produce candidate effects for non-additivity.

So, when two or more substances are related through a common toxic, therapeutic, or other biological effect, yet their effect is non-additive with respect to a measured parameter, this interaction is described by several different terms. In the case where the effect of one substance is diminished by the presence of a second, the situation is fairly straight forward. The Glossary defines antagonism as the "combined effect of two or more factors which is smaller than the solitary effect of any one of those factors". A straightforward addendum in the Glossary is that "in bioassays, the term antagonism may be used when a specified effect is produced by exposure to either of two factors but not by exposure to both together". In this case, it is not necessary to specify which of the two factors is decreasing the activity of the other; both are assumed to have a certain activity, which when they are present together is less than additive. This distinguishes antagonism from inhibition, where one substance may or may not elicit an effect common with another, but is nevertheless capable of antagonizing that response. When exposure is to more than two substances, i.e., to mixtures, outcomes are often quite difficult to predict.

Perhaps a more challenging distinction is between potentiation and synergism, situations where the measured effect of two or more agents is greater than that attributed to either alone. The Glossary entries list potentiation as "Dependent action in which a substance or physical agent at a concentration or dose that does not itself have an adverse effect enhances the harm done by another substance or physical agent", and synergism as "(in toxicology) Pharmacological or toxicological interaction in which the combined biological effect of two or more substances is greater than expected on the basis of the simple summation of the toxicity of each of the individual substances". In essence, potentiation refers to an effect of substance A to increase the effect of B, when A itself does not cause the same effect as B, whereas synergism means that A and B share a common effect, which is greater than additive when both are present.

Maintaining this distinction between potentiation and synergy may not be very useful. One argument would be that if B has no influence on an effect that is elicited by A, but when present increases the effect of A, then it would be said to potentiate the effect of A. On the other hand, if B has an effect in common with A, and both when present together give an effect that is greater than the sum produced by both alone, then we would call that synergism. But this distinction is not straightforward, because it is often experimentally difficult to determine whether the very effect measured may be elicited by one substance only in the presence of the other. Suppose that A and B do not interact but elicit a common effect, E, with contributions from isolated exposures of E_A and E_B, respectively, such that when given together $E_{AB} = E_A + E_B$ (additivity). Now suppose, on the other hand, that B can influence the effect of A (E_A), so that this effect of A has a "pure" component, E_{Aa}, and a component dependent on the presence of B, E_{Ab}. Then $E_A = E_{Aa} + E_{Ab}$. Ignoring for the moment any effect of A on E_B, we can write $E_{AB} = E_{Aa} + E_{Ab} + E_B$. If $E_{Ab} = 0$, we have additivity. If $E_{Ab} < 0$, we have antagonism. But if $E_{AB} > E_A + E_B$, is this because $E_{Ab} > 0$ with $E_B = 0$ (potentiation) or with $E_B > 0$ (synergy)? The decision may be difficult experimentally, when one considers that A may also have an influence on E_B (i.e., $E_{AB} = E_{Aa} + E_{Bb} + E_{Ab} + E_{Ba}$). Even if B has no effect in isolation, E_B may be non-zero only in the presence of A.

Finally, it should be noted that some textbooks of pharmacology recommend against the use of potentiation, referring to any increase above additivity ($E_{AB} > E_A + E_B$) as synergism, regardless of whether either E_A or E_B is zero.

16. LOCAL EFFECT AND SYSTEMIC EFFECT

IUPAC definitions

local effect
Change occurring at the site of contact between an organism and a toxicant [3].

systemic
Relating to the body as a whole [3].

systemic effect
Consequence that is either of a generalized nature or that occurs at a site distant from the point of entry of a substance [3].

Note: A systemic effect requires absorption and distribution of the substance in the body.

Explanatory comment

local effect
Local effects occur at the first site of contact on or in the body where application of the toxicant or exposure to it takes place. Examples are the immediate damage to the skin of contact with alkalis or acids, their similar corrosive effects on the intestine following ingestion, or the direct effects of inhaled gases such as chlorine on the lungs. Chlorine causes swelling of the lung tissues, which may be fatal even if little or none is taken into the bloodstream.

Local effects generally occur quite rapidly after exposure, although consequences such as lung edema may be prolonged, and they may precede systemic effects which then are of different nature. An example of that is cadmium, which upon inhalation of high doses gives rise to lung edema as an acute effect and renal tubular damage as a systemic effect. Thus, the identification of local effects can help to ensure rapid removal of anyone at risk from the exposure situation and also to provide prompt treatment of the intoxication, which may help to prevent systemic effects occurring. In some cases, the systemic effects observed may be secondary biological consequences of the local damage and not due directly to the harmful substance, for example, kidney damage following severe acid destruction of the skin.

systemic effect
Systemic effects occur when a substance is absorbed, through the skin, from the gut, from the lungs, by injection, or is taken up by any other route, enters into the general blood circulation, is transported to various organs throughout the body and gives rise to effects on these organs. Once taken up in an organ, redistribution can occur by a release to the bloodstream and the agent can be taken up in another organ. This is seen for many metals, such as cadmium and lead. Most substances which are not highly reactive at body surfaces tend to produce systemic effects. Some substances produce both serious local and systemic effects. Tetraethyl lead produces effects on skin at the site of absorption, which means that there is a local effect and then, after absorption, an effect on the central nervous system and other organs. Another example is phenol.

In general, while systemic toxicity may produce effects throughout the body, the major effects are on only one or more organs. These organs are referred to as target organs. However, the first organ that develops an adverse effect is called the critical organ for that particular toxicant. Many times, it is discussed whether the damage is reversible or irreversible. Although target organs show the most serious toxic effects, they are not necessarily the sites of highest accumulation of the toxicant. For example,

lead is concentrated in bone, but its most serious effects are probably those on the brain. Common target organs are the brain and central nervous system, the circulatory system, the blood and hemopoietic system, the liver, kidneys, lungs, and skin. Systemic effects often have target organ-specific names, for example, neurotoxic (affecting the central nervous system), cardiotoxic (affecting the heart), hepatotoxic (affecting the liver), or nephrotoxic (affecting the kidney).

Increasing dose may permit a substance which causes a local effect to enter the affected organism and cause a systemic effect on a particular target organ. Increasing dose further will increase the number of target organs and effects until the whole organism is affected if it is not already dead.

17. RATE IN EPIDEMIOLOGY

IUPAC definitions

rate (in epidemiology)
Measure of the frequency with which an event occurs in a defined population in a specified period of time [3].

Note 1: Most such rates are ratios, calculated by dividing a numerator, e.g., the number of deaths, or newly occurring cases of a disease in a given period, by a denominator, e.g., the average population during that period.

Note 2: Some rates are proportions, i.e., the numerator is contained within the denominator.

rate constant, k
Proportionality that relates the rate of a chemical reaction to some function of reactant concentrations [3].

Explanatory comment

The rate of a chemical reaction is a straightforward concept and refers to the amount of substance that reacts, i.e., is converted to another chemical species, in a given period of time. Such rates are preferentially expressed in molar units divided by time. Thus, the IUPAC "Gold Book" defines rate as a derived quantity in which time is a denominator quantity, adding that the rate of x is $\mathrm{d}x/\mathrm{d}t$. A rate constant in chemical kinetics is, then, a proportionality constant that adds the dimension of time to the relationship in concentration between two species. This fairly obvious point is made because we will distinguish two different uses of rate that are important in toxicology and toxicokinetics. One meaning which is consistent with the time base refers to the rate of transfer of a substance between different compartments, pools, sources, or sinks with time as the denominator. Formally, we can treat a substance with the same mathematical formalism whether it is reacting chemically to produce a new product, or being shuttled between compartments. Another meaning of rate is perhaps less obvious to the chemist: in epidemiology, a rate is more generally a frequency where the denominator may be a population. In the following paragraphs, we introduce a number of important rates that can be discussed based on intercompartment trafficking. Then we return to the idea of rate as a frequency.

A discussion of some important rates could begin with mention of the rate of uptake from external media. Factors affecting the uptake of a substance into the body invoke discussion of bioavailability, and then the substance is transported (described by formal rates in compartmental analysis), metabolized (metabolic rate, and see biotransformation or bioconversion), or eliminated (compare with rates of clearance or elimination). Regarding clearance, the GFR is an important concept. It refers to the volume of serum ultrafiltrate cleared of a substance by passage through the glomerular capillaries, again per time.

Clearance from a compartment may reflect more generally metabolism, excretion, or transfer to another compartment. For instance, the rate of clearance of a reactive organic intermediate would contribute to the clearance of the substance. But, we also speak of a rate of biotransformation. This concept is closest to chemical terminology, where the biotransformation usually reflects an enzymatically catalyzed phase I or phase II reaction. The rate here is a clear chemical rate constant, where the mechanism is known or presumed.

The somewhat different use of rate in epidemiology requires further explanation. We often think of a rate as a change with time, and the concept of a rate as a proportionality factor is not intuitively obvious. In its first use, the term is closely connected with the chemical (arithmetical) concept of the rate constant. When we add the qualification that "the numerator is contained within the denominator" we move out of the strict dimension of time to allow a rate or proportionality relative to another denominator. For example, prevalence "rate" in epidemiology is the total number of all individuals who have an attribute or disease at a particular time divided by the population at risk of having the attribute or disease at this point in time. Thus, the numerator, the number of individuals with the attribute, is included in the denominator, the total population at risk. In this epidemiological usage, we are calculating a proportion and not a temporal rate in the physicochemical sense.

Rate is defined in the *Shorter Oxford English Dictionary* [5] as "a stated numerical proportion between two sets of things (the second usually expressed as unity), especially as a measure of amount or degree (*moving at a rate of 50 miles per hour*) or as the basis of calculating an amount or value (*rate of taxation*) or rapidity of movement or change (*travelling at a great rate*)". In the past, a mathematical derivative was described in words as, e.g., the *rate* of change of y with respect to x, with no implication that time has to be involved. We can also say that the Gaussian probability distribution decreases at a rapid rate on each side of its maximum, among many other examples. "Rate" in chemistry should always be coupled with "of reaction" or "of diffusion", etc., to indicate that we refer explicitly to a particular *time* dependence.

Use of the word "frequency" in the definition of the epidemiological sense of rate again introduces the concept of time—though in a different sense—as here frequency involves a certain number of occurrences in a fixed period of time. We would speak of the number of events (e.g., deaths) from a particular cause, based on the whole population, as a rate. Here, the denominator would logically be a number of people, and the resulting rate would be a dimensionless proportionality. The rate, then, is the number of affected or identified individuals divided by the whole population. We define "incidence" as the number of new individuals succumbing to a particular event or illness in a period of time. Prevalence is the number of events existing per unit population at a given time.

So, in epidemiological terms, both incidence and prevalence refer to time as a base, and thus are rates, though they express somewhat different concepts. Incidence is the number of new cases, e.g., of individuals falling ill, normalized by population, in a period of time, whereas prevalence is defined as the number of incidences of a disease or other events existing at a given point in time. Rates are affected by environmental and physiological conditions. In conclusion, knowledge of rates of exchange among body compartments is the key to producing effective toxicokinetic models. Knowledge of rates in epidemiology as proportions is central to developing environmental models that are needed for risk assessment.

18. REACTIVE OXYGEN SPECIES

IUPAC definition

reactive oxygen species (ROS)
Intermediates in the reduction of molecular O_2 to water [3].
Examples: superoxide $O_2^{-\bullet}$, hydrogen peroxide H_2O_2, and hydroxyl $HO^{\bullet}$.

Explanatory comment

Cells produce energy from mitochondrial respiration, which involves a stepwise, four-electron reduction of molecular oxygen. In defining "reactive oxygen species" (ROS) as the intermediates that occur in the biological reduction of molecular O_2 to water, we acknowledge the central role of aerobic respiration in human biology. At the same time, we restrict the term to several oxygen species, each with its own distinct chemistry and important role in biology. Because no chemical process is completely efficient, the intermediates in respiration necessarily "leak" from the reaction pathway to some extent, and serve as the cell's major source of exposure to ROS.

The stepwise reduction is depicted in Fig. 7. Molecular oxygen, or dioxygen (O_2) exists in a double-bonded triplet ground state with an excess of two π-bonding electrons. Acceptance of the first electron into an antibonding orbital reduces the bond strength and creates the radical anion species superoxide ($O_2^{-\bullet}$). A second electron, also accepted into a π antibonding orbital, further reduces the bond strength, and produces the singly bonded peroxide species (O_2^{2-}). Because the pK_a values of this species are >14 and 11.8, it exists as H_2O_2 under biological conditions. The next electron, also accepted into an antibonding orbital, breaks the single bond, and the resulting $HO^\bullet$ is reduced to hydroxide and water. The one-electron redox potentials shown in Fig. 7 are useful for determining whether a step will occur spontaneously in the presence of a redox-active metal complex. This principle has been used, for example, in designing chelating agents for iron that will avoid catalytic generation of $HO^\bullet$.

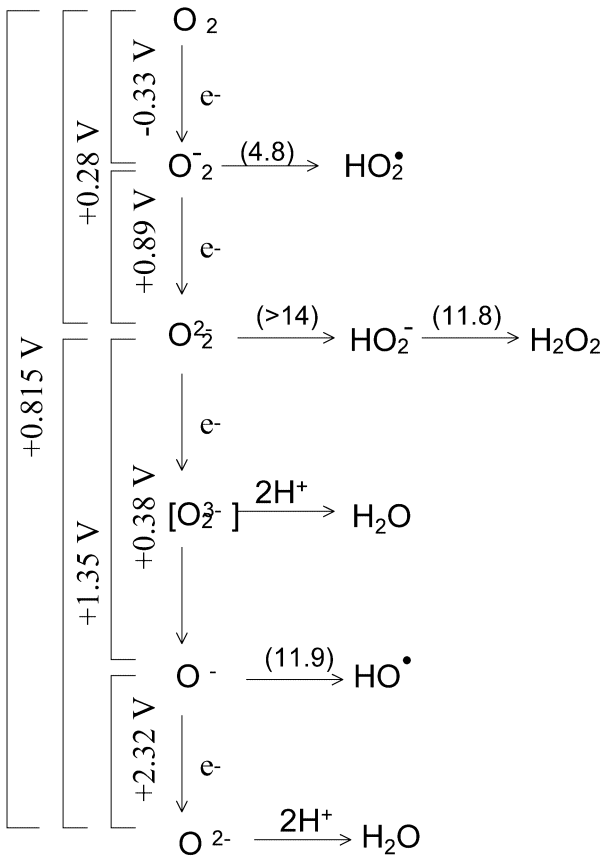

Fig. 7 Four-electron stepwise reduction of molecular oxygen to water. Numbers in parentheses are pK_a values for protonation of the intermediates. Redox potentials for various reductions are shown to the left in volts at pH = 7.

The traditional descriptor "reactive" is somewhat misleading; for a radical, superoxide, is relatively stable and persists to diffuse into the extracellular space. For instance, reperfusion injury (see below) can be diminished by scavenging superoxide from the extracellular space. The $HO^{\bullet}$ radical, on the other hand, reacts with carbon–carbon bonds at diffusion-controlled rates, and so is an extremely harmful species. Peroxide is mainly harmful due to generation of $HO^{\bullet}$ either through homolytic ($HOOH \rightarrow 2HO^{\bullet}$) or reductive heterolytic ($HOOH + e^{-} \rightarrow HO^{\bullet} + HO^{-}$) cleavage of its O–O bond.

The reduction of molecular oxygen to water occurs not only in the mitochondrion where it is coupled to oxidative phosphorylation, but also by the action of oxidative enzymes in the endoplasmic reticulum, lysosomes, peroxisomes, and even in the cytosol. While leakage of intermediates from respiration is a major source of intracellular ROS in aerobic organisms, other sources are also significant. Absorption of high-energy electromagnetic radiation (e.g., X-ray or UV) can permit radiolysis of water to yield $HO^{\bullet} + H^{\bullet}$. Bursts of superoxide occur when neutrophils are activated by appropriate stimuli that occur during inflammation. This involves an NADPH oxidase activity that uses a protein complex known as cytochrome b_{558} to shuttle electrons from within the cell to reduce O_2 at the cell surface. This machinery also exists in some other cells. In addition, during purine metabolism, the enzyme xanthine oxidase uses O_2 as an electron acceptor in the conversion of hypoxanthine to uric acid, thus generating superoxide.

Transition metals with redox potentials in a biologically accessible range, such as iron and copper, can accept and donate electrons in a catalytic fashion. The Fenton reaction with ROS (Fig. 8) is extremely important because it generates $HO^{\bullet}$; it is a catalytic cycle, and available iron can generate large amounts of reactive $HO^{\bullet}$ quickly.

$$1)\quad O_2^{-} + Fe^{3+} \longrightarrow O_2 + Fe^{2+}$$

$$2)\quad Fe^{2+} + H_2O_2 + H^{+} \longrightarrow Fe^{3+} + H_2O + HO^{\bullet}$$

$$O_2^{-} + H_2O_2 + H^{+} \longrightarrow O_2 + H_2O + HO^{\bullet}$$

Fig. 8 The iron-catalyzed Fenton reaction. The overall reaction (below the line) is the sum of reactions 1 and 2.

ROS are important in toxicology because of the cellular and molecular structures they target. On the structural level, major targets are the cell membrane (determining cellular integrity), mitochondria (not only providing the cell with energy, but determining its fate through the role the mitochondrial permeability transition pore and cytochrome c ?? release play in apoptosis), and the nucleus. On a molecular level, this is characterized by damage targeted to lipids, proteins, and nucleic acids. Double bonds in lipids of the bilayer membranes of cells and organelles are subject to attack by ROS (especially $HO^{\bullet}$). The lipids then form peroxides that themselves propagate the injury. A consequence of losing internal membrane integrity is the inability to control ion and water fluxes. Oxidative damage to proteins ranges from indirect effects such as the formation of unnatural disulfide bonds in an oxidizing atmosphere, to direct poisoning of enzymes through attack of essential residues. Oxidation of many proteins also targets them for proteosomal degradation. Nucleic acid mutations can lead to short- and long-term effects. Short-term effects include changes in gene expression and cell phenotype that may be overcome with rapid activation of DNA repair mechanisms. Longer-term effects include either apoptosis or the potential for malignant transformation if genetic defects are not repaired.

ROS are rarely discussed without reference to the many defense mechanisms that have evolved to protect cellular structures against them. These include: (i) enzymes such as superoxide dismutase and catalase that eliminate superoxide and peroxide, respectively; (ii) antioxidants such as ascorbate and vitamins E and A; (iii) the glutathione/glutathione peroxidase system; and (iv) proteins that sequester po-

tentially Fenton-active metals, such as ferritin for iron or metallothionein for copper. Despite these defenses, excess production of ROS is harmful. It is a major contributor to long-term tissue damage in diseases such as hemochromatosis, where excess accumulation of iron results in increased Fenton activity. Another process in which overproduction of ROS causes extensive tissue damage is reperfusion injury. Following infarction of a tissue, restoration of perfusion with oxygenated blood results in a rapid production of ROS, notably superoxide, and tissue damage is exacerbated. Damage following a myocardial infarction, for example, can be decreased with radical scavengers, antioxidants, metal chelators, superoxide dismutase, and catalase.

Though generally thought of as harmful in the field of toxicology, ROS are an essential part of normal cell function; they serve as rapid, diffusible signalling molecules. For example, basal levels of H_2O_2 are required for signalling by the platelet-derived growth factor. And generation of superoxide at the surface of activated neutrophils is necessary for efficient killing of invading bacteria. Patients with genetic defects in the superoxide-generating cytochrome b_{558} machinery develop constrictive granulomas, especially in the lungs (chronic granulomatous disease) as a result of repeated and persistent infections.

The term "oxygen-centered radical" should be avoided. Simple molecular radical species containing nitrogen are also of biological importance, and include the NO^-, $NO^\bullet$, and NO^+ species. Without distinction of where electron density is centered, these are sometimes referred to as reactive nitrogen species. They are important in regulating vascular physiology by eliciting smooth muscle relaxation, and their metabolism includes protein thionitrosylation and formation of peroxynitrite.

19. RISK

IUPAC definitions

risk

1. Probability of adverse effects caused under specified circumstances by an agent in an organism, a population, or an ecological system [3].
2. Expected frequency of occurrence of a harmful event arising from such an exposure [3].

risk assessment

Identification and quantification of the risk resulting from a specific use or occurrence of a chemical or physical agent, taking into account possible harmful effects on individuals or populations exposed to the agent in the amount and manner proposed and all the possible routes of exposure. After [3].

Note: Quantification ideally requires the establishment of dose–effect and dose–response relationships in likely target individuals and populations.

exposure assessment

Process of measuring or estimating concentration (or intensity), duration, and frequency of exposures to an agent present in the environment or, if estimating hypothetical exposures, that might arise from the release of a substance, or radionuclide, into the environment [3].

Explanatory comments

General considerations

Emphasis is placed on the concept of risk as a measure of probability. There is no mention here of the severity of the adverse effects which is sometimes incorporated in definitions of risk such as risk = (probability of unwanted event) × (severity of event). This is because assessment of severity, except at extremes, is essentially a subjective judgment and is part of the definition of hazard. It is important to keep considerations of risk as objective as possible because they determine what management decisions are to be taken following risk assessment (see below). If management decisions are to be effective, they

must be accepted by those to whom they apply. Acceptance depends first of all on agreement on the level of risk. It is therefore important to eliminate subjective elements as far as possible from this first stage of risk assessment. The second stage involves the subjective determination of risk acceptability (see below) and this depends, amongst other things on perception of the severity of the adverse effect for which the risk has been determined.

risk assessment
Identification and quantification of the risk resulting from a specific use or occurrence of an agent, taking into account possible harmful effects on individuals exposed to the agent in the amount and manner proposed and all the possible routes of exposure. Quantification requires the establishment of dose–effect and dose–response relationships in likely target individuals and populations. The process includes four steps: hazard identification, dose–response assessment, exposure assessment, and risk characterization.

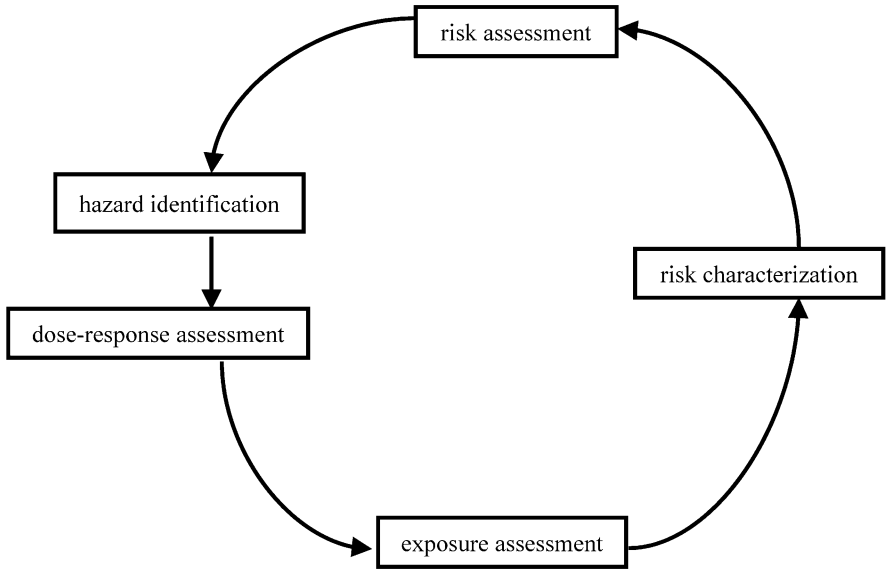

Fig. 9 Risk assessment concept diagram.

hazard identification
Already described under "hazard" above, but the other concepts in the diagram are defined as below.

dose–response assessment
Second of four steps in risk assessment, consisting of the analysis of the relationship between the total amount of an agent absorbed by each of a group of organisms and the changes developed in the group in reaction to the agent, and inferences derived from such an analysis with respect to the entire population.

It should be noted that dose–response assessment always involves extrapolation of results from an experimental or observational group (a sample) to an entire population. Thus, there is a degree of uncertainty resulting from this procedure. Such uncertainty should be determined statistically and clearly stated to inform subsequent management decisions.

exposure assessment
Third step in the process of risk assessment.

1. quantitative and qualitative analysis of the presence of an agent (including its derivatives) that may be present in a given environment and the inference of the possible consequences it may have for a given population of particular concern. Exposure often changes with time and so a calculated mean exposure over a given time interval may be used as the basis for risk assessment. In this case, special attention may need to be given to the possible occurrence of short term peaks of extreme exposure.
2. determination, using a range of different techniques, of the amount of a chemical, physical, or biological agent that could be present in a given medium and the fate of such agent under a number of potential circumstances, and the inference of possible consequences for a hypothetical system that could be affected by this agent. This determination may be based on theoretical considerations and use computer modeling to predict movement and distribution of a substance within one or more environmental compartments at risk.

risk characterization

Preferred definition

Integration of evidence, reasoning, and conclusions collected in hazard identification, dose–response assessment, and exposure assessment and the estimation of the probability, including attendant uncertainties, of occurrence of an adverse effect if an agent is administered to, taken by, or absorbed by a particular organism or population. It is the last step of risk assessment.

Note: In ecological risk assessment, concentration–response assessment is carried out instead of dose–response assessment.

Alternative definition

Qualitative and/or quantitative estimation, including attendant uncertainties, of the severity and probability of occurrence of known and potential adverse effects of a substance in a given population. Compare this with "risk evaluation". This definition requires consideration of all possible effects and their severity. Although this is a fine objective, it is unlikely to be attainable in practice because of its complexity. In the end, the objective can be most nearly reached by combining risk characterizations carried out according to the preferred definition.

risk evaluation

Establishment of a qualitative or quantitative relationship between risks and benefits, involving the complex process of determining the significance of the identified hazards and estimated risks to those organisms or people concerned with or affected by them. It is the first step in risk management and includes economic, ethical, and other nonscientific considerations.

Note: Synonymous with risk–benefit evaluation.

In order to compare risk and benefit, these concepts must be defined in a compatible manner. Since risk is defined in terms of probability of harm, benefit must here be defined in terms of probability of a good outcome. It will also help if "harm" and "good outcome" can be quantitatively defined in the same units.

emission and exposure control

Regulation of emission of potentially toxic substances in order to ensure that exposure to these substances is kept below a level likely to be harmful to humans or other species at risk. This usually involves establishing and agreeing guidelines or legal standards for acceptable ambient concentrations in environmental media together with a system for monitoring exposure and enforcement of standards.

risk monitoring

Process of following up the decisions and actions within risk management in order to ascertain that risk containment or reduction with respect to a particular hazard is assured.

acceptable risk
Type of risk such that the perceived benefits derived by an organism, a population, or an ecological system outweigh the adverse effects that might affect them as a result of administration or exposure to a particular agent.

Acceptance of risk is subjective and dependent upon perception. Different people and groups of people may have very different perceptions, and thus "acceptable risk" can have no absolute definition, for example, in terms of a certain level of probability.

risk management
Decision-making process involving considerations of political, social, economic, and technical factors with relevant risk assessment information relating to a hazard so as to develop, analyze, and compare regulatory and nonregulatory options and to select and implement the optimal decisions and actions for safety from that hazard.

Essentially, risk management is the combination of three steps: risk evaluation, emission and exposure control, and risk monitoring. These steps have been defined above.

20. STRUCTURE–ACTIVITY RELATIONSHIP (SAR)

IUPAC definitions

structure–activity relationship (SAR)
Association between specific aspects of molecular structure and defined biological action.

See also quantitative structure–activity relationship [3].

structure–metabolism relationship (SMR)
Association between the physicochemical and/or the structural properties of a substance and its metabolic behavior [3].

quantitative structure–activity relationship (QSAR)
Quantitative structure–biological activity models derived using regression analysis and containing as parameters physicochemical constants, indicator variables, or theoretically calculated values [3].

Note: The term is extended by some authors to include chemical reactivity, where activity and reactivity are regarded as synonyms. The extension is discouraged.

quantitative structure–metabolism relationship (QSMR)
Quantitative association between the physicochemical and (or) the structural properties of a substance and its metabolic behavior [3].

Explanatory comment

SAR
In toxicology, SAR methods apply various mathematical and statistical models to predict the adverse effects of chemicals based upon their structure. The prediction may be qualitative (e.g., is a substance likely to cause cancer?) or quantitative, QSAR (what level of dose will produce a given effect?). Such methods give results which can be used in various ways. They may indicate a need for further experimentation and evaluation and can be used to select toxicity tests for predicted end-points of concern. This includes prioritizing tests so that likely effects are tested first, which may eliminate the need for further testing.

There is a hope that SAR methods will eventually be an adequate replacement for animal testing, but the current state of the art is not good enough to permit this. In particular, the more possible mechanisms that are associated with an effect, the more difficult and consequently less accurate is any pre-

diction. However, in drug development, animal testing may be avoided for certain compounds for which SAR clearly indicates the potential for serious adverse effects.

Elucidation of SARs is best developed for organic compounds and is still poorly developed for inorganic compounds. For organic compounds, identification of SARs requires knowledge of the biological activities of defined chemical structures, of biological interactions when structures occur in the same molecule, and the derivation of models which can be used to relate total molecular structure to biological effects.

Creation of models uses physicochemical data along with manual pattern recognition methods, cluster analysis, and regression analysis. For meaningful models, data from a substantial number of compounds with differing substituent combinations and well-defined biological effects are required. The main difficulty is in analyzing the data in order to identify particular structural fragments responsible for the production of a defined effect. Even if such fragments are identified, the question remains as to whether these fragments are sufficient in themselves to produce the effect, whether they are always necessary for this effect, and whether the effect is modified by the molecular environment.

SAR methods are available for organic molecules to predict genotoxicity, carcinogenesis, dermal irritation and sensitisation, lethality, biological oxygen demand, and teratogenicity, with varying degrees of accuracy. USEPA and USFDA use models for mutagenicity/carcinogenicity to screen for possible problem compounds.

QSAR

The common view of toxicologists is that QSAR is a screening tool that will catch approximately 60 to 70 % of the tested end-point. QSAR has been validated in a number of studies which show that two-thirds of expected toxicity will be predicted. The corresponding figure for genotoxicity and carcinogenicity can be more than 70 %. This has been found for a restricted number of chemicals. For reproductive toxicity, the predictive value is low. It is important to keep in mind that QSAR is based on available databases, which means that there is a limited knowledge with regard to new chemicals and also to chemicals with presently uncharacterized effects. These attempts at quantitative predictions are currently imprecise and inaccurate, but the field is developing rapidly and QSAR is a valuable tool when used with caution.

21. TERMS APPLIED TO TOXIC SUBSTANCES

IUPAC definitions with comment

biocide

Substance intended to kill living organisms [2].

Comment: This term as defined above includes all pesticides and related substances. Somewhat confusingly, in European legislation the term "biocides" excludes agricultural pesticides, plant protection products, medicines, and cosmetics, which are covered by other more specific legislation, and includes only those pesticides which are used for certain restricted purposes such as preserving wood, preventing ship fouling, disinfection, controlling mice and rats, and controlling domestic insects such as cockroaches and ants. However, this still leaves in the European legislation a large number of potentially harmful substances of the types listed below:

MAIN GROUP 1	Disinfectants and general biocidal products
Product-type 1	Human hygiene biocidal products
Product-type 2	Private area and public health area disinfectants and other biocidal products
Product-type 3	Veterinary hygiene biocidal products
Product-type 4	Food and feed area disinfectants
Product-type 5	Drinking water disinfectants

MAIN GROUP 2	Preservatives
Product-type 6	In-can preservatives
Product-type 7	Film preservatives
Product-type 8	Wood preservatives
Product-type 9	Fiber, leather, rubber, and polymerized materials preservatives
Product-type 10	Masonry preservatives
Product-type 11	Preservatives for liquid-cooling and processing systems
Product-type 12	Slimicides
Product-type 13	Metalworking-fluid preservatives
MAIN GROUP 3	Pest control
Product-type 14	Rodenticides
Product-type 15	Avicides
Product-type 16	Molluscicides
Product-type 17	Piscicides
Product-type 18	Insecticides, acaricides, and products to control other arthropods
Product-type 19	Repellents and attractants
MAIN GROUP 4	Other biocidal products
Product-type 20	Preservatives for food or feedstocks
Product-type 21	Antifouling products
Product-type 22	Embalming and taxidermist fluids
Product-type 23	Control of other vertebrates

drug
Any substance that, when absorbed into a living organism, may modify one or more of its functions [2].

Comment: The term is generally accepted for a substance taken for a therapeutic purpose, but is also commonly used for substances of abuse. Just as any substance can be a toxicant, so any substance can be a drug. The term carries with it the implication of use for medical purposes, but also the potential for abuse to produce an effect desired by the abuser, but which is ultimately harmful.

pesticide
Substance intended to kill pests: in common usage, any substance used for controlling, preventing, or destroying animal, microbiological, or plant pests [1].

Comment: As with most of the terms in this group, the definition can only be applied with knowledge of the intended use of the substance. Almost any substance can be a pesticide if it is used for that purpose. For example, acetone can be used to kill most insects, but it is unusual to use it for this purpose and so it is not normally classified as a pesticide. Similarly, sodium chloride can kill most plants, but is not classified as a pesticide. Thus, the term as used in practice is usually based on some official list produced for regulatory purposes and has little scientific logic behind it. This may lead to careless use by people who do not understand that many pesticides are not specific for the pests to which they are applied but can harm people as well. This may be a particular problem with herbicides, which are named as though they were specific for killing plants although they may be—like paraquat—extremely toxic to humans.

poison (in toxicology)
Substance that, taken into or formed within the organism, impairs the health of the organism and may kill it [3].

Comment: This word comes from the Greek *potein* to drink and hence has the same root as the word "potion". When love potions were devised, their use to affect other people, often to their harm, gradually led to the idea of poison as we use the word today (see "venom" below). It may be related to the Irish word "poteen", which means illegally distilled Irish whisky.

toxicant
This is the preferred term for a substance that is considered to be toxic under circumstances which are thought likely to happen [3].

Comment: This word comes from the Greek *toxikos* = of or for the bow and was originally applied to the poison used to tip arrows. The term "poison" is nearly a synonym, but tends to be applied to substances that may be deliberately used for poisoning, such as pesticides, and often has overtones of criminal use.

toxic substance (agent, chemical, material)
Material causing injury to living organisms as a result of physicochemical interactions.
After [3]

Comment: To the toxicologist, any substance is potentially toxic since it is a matter of dose and so the distinction between toxic and nontoxic is arbitrary. For regulatory purposes, it has been historically based on the short term (acute) LD_{50}, but this is a very unsatisfactory basis for such classification as it is essentially a rather poor (single point) measure of the capacity to kill mammals as surrogates for humans and it is not an absolute measure since, even with similar test populations, it can vary considerably. Many animals have died for this classification, which tells us little about doses causing sublethal or chronic effects, for example, mutations or cancer, which are of major concern for human health. Thus, labelling substances as harmful, toxic, or very toxic on the basis of the LD_{50} is of limited value as substances which cause serious sublethal and/or chronic effects may not be labelled as harmful or toxic in spite of their significant potential for harm.

toxin
Poisonous substance produced by a biological organism such as a microbe, animal, or plant [3].

Comment: Like "toxicant", this word comes from the Greek *toxikos* = of or for the bow and was originally applied to the poison, usually extracted from plants, which was used to tip the arrows. In turn, it may derive from *taxus*, the yew tree, from which arrows were made and which has berries that are poisonous. "Toxin" has been commonly used as a synonym for "toxicant", but this usage is unacceptable since the distinction between naturally occurring toxicants produced by living organisms (true "toxins") and synthetic toxicants is an important one. It is particularly inappropriate to apply the term to inorganic toxicants since no inorganic toxin is known.

venom
Animal toxin generally used for self-defense or predation and usually delivered by a bite or sting [2].

Comment: This term derives from the word "wen" meaning to wish, from which developed "venus", "venery", and venerate", all related to concepts of love. A love potion became a "venin", and this became "venom".

xenobiotic
Compound with a chemical structure foreign to a given organism [3].

Comment: The term is usually restricted to manmade compounds. It originates from the Greek words *xenos* = foreign and *biotikos* = living. True toxins as defined above are never referred to as xenobiotics although they may occur in circumstances which satisfy the above definition.

Other related definitions

medicine
Any drug or remedy.

Comment: Again this definition depends on usage. Any substance, e.g., herbs, willow bark, or honey, may be used as a drug or a remedy and, as always, the end effect will depend on the dose.

pharmaceutical
Medicinal drug.

Comment: The Greek root *pharmakon* also means "enchanted potion" or "poison". This term may have a legal definition in national legislation.

22. VOLUME OF DISTRIBUTION

IUPAC definition

volume of distribution
Apparent (hypothetical) volume of fluid required to contain the total amount of a substance in the body at the same concentration as that present in the plasma, assuming equilibrium has been attained [3].

Explanatory comment

Volume of distribution can be expressed as:

V_d/litre = (dose/mg)/(plasma concentration/mg L^{-1})

or = (dose/mol)/(plasma concentration/mol L^{-1})

From this relationship, it can be seen that lower plasma concentrations imply a higher volume of distribution of the substance while higher plasma concentrations imply a lower V_d. A value for the V_d for a given substance of about 5 L would imply the substance is primarily in the plasma. On the other hand, a V_d of much more than 5 L implies that the substance is more widely distributed through the body. A value of more than 50 L indicates that the compound is accumulated in the body.

If a toxic substance is mostly bound to plasma proteins such as albumin, the V_d will approximate to the plasma volume. If a toxic substance is highly lipid soluble, and distributes mainly to adipose tissue, the plasma concentration will be low and the V_d will be larger than the plasma volume and may even exceed the volume of total body water.

The V_d has certain limitations. The volume of distribution is a theoretical measurement, and the possibility that it may exceed the volume of total body water emphasizes this fact. Toxic substances have different affinities for different body tissues, and the observation of a large V_d does not indicate the location of the relevant toxic substance in the body. Even where this is known, it must be remembered that the main location of the substance may not be its site of action. For example, organochlorines

accumulate in fatty tissue, but their site of action may be on the nervous system or on the reproductive system.

Plasma concentration and hence volume of distribution changes over time, and so a single determination of V_d gives much less information than a time course study.

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ANNEX 1: ABBREVIATIONS AND ACRONYMS

AIC	Akaike Information Criterion: a statistical procedure that provides a measure of the goodness-of-fit of a dose–response model to a set of data. The AIC is calculated from the equation AIC = $2k - 2\ln(L)$, where k is the number of parameters, and L is the likelihood function. Usually, normally distributed errors are assumed and AIC is computed as AIC = $2k + n\ln(RSS/n)$, where n is the number of observations and RSS is the residual sum of squares.
ADME	absorption, distribution, metabolism, and excretion
ANSI	American National Standards Institute
ATP	adenosine triphosphate
BMC	benchmark concentration
BMCL	confidence limit for BMC
BMD	benchmark dose
BMDL	confidence limit for BMD
BMDS	benchmark dose at a given standard deviation
BMR	benchmark rate
CL	confidence limit
DNA	deoxyribonucleic acid
EEA	European Environmental Agency
EC	effective concentration
ECB	European Chemicals Bureau

ED_x	effective dose for a biological effect in x % of the individuals in the test population
GFR	glomerular filtration rate
HQ	hazard quotient
IAEA	International Atomic Energy Authority
IEEE	Institute of Electrical and Electronics Engineers
IPCS	International Programme on Chemical Safety
IUPAC	International Union of Pure and Applied Chemistry
LC_{50}	median concentration lethal to 50 % of a test population
LD_{50}	median dose lethal to 50 % of a test population
LED_x	lowest effective dose for a biological effect in x % of the individuals in the test population
LOAEL	lowest-observed-adverse-effect level
MFO	mixed function oxidase
NADPH	nicotinamide adenine dinucleotide phosphate (reduced)
NAG	*N*-acetyl-D-glycosaminidase
NAS	National Academy of Science
NIOSH	U.S. National Institute of Occupational Safety and Health
NOAEL	no-observed-adverse-effect level
PBPK	physiologically based pharmacokinetic modeling
PBPD	physiologically based pharmacodynamic modeling
PBTK	physiologically based toxicokinetic modeling
PEL	permissible exposure limit
PIPS	persistant inorganic pollutant
PK	pharmacokinetic
POP	persistant organic pollutant
QSAR	quantitative structure–activity relationship
QSMR	quantitative structure–metabolism relationship
REACH	Registration, Evaluation and Authorisation of CHemicals
RfC	reference concentration
RfD	reference dose
ROS	reactive oxygen species
SAR	structure–activity relationship; specific (standard) absorption rate
SD	standard deviation
SMR	structure–metabolism relationship
SE	standard error
TEF	toxicity equivalency factor
TEQ	toxicity equivalent
UDP	uridine diphosphate
USEPA	United States Environment Protection Agency
USFDA	United States Food and Drug Agency
W_R	quality factor (radiation)